

CARNEGIE INSTITUTION OF WASHINGTON

YEAR BOOK No. 48

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With Administrative Reports through December 9, 1949



CARNEGIE INSTITUTION OF WASHINGTON
WASHINGTON, D. C.

1949

THE LCMO 1A(TiM0»E FuSS, BALTIMORE, MARYLAND

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PRESIDENT AND TRUSTEES

PRESIDENT

VANNEVAR BUSH

BOARD OF TRUSTEES

WALTER S. GIFFORD, *Chairman*

ELIHU ROOT, JR., *Vice-Chairman*

LEWIS H. WEED, *Secretary*

JAMES F. BELL	CARYL P. HASKINS	SEELEY G. MUDD
ROBERT WOODS BLISS	BARKLIE HENRY	WILLIAM I. MYERS
LINDSAY BRADFORD	{HERBERT HOOVER	HENNING W. PRENTIS, JR.
OMAR N. BRADLEY	§FRANK B. JEWETT	ELIHU ROOT, JR.
^FREDERIC A. DELANO	ERNEST O. LAWRENCE	HENRY R. SHEPLEY
HOMER L. FERGUSON	ALFRED L. LOOMIS	CHARLES P. TAFT
W. CAMERON FORBES	ROBERT A. LOVETT	JUAN T. TRIPPE
fjAMES FORRESTAL	ROSWELL MILLER	JAMES W. WADSWORTH
WALTER S. GIFFORD	HENRY S. MORGAN	LEWIS H. WEED

Executive Committee

WALTER S. GIFFORD, *Chairman*

ROBERT WOODS BLISS
VANNEVAR BUSH

HENRY S. MORGAN

HENNING W. PRENTIS, JR.

ELIHU ROOT, JR.

HENRY R. SHEPLEY

LEWIS H. WEED

finance Committee

LINDSAY BRADFORD, *Chairman*

ALFRED L. LOOMIS

HENRY S. MORGAN

HENNING W. PRENTIS, JR.

ELIHU ROOT, JR.

Auditing Committee

ROSWELL MILLER, *Chairman*

HOMER L. FERGUSON

JAMES W. WADSWORTH

Nominating Committee

HENNING W. PRENTIS, JR., *Chairman*

JAMES F. BELL

LINDSAY BRADFORD

WALTER S. GIFFORD

Committee on Astronomy

SEELEY G. MUDD, *Chairman*

ROSWELL MILLER

ELIHU ROOT, JR.

JUAN T. TRIPPE

Committee on Terrestrial Sciences

ERNEST O. LAWRENCE, *Chairman*

HOMER L. FERGUSON

BARKLIE HENRY

Committee on Biological Sciences

LEWIS H. WEED, *Chairman*

CARYL P. HASKINS

ALFRED L. LOOMIS

CHARLES P. TAFT

WILLIAM I. MYERS

Committee on Historical Research

HENRY R. SHEPLEY, *Chairman*

JAMES F. BELL

ROBERT WOODS BLISS

JAMES W. WADSWORTH

* Resigned October 13, 1949.
t Deceased May 22, 1949.

X Resigned September 15, 1949.
§ Deceased November 18, 1949.

FORMER PRESIDENTS AND TRUSTEES

PRESIDENTS

DANIEL COIT GILMAN, 1902-1904 ROBERT SIMPSON WOODWARD, 1904-1920
 JOHN CAMPBELL MERRIAM, *President* 1921-1938; *President Emeritus* 1939-1945

TRUSTEES

ALEXANDER AGASSIZ	1904-05	SETH LOW	1902-16
GEORGE J. BALDWIN	1925-27	WAYNE MACVEAGH	1902-07
THOMAS BARBOUR	1934-46	ANDREW J. MELLON	19 ² 4"37
JOHN S. BILLINGS	1902-13	DARIUS O. MILLS	1902-09
ROBERT S. BROOKINGS	1910—29	S. WEIR MITCHELL	1902-14
JOHN L. CADWALADER	1903-14	ANDREW J. MONTAGUE	1907-35
WILLIAM W. CAMPBELL	1929-38	WILLIAM W. MORROW	1902-29
JOHN J. CARTY	1916-32	WILLIAM CHURCH OSBORN	19 ² 7-34
WHITEFOORD R. COLE	19 ² 5"34	JAMES PARMELEE	1917-31
FREDERIC A. DELANO	1927-49	WM. BARCLAY PARSONS	1907-32
CLEVELAND H. DODGE	1903-23	STEWART PATON	1916-42
WILLIAM E. DODGE	1902—03	GEORGE W. PEPPER	1914-19
CHARLES P. FENNER	1914-24	JOHN J. PERSHING	193 ^o -43
SIMON FLEXNER	1910—14	HENRY S. PRITCHETT	1906-36
JAMES FORRESTAL	1948-49	GORDON S. RENTSCHLER	1946-48
WILLIAM N. FREW	1902—15	ELIHU ROOT	1902-37
LYMAN J. GAGE	1902—12	JULIUS ROSENWALD	1929-31
CASS GILBERT	1924-34	MARTIN A. RYERSON	1908-28
FREDERICK H. GILLETT	1924-35	THEOBALD SMITH	1914-34
DANIEL C. GILMAN	1902—08	JOHN C. SPOONER	1902—07
JOHN HAY	1902-05	WILLIAM BENSON STOREY	19 ² 4-39
MYRON T. HERRICK	1915-29	RICHARD P. STRONG	1934-48
ABRAM S. HEWITT	1902—03	WILLIAM H. TAFT	1906-15
HENRY L. HIGGINSON	1902—19	WILLIAM S. THAYER	1929-32
ETHAN A. HITCHCOCK	1902-09	CHARLES D. WALCOTT	1902-27
HENRY HITCHCOCK	1902—02	FREDERIC C. WALCOTT	1931-48
HERBERT HOOVER	1920—49	HENRY P. WALCOTT	1910-24
WILLIAM WIRT HOWE	1903—09	WILLIAM H. WELCH	1906-34
CHARLES L. HUTCHINSON	1902—04	ANDREW D. WHITE	1902-03
WALTER A. JESSUP	*93 ⁸ "44	EDWARD D. WHITE	1902-03
FRANK B. JEWETT	1933-49	HENRY WHITE	1913-27
SAMUEL P. LANGLEY	1904—06	GEORGE W. WICKERSHAM	1909-36
CHARLES A. LINDBERGH	*934-39	ROBERT S. WOODWARD	1905-24
WILLIAM LINDSAY	1902—09	CARROLL D. WRIGHT	1902—08
HENRY CABOT LODGE	1914-24		

Besides the names enumerated above, the following were *ex-officio* members of the Board of Trustees under the original charter, from the date of organization until April 28, 1904: the President of the United States, the President of the Senate, the Speaker of the House of Representatives, the Secretary of the Smithsonian Institution, the President of the National Academy of Sciences.

STAFF OF INVESTIGATORS FOR THE YEAR 1949

ASTRONOMY

MOUNT WILSON AND PALOMAR OBSERVATORIES

8JJ Santa Barbara Street, Pasadena 4, California

Mount Wilson Observatory organized in 1904; George E. Hale, Director 1904—1923, Honorary Director 1923-1936; Walter S. Adams, Director 1924—1945. Unified operation with the Palomar Observatory of the California Institute of Technology began April 1, 1948.

IRA S. BOWENJ *Director*
WALTER BAADE
HORACE W. BABCOCK
JESSE L. GREENSTEIN
EDWIN P. HUBBLE
MILTON L. HUMASON
JOSEF J. JOHNSON
PAUL W. MERRILL
RUDOLPH L. MINKOWSKI

SETH B. NICHOLSON
EDISON PETTIT
ROBERT S. RICHARDSON
ROSCOE F. SANFORD
ALBERT G. WILSON
OLIN C. WILSON
RALPH E. WILSON
FRITZ ZWICKY

TERRESTRIAL SCIENCES

GEOPHYSICAL LABORATORY

2801 Upton Street, N.W., Washington 8, D. C.

Organized in 1906, opened in 1907; Arthur L. Day, Director 1909-1936

LEASON H. ADAMS, *Director*
NORMAN L. BOWEN
JOHN S. BURLEW
FELIX CHAYES
GORDON L. DAVIS
JOSEPH L. ENGLAND
*ROY W. GORANSON
JOSEPH W. GREIG
FRANK C. KRACEK
GEORGE W. MOREY

J. FRANK SCHAIRER
O. FRANK TUTTLE
WILLIAM D. URRY
HATTEN S. YODER
JEMANUEL G. ZIES

Visiting Investigators

MACKENZIE LAWRENCE KEITH
KAARLO J. NEUVONEN
§THURE G. SAHAMA

DEPARTMENT OF TERRESTRIAL MAGNETISM

5241 Broad Branch Road, N.W., Washington 15, D. C.

Organized in 1904; Louis A. Bauer, Director 1904-1929; John A. Fleming, Acting Director 1929-1934, Director 1935—1946.

MERLE A. TUVE, *Director*
PHILIP H. ABELSON
LLOYD V. BERKNER
DEAN B. COWIE
SCOTT E. FORBUSH
NORMAN P. HEYDENBURG
*ELLIS A. JOHNSON
RICHARD B. ROBERTS
11 WILLIAM J. ROONEY
HOWARD E. TATEL
OSCAR W. TORRESON
ERNEST H. VESTINE
GEORGE R. WAIT
HARRY W. WELLS

Research Associate

HUGH H. DARBY

Visiting Investigators

EDGAR O. BOWLES
WILLIAM R. DURYEE
JOHN W. GRAHAM
ARTHUR T. NESS
ROBERT T. NIESET
IRENA Z. ROBERTS
MANUEL S. VALLARTA

* On leave of absence,
f Resigned in 1949.
% Retired in 1949.

§Term of appointment completed in 1949.
‡Deceased August 31, 1949.

CARNEGIE INSTITUTION OF WASHINGTON

BIOLOGICAL SCIENCES

DIVISION OF PLANT BIOLOGY

Central Laboratory, Stanford, California

Desert Laboratory, opened in 1903, became headquarters of Department of Botanical Research in 1905. Name changed to Laboratory for Plant Physiology in 1923; reorganized in 1928 as Division of Plant Biology, including Ecology. Herman A. Spoehr, Chairman 1927-1930 and 1931-1947, Chairman Emeritus 1947--.

C. STACY FRENCH, <i>Director</i>	HAROLD W. MILNER
JENS C. CLAUSEN	JAMES H. C. SMITH
PAUL GRUN	HERMAN A. SPOEHR
WILLIAM M. HIESEY	*HAROLD H. STRAIN
DAVID D. KECK	

DEPARTMENT OF EMBRYOLOGY

Wolfe and Madison Streets, Baltimore 5, Maryland

Organized in 1914; Franklin P. Mall, Director 1914-1917; George L. Streeter, Director 1918-1940

GEORGE W. CORNER, <i>Director</i>	SAMUEL R. M. REYNOLDS
ROBERT K. BURNS	DAVID B. TYLER
LOUIS B. FLEXNER	
CHESTER H. HEUSER, <i>Curator of the Embryological Collection</i>	<i>Research Associate</i> ELIZABETH M. RAMSEY

DEPARTMENT OF GENETICS

Cold Spring Harbor, Long Island, New York

Station for Experimental Evolution opened in 1904; name changed to Department of Experimental Evolution in 1906; combined with Eugenics Record Office in 1921 to form Department of Genetics. Charles B. Davenport, Director 1904-1934; Albert F. Blakeslee, Director 1935-1941.

MILISLAV DEMEREC, <i>Director</i>	MARGARET R. MCDONALD
BERWIND P. KAUFMANN	
EDWIN C. MACDOWELL	<i>Research Associate</i>
BARBARA MCCLINTOCK	ERNEST W. CASPARI

HISTORICAL RESEARCH

DIVISION OF HISTORICAL RESEARCH

10 Frisbie Place, Cambridge 38, Massachusetts

Department of Historical Research organized in 1903; Andrew C. McLaughlin, Director 1903-1905; J. Franklin Jameson, Director 1905-1928. In 1930 this Department was incorporated as a section of United States history in a new Division of Historical Research.

ALFRED V. KIDDER, <i>Chairman</i>	GEORGE SARTON
HARRY E. D. POLLOCK, <i>Executive Officer</i>	ANNA O. SHEPARD
MARGARET W. HARRISON, <i>Editor</i>	EDWIN M. SHOOK
EARL H. MORRIS	A. LEDYARD SMITH
ALEXANDER POGO	ROBERT E. SMITH
TATIANA PROSKOURIAKOFF	GUSTAV STRÖMSVIK
RALPH L. ROYS	J. ERIC S. THOMPSON
KARL RUPPBRT	

* On leave of absence.

+ Retired in 1949.

STAFF OF INVESTIGATORS FOR THE YEAR 1949

RESEARCH ASSOCIATES

RESEARCH ASSOCIATES ENGAGED IN POST-RETIREMENT STUDIES

WALTER S. ADAMS, Astronomy

HERBERT E. MERWIN, Geophysics

ALFRED H. JOY, Astronomy

EMANUEL G. ZIES, Geophysics

RESEARCH ASSOCIATES CONNECTED WITH OTHER INSTITUTIONS

JOSEPH C. BOYCE (New York University), Physics

RALPH W. CHANEY (University of California), Paleobotany

T. H. DOBZHANSKY (Columbia University), Genetics

FRANK T. GUCKER, JR. (Indiana University), Chemistry

CARYL P. HASKINS (Haskins Laboratories), Biology

ARTHUR T. HERTIG (Boston Lying-in Hospital), Embryology

E. A. LOWE (The Institute for Advanced Study), Paleogeography

ROBERT REDFIELD (University of Chicago), Anthropology

FRANCE V. SCHOLLES (University of New Mexico), History

OFFICES OF ADMINISTRATION

Office of the President

VANNEVAR BUSH, *President*
PAUL A. SCHERER, *Executive Officer*
SAMUEL CALLAWAY, *President's Secretary*

Office of Publications and Public Relations

FREDERICK G. FASSETT, JR., *Director*
AILENE J. BAUER, *Assistant to the Director*
DOROTHY R. SWIFT, *Editor*

Adviser in International Scientific Relations

JOHN A. FLEMING

Office of the Bursar

EARLE B. BIESECKER, *Bursar*
J. STANLEY LINGEBACH, *Assistant Bursar*
JAMES F. SULLIVAN, *Assistant to the Bursar*

Investment Office (New York City)

PARKER MONROE, *Investment Officer*
RICHARD F. F. NICHOLS, *Assistant Investment Officer*

ORGANIZATION, PLAN, AND SCOPE

The Carnegie Institution of Washington was founded by Andrew Carnegie, January 28, 1902, when he gave to a board of trustees an endowment of registered bonds of the par value of ten million dollars. To this fund an addition of two million dollars was made by Mr. Carnegie on December 10, 1907, and a further addition of ten million dollars was made by him on January 19, 1911. Furthermore, the income of a reserve fund of about three million dollars, accumulated in accordance with the founder's specifications in 1911, is now available for general use, and in recent years a total of ten million dollars has been paid by the Carnegie Corporation of New York as increase to the Endowment Fund of the Institution. The Institution was originally organized under the laws of the District of Columbia and incorporated as the *Carnegie Institution*, articles of incorporation having been executed on January 4, 1902. The Institution was reincorporated, however, by an act of the Congress of the United States, approved April 28, 1904, under the title of the *Carnegie Institution of Washington*. (See existing Articles of Incorporation on following pages.)

Organization under the new Articles of Incorporation was effected May 18, 1904, and the Institution was placed under the control of a board of twenty-four trustees, all of whom had been members of the original corporation. The trustees meet annually in December to consider the affairs of the Institution in general, the progress of work already undertaken, and the initiation of new projects, and to make the necessary appropriations for the ensuing year. During the intervals between the meetings of the trustees the affairs of the Institution are conducted by an Executive Committee chosen by and from the Board of Trustees and acting through the President of the Institution as chief executive officer.

The Articles of Incorporation of the Institution declare in general "that the objects of the corporation shall be to encourage, in the broadest and most liberal manner, investigation, research, and discovery, and the application of knowledge to the improvement of mankind."

The Institution is essentially an operating organization. It attempts to advance fundamental research in fields not normally covered by the activities of other agencies, and to concentrate its attention upon specific problems, with the idea of shifting attack from time to time to meet the more pressing needs of research as they develop with increase of knowledge. Some of these problems require the collaboration of several investigators, special equipment, and continuous effort. Many close relations exist among activities of the Institution, and a type of organization representing investigations in astronomy, in terrestrial sciences, in biological sciences, and in historical research has been effected. Conference groups on various subjects have played a part in bringing new vision and new methods to bear upon many problems. Constant efforts are made to facilitate interpretation and application of results of research activities of the Institution, and an Office of Publications and Public Relations provides means for appropriate publication.

ARTICLES OF INCORPORATION

PUBLIC NO. 260. An Act to incorporate the Carnegie Institution of Washington.

Be it enacted by the Senate and House of Representatives of the United States of America in Congress assembled, That the persons following, being persons who are now trustees of the Carnegie Institution, namely, Alexander Agassiz, John S. Billings, John L. Cadwalader, Cleveland H. Dodge, William N. Frew, Lyman J. Gage, Daniel C. Gilman, John Hay, Henry L. Higginson, William Wirt Howe, Charles L. Hutchinson, Samuel P. Langley, William Lindsay, Seth Low, Wayne MacVeagh, Darius O. Mills, S. Weir Mitchell, William W. Morrow, Ethan A. Hitchcock, Elihu Root, John C. Spooner, Andrew D. White, Charles D. Walcott, Carroll D. Wright, their associates and successors, duly chosen, are hereby incorporated and declared to be a body corporate by the name of the Carnegie Institution of Washington and by that name shall be known and have perpetual succession, with the powers, limitations, and restrictions herein contained.

SEC. 2. That the objects of the corporation shall be to encourage, in the broadest and most liberal manner, investigation, research, and discovery, and the application of knowledge to the improvement of mankind; and in particular—

(a) To conduct, endow, and assist investigation in any department of science, literature, or art, and to this end to cooperate with governments, universities, colleges, technical schools, learned societies, and individuals.

(b) To appoint committees of experts to direct special lines of research.

(c) To publish and distribute documents.

(d) To conduct lectures, hold meetings, and acquire and maintain a library.

(e) To purchase such property, real or personal, and construct such building or buildings as may be necessary to carry on the work of the corporation.

(f) In general, to do and perform all things necessary to promote the objects of the institution, with full power, however, to the trustees hereinafter appointed and their successors from time to time to modify the conditions and regulations under which the work shall be carried on, so as to secure the application of the funds in the manner best adapted to the conditions of the time, provided that the objects of the corporation shall at all times be among the foregoing or kindred thereto.

SEC. 3. That the direction and management of the affairs of the corporation and the control and disposal of its property and funds shall be vested in a board of trustees, twenty-two in number, to be composed of the following individuals: Alexander Agassiz, John S. Billings, John L. Cadwalader, Cleveland H. Dodge, William N. Frew, Lyman J. Gage, Daniel C. Gilman, John Hay, Henry L. Higginson, William Wirt Howe, Charles L. Hutchinson, *Samuel P. Langley*, William Lindsay, Seth Low, Wayne MacVeagh, Darius O. Mills, S. Weir Mitchell, William W. Morrow, *Ethan A. Hitchcock*, Elihu Root, John C. Spooner, Andrew D. White, Charles D. Walcott, Carroll D. Wright, who shall constitute the first board of trustees. The board of trustees shall have power from time to time to increase its membership to not more than twenty-seven members. Vacancies occasioned by death, resignation, or otherwise shall be filled by the remaining trustees in such manner as the by-laws

ARTICLES OF INCORPORATION

shall prescribe; and the persons so elected shall thereupon become trustees and also members of the said corporation. The principal place of business of the said corporation shall be the city of Washington, in the District of Columbia.

SEC. 4. That such board of trustees shall be entitled to take, hold, and administer the securities, funds, and property so transferred by said Andrew Carnegie to the trustees of the Carnegie Institution and such other funds or property as may at any time be given, devised, or bequeathed to them, or to such corporation, for the purposes of the trust; and with full power from time to time to adopt a common seal, to appoint such officers, members of the board of trustees or otherwise, and such employees as may be deemed necessary in carrying on the business of the corporation, at such salaries or with such remuneration as they may deem proper; and with full power to adopt by-laws from time to time and such rules or regulations as may be necessary to secure the safe and convenient transaction of the business of the corporation; and with full power and discretion to deal with and expend the income of the corporation in such manner as in their judgment will best promote the objects herein set forth and in general to have and use all powers and authority necessary to promote such objects and carry out the purposes of the donor. The said trustees shall have further power from time to time to hold as investments the securities hereinabove referred to so transferred by Andrew Carnegie, and any property which has been or may be transferred to them or such corporation by Andrew Carnegie or by any other person, persons, or corporation, and to invest any sums or amounts from time to time in such securities and in such form and manner as are permitted to trustees or to charitable or literary corporations for investment, according to the laws of the States of New York, Pennsylvania, or Massachusetts, or in such securities as are authorized for investment by the said deed of trust so executed by Andrew Carnegie, or by any deed of gift or last will and testament to be hereafter made or executed.

SEC. 5. That the said corporation may take and hold any additional donations, grants, devises, or bequests which may be made in further support of the purposes of the said corporation, and may include in the expenses thereof the personal expenses which the trustees may incur in attending meetings or otherwise in carrying out the business of the trust, but the services of the trustees as such shall be gratuitous.

SEC. 6. That as soon as may be possible after the passage of this Act a meeting of the trustees hereinbefore named shall be called by Daniel C. Gilman, John S. Billings, Charles D. Walcott, S. Weir Mitchell, John Hay, Elihu Root, and Carroll D. Wright, or any four of them, at the city of Washington, in the District of Columbia, by notice served in person or by mail addressed to each trustee at his place of residence; and the said trustees, or a majority thereof, being assembled, shall organize and proceed to adopt by-laws, to elect officers and appoint committees, and generally to organize the said corporation; and said trustees herein named, on behalf of the corporation hereby incorporated, shall thereupon receive, take over, and enter into possession, custody, and management of all property, real or personal, of the corporation heretofore known as the Carnegie Institution, incorporated, as hereinbefore set forth under "An Act to establish a Code of Law for the District of Columbia, January fourth, nineteen hundred and two," and to all its rights, contracts, claims,

CARNEGIE INSTITUTION OF WASHINGTON

and property of any kind or nature; and the several officers of such corporation, or any other person having charge of any of the securities, funds, real or personal, books, or property thereof, shall, on demand, deliver the same to the said trustees appointed by this Act or to the persons appointed by them to receive the same; and the trustees of the existing corporation and the trustees herein named shall and may take such other steps as shall be necessary to carry out the purposes of this Act.

SEC. 7. That the rights of the creditors of the said existing corporation known as the Carnegie Institution shall not in any manner be impaired by the passage of this Act, or the transfer of the property hereinbefore mentioned, nor shall any liability or obligation for the payment of any sums due or to become due, or any claim or demand, in any manner or for any cause existing against the said existing corporation, be released or impaired; but such corporation hereby incorporated is declared to succeed to the obligations and liabilities and to be held liable to pay and discharge all of the debts, liabilities, and contracts of the said corporation so existing to the same effect as if such new corporation had itself incurred the obligation or liability to pay such debt or damages, and no such action or proceeding before any court or tribunal shall be deemed to have abated or been discontinued by reason of the passage of this Act.

SEC. 8. That Congress may from time to time alter, repeal, or modify this Act of incorporation, but no contract or individual right made or acquired shall thereby be divested or impaired.

SEC. 9. That this Act shall take effect immediately.

Approved, April 28, 1904.

BY-LAWS OF THE INSTITUTION

Adopted December 13, 1904. Amended December 13, 1910, December 13, 1912, December 10, 1937, December 15, 1939, December 13, 1940, December 18, 1942, and December 12, 1947

ARTICLE I

THE TRUSTEES

1. The Board of Trustees shall consist of twenty-four members, with power to increase its membership to not more than twenty-seven members. The Trustees shall hold office continuously and not for a stated term.
2. In case any Trustee shall fail to attend three successive annual meetings of the Board he shall thereupon cease to be a Trustee.
3. No Trustee shall receive any compensation for his services as such.
4. All vacancies in the Board of Trustees shall be filled by the Trustees by ballot at an annual meeting, but no person shall be declared elected unless he receives the votes of two-thirds of the Trustees present.

ARTICLE II

OFFICERS OF THE BOARD

1. The officers of the Board shall be a Chairman of the Board, a Vice-Chairman, and a Secretary, who shall be elected by the Trustees, from the members of the Board, by ballot to serve for a term of three years. All vacancies shall be filled by the Board for the unexpired term; provided, however, that the Executive Committee shall have power to fill a vacancy in the office of Secretary to serve until the next meeting of the Board of Trustees.
2. The Chairman shall preside at all meetings and shall have the usual powers of a presiding officer.
3. The Vice-Chairman, in the absence or disability of the Chairman, shall perform the duties of the Chairman.
4. The Secretary shall issue notices of meetings of the Board, record its transactions, and conduct that part of the correspondence relating to the Board and to his duties.

ARTICLE III

EXECUTIVE ADMINISTRATION

The President

1. There shall be a President who shall be elected by ballot by, and hold office during the pleasure of, the Board, who shall be the chief executive officer of the Institution. The President, subject to the control of the Board and the Executive Committee, shall have general charge of all matters of administration and supervision of all arrangements for research and other work undertaken by the Institution or with its funds. He shall prepare and submit to the Board of Trustees and to the Executive

CARNEGIE INSTITUTION OF WASHINGTON

Committee plans and suggestions for the work of the Institution, shall conduct its general correspondence and the correspondence with applicants for grants and with the special advisers of the Committee, and shall present his recommendations in each case to the Executive Committee for decision. AH proposals and requests for grants shall be referred to the President for consideration and report. He shall have power to remove, appoint, and, within the scope of funds made available by the Trustees, provide for compensation of subordinate employees and to fix the compensation of such employees within the limits of a maximum rate of compensation to be established from time to time by the Executive Committee. He shall be *ex officio* a member of the Executive Committee*

2. He shall be the legal custodian of the seal and of all property of the Institution whose custody is not otherwise provided for. He shall sign and execute on behalf of the corporation all contracts and instruments necessary in authorized administrative and research matters and affix the corporate seal thereto when necessary, and may delegate the performance of such acts and other administrative duties in his absence to the Executive Officer. He may execute all other contracts, deeds, and instruments on behalf of the corporation and affix the seal thereto when expressly authorized by the Board of Trustees or Executive Committee. He may, within the limits of his own authorization, delegate to the Executive Officer authority to act as custodian of and affix the corporate seal. He shall be responsible for the expenditure and disbursement of all funds of the Institution in accordance with the directions of the Board and of the Executive Committee, and shall keep accurate accounts of all receipts and disbursements. Following approval by the Executive Committee he shall transmit to the Board of Trustees before its annual meeting in December a written report of the operations and business of the Institution for the preceding fiscal year with his recommendations for work and appropriations for the succeeding calendar year.

3. He shall attend all meetings of the Board of Trustees.

4. There shall be an officer designated Executive Officer who shall be appointed by and hold office at the pleasure of the President, subject to the approval of the Executive Committee. His duties shall be to assist and act for the President as the latter may duly authorize and direct.

5. The President shall retire from office at the end of the calendar year in which he becomes sixty-five years of age.

ARTICLE IV

MEETINGS

1. The annual meeting of the Board of Trustees shall be held in the City of Washington, in the District of Columbia, on the first Friday following the second Thursday of December in each year unless the date and place of meeting are otherwise ordered by the Executive Committee.

2. Special meetings of the Board may be called by the Executive Committee by notice served personally upon, or mailed to the usual address of, each Trustee twenty days prior to the meeting.

3. Special meetings shall, moreover, be called in the same manner by the Chairman upon the written request of seven members of the Board.

BY-LAWS OF THE INSTITUTION

ARTICLE V

COMMITTEES

1. There shall be the following standing Committees, *viz.* an Executive Committee, a Finance Committee, an Auditing Committee, and a Nominating Committee.

2. All vacancies occurring in the Executive Committee, the Finance Committee, the Auditing Committee, and the Nominating Committee shall be filled by the Trustees at the next regular meeting. In case of vacancy in the Finance Committee, the Auditing Committee, or the Nominating Committee, upon request of the remaining members of such committee, the Executive Committee may fill such vacancy by appointment until the next meeting of the Board of Trustees.

3. The terms of all officers and of all members of committees, as provided for herein, shall continue until their successors are elected or appointed.

Executive Committee

4. The Executive Committee shall consist of the Chairman and Secretary of the Board of Trustees and the President of the Institution *ex officio* and, in addition, five trustees to be elected by the Board by ballot for a term of three years, who shall be eligible for re-election. Any member elected to fill a vacancy shall serve for the remainder of his predecessor's term.

5. The Executive Committee shall, when the Board is not in session and has not given specific directions, have general control of the administration of the affairs of the corporation and general supervision of all arrangements for administration, research, and other matters undertaken or promoted by the Institution. It shall also submit to the Board of Trustees a printed or typewritten report of each of its meetings, and at the annual meeting shall submit to the Board a report for publication.

6. The Executive Committee shall have power to authorize the purchase, sale, exchange, or transfer of real estate.

Finance Committee

7. The Finance Committee shall consist of five members to be elected by the Board of Trustees by ballot for a term of three years.

8. The Finance Committee shall have custody of the securities of the corporation and general charge of its investments and invested funds, and shall care for and dispose of the same subject to the directions of the Board of Trustees. It shall have power to authorize the purchase, sale, exchange, or transfer of securities and to delegate this power. It shall consider and recommend to the Board from time to time such measures as in its opinion will promote the financial interests of the Institution, and shall make a report at each meeting of the Board.

Auditing Committee

9. The Auditing Committee shall consist of three members to be elected by the Board of Trustees by ballot for a term of three years.

CARNEGIE INSTITUTION OF WASHINGTON

10. Before each annual meeting of the Board of Trustees, the Auditing Committee shall cause the accounts of the Institution for the preceding fiscal year to be audited by public accountants. The accountants shall report to the Committee, and the Committee shall present said report at the ensuing annual meeting of the Board with such recommendations as the Committee may deem appropriate.

Nominciting Committee

11. The Nominating Committee shall consist of the Chairman of the Board of Trustees *ex officio* and, in addition, three trustees to be elected by the Board by ballot for a term of three years, who shall not be eligible for re-election until after the lapse of one year. Any member elected to fill a vacancy shall serve for the remainder of his predecessor's term, provided that of the Nominating Committee first elected after adoption of this By-Law one member shall serve for one year, one member shall serve for two years, and one member shall serve for three years, the Committee to determine the respective terms by lot.

12. Sixty days prior to an annual meeting of the Board the Nominating Committee shall notify the Trustees by mail of the vacancies to be filled in membership of the Board. Each Trustee may submit nominations for such vacancies. Nominations so submitted shall be considered by the Nominating Committee, and ten days prior to the annual meeting the Nominating Committee shall submit to members of the Board by mail a list of the persons so nominated, with its recommendations for filling existing vacancies on the Board and its Standing Committees. No other nominations shall be received by the Board at the annual meeting except with the unanimous consent of the Trustees present.

ARTICLE VI

FINANCIAL ADMINISTRATION

1. No expenditure shall be authorized or made except in pursuance of a previous appropriation by the Board of Trustees, or as provided In Article V, paragraph 8, hereof.

2. The fiscal year of the Institution shall commence on the first day of July in each year.

3. The Executive Committee shall submit to the annual meeting of the Board a full statement of the finances and work of the Institution for the preceding fiscal year and a detailed estimate of the expenditures of the succeeding calendar year.

4. The Board of Trustees, at the annual meeting in each year, shall make general appropriations for the ensuing calendar year; but nothing contained herein shall prevent the Board of Trustees from making special appropriations at any meeting.

5. The Executive Committee shall have general charge and control of all appropriations made by the Board. Following the annual meeting each year, the Executive Committee may make allotment of funds for the period from January 1 to termination of the fiscal year on June 30. It may also make allotment of funds for the period from July 1 to December 31 in advance of July 1. The Committee shall, however, have full authority for allotment of available funds to meet necessary

BY-LAWS OF THE INSTITUTION

expenditures by other methods, if desirable, and for transfer of balances to meet special needs. It shall make provision for outstanding obligations and for reversion of unexpended balances at termination of the fiscal year.

6. The securities of the Institution and evidences of property, and funds invested and to be invested, shall be deposited in such safe depository or in the custody of such trust company and under such safeguards as the Finance Committee shall designate, subject to directions of the Board of Trustees. Income of the Institution available for expenditure shall be deposited in such banks or depositories as may from time to time be designated by the Executive Committee.

7. Any trust company entrusted with the custody of securities by the Finance Committee may, by resolution of the Board of Trustees, be made Fiscal Agent of the Institution, upon an agreed compensation, for the transaction of the business coming within the authority of the Finance Committee.

ARTICLE VII

AMENDMENT OF BY-LAWS

1. These by-laws may be amended at any annual or special meeting of the Board of Trustees by a two-thirds vote of the members present, provided written notice of the proposed amendment shall have been served personally upon, or mailed to the usual address of, each member of the Board twenty days prior to the meeting.

ABSTRACT OF MINUTES OF THE FIFTY-FIRST MEETING OF THE BOARD OF TRUSTEES

The annual meeting of the Board of Trustees was held in Washington, D. C, in the Board Room of the Administration Building, on Friday, December 9, 1949. It was called to order at 10:35 A.M. by the Chairman, Mr. Gifford.

Upon roll call, the following Trustees responded: James F. Bell, Robert Woods Bliss, Lindsay Bradford, W. Cameron Forbes, Walter S. Gifford, Ernest O. Lawrence, Alfred L. Loomis, Robert A. Lovett, Roswell Miller, Henry S. Morgan, Seeley G. Mudd, Henning W. Prentis, Jr., Elihu Root, Jr., Henry R. Shepley, Charles P. Taft, Juan T. Trippe, and James W. Wadsworth. The President of the Institution, Vannevar Bush, was also present.

The minutes of the fiftieth meeting were approved as printed and submitted to the members of the Board.

Reports of the President, the Executive Committee, the Finance Committee, the Auditor, the Auditing Committee, and the Nominating Committee, and of the heads of Divisions and Departments and Research Associates of the Institution were presented and considered.

The sum of \$1,630,478 was appropriated for expenditure by the Institution under the general charge and control of the Executive Committee.

Vacancies in the membership of the Board of Trustees caused by the death of James Forrestal and by the resignations of Frederic A. Delano and Herbert Hoover were filled by the election of Omar N. Bradley, Caryl P. Haskins, and Barklie Henry.

Henning W. Prentis, Jr., was elected a member of the Executive Committee for the term ending in 1950 to succeed the late Frank B. Jewett. Roswell Miller was elected Chairman of the Auditing Committee to succeed Mr. Delano for the term ending in 1951. Lindsay Bradford was elected a member of the Nominating Committee for a period of three years, succeeding Henry S. Morgan.

The meeting adjourned at 12:08 P.M.

REPORT OF THE EXECUTIVE COMMITTEE

FOR THE YEAR ENDED JUNE 30, 1949

To the Trustees of the Carnegie Institution of Washington:

GENTLEMEN: In accordance with the provisions of the By-Laws, the Executive Committee submits this report to the annual meeting of the Board of Trustees.

The detailed record of the activities of the Institution is presented in the reports from the Departments and Divisions, which are contained in the Year Book, a review of some of the highlights being given in the report of the President. The estimate of expenditures for the calendar year 1950 contained in the report of the President has been considered and approved by the Executive Committee, and the Committee has also provisionally approved and recommends to the Board the proposed budget based thereon.

The Board of Trustees at its meeting of December 10, 1948, appointed the firm of Haskins & Sells to audit the accounts of the Institution for the fiscal year ending June 30, 1949. The report of the Auditor, including a balance sheet showing assets and liabilities of the Institution on June 30, 1949, is submitted as a part of the report of the Executive Committee.

In addition to the report of the Auditor there is also submitted a financial statement for the fiscal year ended June 30, 1949, showing funds available for expenditure and amounts allotted by the Executive Committee, and the customary statement of aggregate receipts and disbursements, together with a schedule of real estate and equipment. These statements together with the tables in the Auditor's report comprise the statement of the finances of the Institution.

Three vacancies exist in the membership of the Board of Trustees, resulting from the death in May 1949 of James Forrestal and the resignations of Herbert Hoover in September 1949 and Frederic A. Delano in October 1949.

The term of Mr. Morgan as a member of the Nominating Committee will end at the annual meeting, and a vacancy has resulted in the membership of the Auditing Committee because of the resignation of Mr. Delano.

WALTER S. GIFFORD, *Chairman*
ROBERT WOODS BLISS
VANNEVAR BUSH
FRANK B. JEWETT
HENRY S. MORGAN
ELIHU ROOT, JR.
HENRY R. SHEPLEY
LEWIS H. WEED

October 20, 1949

FINANCIAL STATEMENT FOR THE YEAR ENDED JUNE 30, 1949

	Balances available June 30, 1948	Trustees' appropriations	Net allotments and transfers	Other credits	Total available	Expenditures	Balances available June 30, 1949 for	
							Expenditure	Allotment
Administration	\$6,129.85	\$126,750.00	\$10,670.19	\$55.98	\$143,606.02	\$140,407.51	\$3,198.51
Carnegie Corporation Emergency Fund_____	115,900.41	115,900.41	\$115,900.41
General Contingent Fund....	351,460.18	101,600.00	-78,104.63	374,955.55	374,955.55
General Operations	9,032.00	70,790.00	-59,684.29	715.00	20,852.71	20,852.71
Harriet H. Mayor Relief Fund	9,750.00	9,750.00	650.00	9,100.00
Pension Fund	187,974.09	95,000.00	7,100.23	290,074.32	110,763.85	179,310.47
General Publications	47,478.88	30,000.00	9,265.64	86,744.52	31,387.68	45,402.87	9,953.97
Office of Publications	5,711.68	35,550.00	832.71	42,094.39	36,221.86	5,872.53
Research Projects, Fellowships, Grants, etc.	166,892.08	95,024.48	42,200.00	304,116.56	99,053.95	186,090.94	18,971.67
Special Reconversion Fund. . .	12,630.81	-12,630.81
Departmental Research Operations;								
Plant Biology	16,644.62	86,100.00	-4,885.93	97,858.69	87,663.68	10,195.01
Genetics	29,015.95	132,995.00	704.75	32,881.18	195,596.88	175,478.08	20,118.80
Geophysical Laboratory....	18,703.15	201,785.00	2,199.52	150.74	222,838.41	212,179.09	10,659.32
Historical Research	4,774.15	124,213.00	-242.10	128,745.05	118,251.22	10,493.83
Mount Wilson Observatory.	22,317.48	248,400.00	-15,838.24	980.86	255,860.10	238,692.63	17,167.47
Terrestrial Magnetism	28,563.45	272,750.00	25,723.80	12.44	327,049.69	298,639.57	28,410.12
Embryology	9,094.27	100,853.00	-514.59	11,425.00	120,857.68	118,153.73	2,703.95
	<u>\$1,042,073.05</u>	<u>\$1,596,786.00</u>	<u>\$355.09</u>	<u>\$97,686.84</u>	<u>\$2,736,900.98</u>	<u>\$1,667,542.85</u>	<u>\$528,723.82</u>	<u>\$540,634.31</u>

AGGREGATE CASH RECEIPTS AND DISBURSEMENTS FROM ORGANIZATION, JANUARY 28, 1902, TO JUNE 30, 1949

RECEIPTS		DISBURSEMENTS	
<i>Securities Sold or Redeemed</i>	\$141,088,600.74	<i>Securities Purchased</i>	\$156,964,217.81
<i>Income from Securities and Bank Balances</i> ,	59,250,958.94	<i>Accrued Interest on Securities Purchased</i>	795,206.16
<i>Proceeds from Sale of Stock Dividends</i>	124,545.40	<i>Pension Fund</i>	2,052,688.22
<i>Sales of Publications</i>	401,513.16	<i>Bickel Fund</i>	90.38
<i>Bickel Fund (Bequest)</i>	300.00	<i>General Reserve Fund</i>	30,477.43
<i>Colour n Fund (Bequest)</i>	52,015.74	<i>Insurance Fund</i>	140,532.24
<i>Hah Relief Fund (Gift)</i>	2,382.28	<i>Harriman Fund</i>	346.44
<i>Harkavy Fund (Gift)</i>	3,050.00	<i>Harriet H. Mayor Relief Fund</i>	900.00
<i>Harriman Fund (Sale of Land)</i>	4,043.70	<i>Harkavy Fund</i>	221.20
<i>Teeple Fund (Bequest)</i>	10,888.42	<i>Special Emergency Reserve Fund</i>	63,819.41
<i>Van Gelder Fund (Bequest)</i>	1,278.58	<i>National Defense Revolving Fund</i>	3,062,974.97
<i>Carnegie Corporation of New York (Endowment Increase and for Specific Purposes)</i>	13,759,381.24	<i>General Contingent Fund</i>	342,289.26
<i>From Other Organizations and Individuals for Specific Purposes</i>	545,885.98	<i>Carnegie Corporation of New York Emergency Fund. Administration Building and Addition:</i>	
<i>Pension Fund (Refunds)</i>	101,482.47	<i>Construction and Site (Old Building)</i>	309,915.69
<i>General Reserve Fund</i>	79,966.54	<i>Construction (Addition to Administration Bldg.)</i>	416,206.07
<i>Insurance Fund (Refunds)</i>	13,076.02	<i>Site (Addition to Administration Building)</i>	68,570.96
<i>National Defense Revolving Fund (Refunds and Advances)</i>	3,095,347.20	<i>Miscellaneous Expenditures*</i>	40,825.37
<i>Administration Building Addition Account, Rentals and Refunds</i>	18,021.09	<i>Departmental Research Operations:</i>	
<i>Employees' Salary Deductions for the Purchase of U. S. Bonds</i>	99,353.65	<i>Departments of Research, Buildings and Equipment</i>	3,286,525.81
<i>Miscellaneous Refunds and Receipts</i>	1,205,222.32	<i>Departmental Operations</i>	38,703,980.72
	<u>\$219,857,313.47</u>	<i>Research Projects, Fellowships, Grants, etc.</i>	5,731,433.48
		<i>Publication</i>	3,201,947.29
		<i>Administration</i>	3,457,360.70
		<i>Employees' U. S. Bond Purchases</i>	99,224.40
		<i>National Research Council</i>	150,000.00
		<i>Miscellaneous</i>	28,108.36
			<u>\$219,049,307.14</u>
		<i>June 30, 1949, Cash in Banks</i>	808,006.33
			<u>\$219,857,313.47</u>

*Includes Equipment \$7,206.41, Repairs and Alterations to Old Building \$18,599.29.

REAL ESTATE AND EQUIPMENT, ORIGINAL COST

JUNE 30, 1949

<i>Administration</i>		
<i>1530 P Street, N.W., Washington 5, D. C.</i>		
Building and site.....	\$797,633.96	
Equipment.....	19,920.73	\$817,554.69
	<hr/>	
<i>Division of Plant Biology</i>		
<i>Stanford, California</i>		
Buildings and grounds.....	\$73,229.67	
Laboratory apparatus.....	40,913.65	
Library.....	23,340.81	
Operating equipment.....	18,991.99	156,476.12
	<hr/>	
<i>Department of Embryology</i>		
<i>Wolfe and Madison Streets, Baltimore 5, Maryland</i>		
Laboratory apparatus.....	\$25,358.38	
Library.....	7,976.34	
Operating equipment.....	5,311.21	38,645.93
	<hr/>	
<i>Department of Genetics</i>		
<i>Cold Spring Harbor, Long Island, New York</i>		
Buildings and grounds.....	\$275,734.60	
Laboratory apparatus.....	49,448.17	
Library.....	65,256.59	
Operating equipment.....	26,474.74	416,914.10
	<hr/>	
<i>Geophysical Laboratory</i>		
<i>2801 Upton Street, N.W., Washington 8, D. C.</i>		
Buildings and grounds.....	\$170,383.79	
Laboratory apparatus.....	140,232.39	
Library.....	36,510.70	
Operating equipment.....	39,439.34	386,566.22
	<hr/>	
<i>Division of Historical Research</i>		
<i>10 Frisbie Place, Cambridge 38, Massachusetts</i>		
Library.....	\$10,408.05	
Operating equipment.....	18,891.38	29,299.43
	<hr/>	
<i>Mount Wilson Observatory</i>		
<i>813 Santa Barbara Street, Pasadena 4, California</i>		
Buildings and grounds.....	\$268,629.81	
Instruments.....	539,722.36	
Library.....	69,726.52	
Operating equipment.....	62,489.81	
Hooker 100-inch reflector.....	641,070.49	1,581,638.99
	<hr/>	
<i>Department of Terrestrial Magnetism</i>		
<i>5241 Broad Branch Road, N.W., Washington 15, D. C.</i>		
Buildings and grounds.....	\$400,311.53	
Laboratory apparatus.....	176,696.93	
Library.....	35,651.64	
Operating equipment.....	64,324.92	676,985.02
	<hr/>	
		<hr/>
		\$4,104,080.50
		<hr/>

ACCOUNTANTS' CERTIFICATE

To the Board of Trustees of Carnegie Institution of Washington:

We have examined the balance sheet (and supporting schedule of securities owned) of Carnegie Institution of Washington as of June 30, 1949 and the related statements of income and expenditures and current funds surplus for the year then ended. Our examination was made in accordance with generally accepted auditing standards, and accordingly included such tests of the accounting records and such other auditing procedures as we considered necessary in the circumstances, except that we did not examine the records in support of expenditures made (approximately \$193,000) by five of the seven branch offices of the Institution, but we have reviewed internal audit reports of the Bursar's office covering examinations of all branch records during the year.

Effective as of July 1, 1948 the Institution adopted the policy of recording proceeds from sales of non-cash dividends, and in any case where the non-cash dividend is retained as an investment its market value on date of receipt, as income. However, generally accepted accounting principles do not recognize ordinary stock dividends as income. Therefore, proceeds of \$109,071.83 from sales of non-cash dividends during the year under review should not be taken into account as dividend income; the other non-cash distributions sold, amounting to \$15,473.57, are properly considered as dividend income.

In our opinion, subject to the exceptions stated above with respect to the limitation of the scope of our examination and the inclusion of the proceeds from the sales of ordinary stock dividends as income, the accompanying balance sheet and statements of income and expenditures and current funds surplus present fairly the financial position of the Institution at June 30, 1949 and the results of its operations for the year then ended, in conformity with generally accepted accounting principles applied on a basis consistent with that of the preceding period.

HASKINS & SELLS

October 24, 1949

BALANCE SHEET JUNE 30, 1949

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		ASSETS	
<i>Current Funds:</i>			
General:			
Cash in banks and on hand...	\$475,476.01		
Advances—Departmental Research Operations.....	12,424.09		
Accounts receivable—other.....	868.22		
Inventory—books.....	146,366.30		
Deferred charges.....	15,356.88		
Due from Endowment and Other Special Funds.....	377,963.93	\$1,028,455.43	
<hr/>			
Restricted:			
Cash in banks.....	84,533.00	\$1,112,988.43	
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<i>Endowment and Other Special Funds:</i>			
Cash in banks.....	\$263,188.93		
Securities (approximate market value \$41,916,000);			
Bonds:			
U. S. Government.....	\$9,027,873.15		
Foreign and International Bank.....	853,288.88		
Public utility.....	2,300,223.90		
Communication.....	1,168,405.65		
Railroad.....	274,484.45		
Railroad equipment trust.....	877,386.21		
Industrial and miscellaneous.....	5,225,723.79		
Stocks:			
Preferred.....	3,980,855.10		
Common.....	16,930,579.91	40,638,821.04	40,902,009.97
<hr/>			
<i>Plant Funds:</i>			
Invested in real estate and equipment:			
Office of Administration.....	\$817,554.69		
Departments of Research.....	3,286,525.81	4,104,080.50	
<hr/>			
TOTAL.....		\$46,119,078.90	

		LIABILITIES	
<i>Current Funds:</i>			
General:			
Accounts payable.....	\$273.25		
Reserve for valuation of books and accounts receivable.....	147,234.52		
Current Funds surplus:			
Reserves for unexpended appropriations:			
Administration.....	\$3,198.51		
Departmental Research Operations.....	99,748.50		
General Operations.....	20,852.71		
Publication.....	61,229.37		
Research Projects, Fellowships, Grants, etc.....	205,062.61		
<hr/>			
Total.....	\$390,091.70		
Other reserves:			
Carnegie Corporation Emergency Fund.....	115,900.41		
General Contingent Fund.....	374,955.55	880,947.66	
<hr/>			
Restricted:			
Bickel Fund.....	\$209.62		
Harkavy Fund—income account.....	195.31		
Harriman Fund—income account.....	84,128.07	84,533.00	\$1,112,988.43
<hr/>			
<i>Endowment and Other Special Funds:</i>			
Due to Current Funds.....	\$377,963.93		
Capital Funds*:			
Endowment Fund.....	\$32,000,000.00		
Capital Reserve Fund.....	5,645,235.43		
Colburn Fund.....	103,310.80		
Harkavy Fund.....	2,828.80		
Harriman Fund.....	304,043.70		
Teeple Fund.....	10,888.42		
Van Gelder Fund.....	1,278.58		
Special Funds:			
General Reserve Fund.....	2,140,012.86		
George E. Hale Relief Fund.....	3,491.58		
Harriet H. Mayor Relief Fund.....	9,100.00		
Pension Fund.....	179,310.47		
Special Income Reserve.....	124,545.40	40,524,046.04	40,902,009.97
<hr/>			
<i>Plant Funds:</i>			
Income invested in plant*.....	\$3,929,591.43		
Harriman property (gift).....	134,418.07		
Harkavy property (gift).....	2,070.00		
Solar Laboratory (Hale gift) (equipment valued at \$20,803.39 included above*).....	38,000.00		
Bickel property (bequest) (nominal value).....	.100	4,104,080.50	
<hr/>			
TOTAL.....		\$46,119,078.90	

*Represents proceeds from sale of non-cash dividends.

STATEMENT OF INCOME AND EXPENDITURES FOR THE YEAR ENDED JUNE 30, 1949

INCOME:

Investment income:			
Interest and dividends on securities	\$1,694,577.46		
Less—Amortization of bond premiums	<u>11,858.35</u>		
	\$1,682,719.11		
Proceeds from sale of stock dividends	<u>124,545.40</u>	\$1,807,264.51	
Less—Income allocated to:			
Special Income Reserve	\$124,545.40		
General Reserve Fund	85,449.25		
Pension Fund	350.23		
George E. Hale Relief Fund	17.00		
Harkavy Fund	<u>116.63</u>		
			<u>210,478.51</u>

NET INVESTMENT INCOME APPROPRIATED FOR CURRENT

PURPOSES			\$1,596,786.00
Other income:			
Sales of publications	\$9,050.87		
American Cancer Society—grants	23,062.00		
Carnegie Corporation of New York—grants	42,000.00		
Dormitory and mess hall	9,651.68		
Bickel property	\$300.00		
Z*«—Maintenance expenses	<u>90.38</u>	209.62	
Estate of John E. Teepte (deceased)	48.66		
George E. Hale Relief Fund	3,474.58		
Life Insurance Medical Research Fund	1,000.00		
U. S. Public Health Service—grants	9,125.00		
Miscellaneous	<u>9,204.39</u>		
Total	\$106,826.80		
Less—Amounts credited to:			
Restricted funds	\$209.62		
General Reserve Fund	6,402.24		
Special funds	<u>3,523.24</u>	10,135.10	96,691.70
TOTAL INCOME*			<u>\$1,693,477.70</u>

EXPENDITURES:

Pension Fund—annuity and insurance	\$110,763.85		
Harriet H. Mayor Relief Fund	.650.00		
Departmental Research Operations:			
Salaries	\$929,770.45		
Operating expenses	308,353.17		
Dormitory and mess hall—salaries	3,386.00		
Dormitory and mess hall—operating expenses	<u>7,548.35</u>	1,249,058.00	
Research Projects, Fellowships, Grants, etc.:			
Salaries	\$13,536.90		
Grants and miscellaneous	32,125.70		
Fellowship program	<u>53,391.35</u>	99,053.95	
Office of Publications:			
Printing and publishing expenses	\$31,387.68		
Office expenses:			
Salaries	29,376.77		
Stationery, postage, etc.	<u>6,845.09</u>	67,609.54	
Administration		<u>140,407.51</u>	
TOTAL EXPENDITURES			<u>1,667,542.85</u>

Excess of income over expenditures \$25,934.85

*Does not include net gain from sales and redemptions of securities shown in summary of security transactions, page xxxvi.

STATEMENT OF CURRENT FUNDS SURPLUS FOR THE YEAR ENDED JUNE 30, 1949

Balance, July 1, 1948		\$844,348.96
Additions:		
Transfers from:		
Harriman Fund	\$1,000.00	
Pension Fund	9,013.85	
Harriet H. Mayor Relief Fund	650.00	
Excess of income over expenditures	<u>25,934.85</u>	36,598.70
Balance, June 30, 1949		<u>\$880,947.66</u>

SCHEDULE OF SECURITIES OWNED JUNE 30, 1949

Principal amount	Description	Maturity	Cost, amortized cost, or value at date acquired	Approximate market value	*T AVP income
UNITED STATES GOVERNMENT BONDS					
1500,000	U. S. of America Treasury Certf. of Ind. 1 #s	1-1-50	\$500,146.98	\$500,300	—\$34.25
304,000	U. S. of America Treasury 2s	1951-49	304,000.00	304,475	6,080.00
312,000	U. S. of America Treasury 2s	1951-49	312,000.00	313,463	6,240.00
200,000	U. S. of America Treasury 2s	1952-50	200,000.00	201,188	4,000.00
4,500,000	U. S. of America Treasury 2s	1954-52	4,500,000.00	4,599,844	90,000.00
800,000	U. S. of America Treasury 2Ks	1955-52	800,000.00	821,000	18,000.00
400,000	U. S. of America Treasury 2 1/4s	1959-56	420,677.50*	417,750	9,000.00
1,239,000	U. S. of America Treasury 2 Ks	1954-52	1,241,048.67*	1,278,106	30,975.00
50,000	U. S. of America Savings Series "G" 2Ks	1953	50,000.00	48,350	1,250.00
50,000	U. S. of America Savings Series "G" 2Ks	1954	50,000.00	48,200	1,250.00
50,000	U. S. of America Savings Series "G" 2#s	1954	50,000.00	48,050	1,250.00
100,000	U. S. of America Savings Series "G" 2Ks	1955	100,000.00	95,800	2,500.00
100,000	U. S. of America Savings Series "G" 2Ks	1956	100,000.00	95,200	2,500.00
100,000	U. S. of America Savings Series "G" 2Ks	1957	100,000.00	94,700	2,500.00
100,000	U. S. of America Savings Series "G" 2 1/4s	1958	100,000.00	94,800	2,500.00
100,000	U. S. of America Savings Series "G" 2Ks	1959	100,000.00	95,600	2,500.00
100,000	U» S. of America Savings Series "G" 2Ks	1960	100,000.00	98,800	1,250.00
	Income from bonds redeemed				4,245.33
<u>\$9,005,000</u>	Total U. S. Government		<u>\$9,027,873.15</u>	<u>\$9,155,626</u>	<u>\$186,006.08</u>
FOREIGN AND INTERNATIONAL BANK BONDS					
1100,000	Australia, Commonwealth of, S. F. 3 Ks	1956	\$100,000.00	\$94,000	\$3,250.00
50,000	Australia, Commonwealth of, S. F. 3 H%	1957	48,750.00	47,000	1,625.00
90,000	Canadian National Ry. Co., 4Ks Guar	1951	90,091.47*	94,500	4,050.00
100,000	Canadian National Ry. Co., 4Ks Guar	1957	112,000.00	114,000	4,500.00
57,000	Canadian National Ry. Co., 5s Guar	1969	61,008.30*	59,850	2,850.00
35,000	Canadian National Ry. Co., 5s Guar	1970	37,354.52*	37,100	1,750.00
100,000	International Bank for Reconstruction and Development, 2 1/4s	1957	100,000.00	100,750	2,250.00
200,000	Shawinigan Water & Power Co., 1st Mtg. & Coll. Tr. S. F. 3s	1971	207,920.00*	196,000	6,000.00
100,000	City of Toronto Cons. Loan Deb. 5s	1949	96,164.59	101,000	5,000.00
<u>\$832,000</u>	Total Foreign and International Bank		<u>\$853,288.88</u>	<u>\$844,200</u>	<u>\$31,275.00</u>

*After deduction for amortization of premiums on bonds purchased subsequent to January 1, 1940.

SCHEDULE OF SECURITIES OWNED JUNE 30, 1949—Continued

Principal amount	Description	Maturity	Cost, amortized cost, or value at date acquired	Approximate Fair Value	Net Income
PUBLIC UTILITY BONDS					
\$241,000	Columbus & Southern Ohio Electric Co., 1st Mtg. 3Ks.	1970	\$254,705.08*	\$257,870	\$7,832.50
300,000	Consolidated Natural Gas Co., Deb. 2MB.	1968	300,832.60*	306,000	8,250.00
100,000	Detroit Edison Co., Conv. Deb. 3s.	1958	105,242.99*	107,000	1,250.00
37,000	Greyhound Corporation, S. F. Deb. 3s.	1959	37,313.83*	37,370	1,110.00
200,000	Minnesota Power & Light Co., 1st Mtg. 3/8s.	1975	204,338.69*	204,000	6,250.00
100,000	Ohio Power Co., 1st Mtg. 3/8s.	1968	101,500.00	106,000	3,250.00
97,000	Oklahoma Natural Gas Co., 1st Mtg. 2 1/2s.	1961	98,056.57*	97,970	2,788.75
100,000	Panhandle Eastern Pipe Line Co., S. F. Deb. 3 Ks.	1973	101,743.82*	104,000	271.17
50,000	Philadelphia Electric Co., 1st & Ref. Mtg. 2Ms.	1978	49,687.50	51,000	1,437.50
207,000	Philadelphia Electric Power Co., 1st Mtg. 2#s.	1975	211,562.32*	200,790	5,433.75
200,000	Public Service Co. of Indiana, Inc., 1st Mtg. 3/4s.	1975	204,358.12*	206,000	6,250.00
125,000	Puget Sound Power & Light Co., 1st Mtg. 4 1/2s.	1972	129,128.38*	131,250	5,312.50
216,000	Tennessee Gas & Transmission Co., 1st Mtg. Pipe Line 2 1/2s.	1966	218,754.00*	211,680	5,940.00
283,000	United Gas Corp., 1st Mtg. & Coll. Tr. 2Ks.	1967	283,000.00	283,000	7,782.50
	Income from bonds redeemed				280.00
<u>\$2,256,000</u>	Total Public Utility		<u>\$2,300,223.90</u>	<u>\$2,303,930</u>	<u>\$63,438.67</u>
COMMUNICATION BONDS					
\$150,000	American Telephone & Telegraph Co., Conv. Deb. 2KB.	1961	\$155,378.55*	\$153,000	\$4,125.00
150,000	American Telephone & Telegraph Co., Deb. 2KB.	1975	152,418.75*	147,000	4,125.00
45,000	American Telephone & Telegraph Co., Conv. Deb. 3/4s.	1959	45,000.00	49,050	6,250.00
200,000	Mountain States Telephone & Telegraph Co., Deb. 3 1/2e.	1978	201,680.00*	208,000	3,125.00
100,000	New York Telephone Co., Ref. Mtg. 3 1/2e.	1978	101,380.69*	105,000	3,125.00
200,000	Pacific Telephone & Telegraph Co., Deb. 3 1/2s.	1978	205,297.66*	210,000	6,500.00
300,000	Southwestern Bell Telephone Co., Deb. 3Ms.	1983	307,250.00*	318,000	9,375.00
	Income from bonds called				2,600.00
<u>\$1,145,000</u>	Total Communication		<u>\$1,168,405.65</u>	<u>\$1,190,050</u>	<u>\$36,100.00</u>
RAILROAD BONDS					
1100,000	Chesapeake & Ohio Ry. Co., Gen. Mtg. 4 1/2s.	1992	\$99,464.29	\$124,000	\$4,500.00
75,000	Chicago & W. Indiana R. R. Co., Cons. 4s.	1952	70,357.66	77,250	3,000.00
<u>100,000</u>	Pennsylvania R. R. Co., Cons. Mtg. 4#s.	1960	<u>104,662.50</u>	<u>114,000</u>	<u>4,500.00</u>
	Income from bonds sold				6.39
<u>^\$275,000</u>	Total Railroad		<u>\$274,484.45</u>	<u>\$315,250</u>	<u>\$12,006.39</u>

* After deduction for amortization of premiums on bonds purchased subsequent to January 1, 1940.

SCHEDULE OF SECURITIES OWNED JUNE 30, 1949—Continued

Principal amount	Description	Maturity	Cost, amortized cost, or value at date acquired	Approximate market value	Net income
RAILROAD EQUIPMENT TRUST BONDS					
\$150,000	Chesapeake & Ohio Ry. Co., Eq. Tr. 2s.	1956-58	\$146,340.34	\$146,500	\$3,000.00
300,000	Chicago Burlington & Quincy R. R. Co., Eq. Tr. 2 [^] s.	1958-63	292,507.12	293,500	6,750.00
150,000	Pennsylvania R. R. Co., Eq. Tr. 2 ^H s Guar.	1958-62	146,358.96	145,500	3,562.50
150,000	Southern Pacific Co., Eq. Tr. 2 [^] s.	1956-58	146,251.10	146,000	3,187.50
150,000	Southern Railway Co., Eq. Tr. 2 Ms.	1956-58	145,928.69	145,500	3,187.50
<u>\$900,000</u>	Total Railroad Equipment Trust.		<u>\$877,386.21</u>	<u>\$877,000</u>	<u>\$19,687.50</u>
INDUSTRIAL AND MISCELLANEOUS BONDS					
#200,000	American Tobacco Co., Deb. 3s.	1969	\$203,051.32*	\$208,000	\$6,000.00
240,000	Bristol Myers Co., Deb. 3s.	1968	241,146.90*	247,200	7,200.00
64,000	Devco & Reynolds Co., Inc., S. F. Deb. 2 ^{pis} .	1965	65,212.16*	64,640	1,840.00
192,000	Eastern Gas & Fuel Associates, 1st Mtg. & Coll. Tr. 3#s.	1965	195,428.99*	188,160	6,720.00
153,000	Food Machinery Corp., S. F. Deb. 2Ks.	1962	152,308.98	151,470	3,825.00
300,000	Goodrich (B. F.) Company, 1st Mtg. 2Ks.	1965	301,184.24*	303,000	8,250.00
291,000	P. Lorillard Co., Deb. 3s.	1963	300,041.08*	299,730	8,730.00
300,000	National Dairy Products Corp., Deb. 2Ks.	1970	304,403.52*	300,000	8,250.00
400,000	Phillips Petroleum Co., S. F. Deb. 2 ^H s.	1964	403,333.13*	407,960	11,000.00
23,000	Pittsburgh Consolidation Coal Co., Deb. 3 [^] s.	1965	23,156.43*	23,460	805.00
150,000	Quaker Oats Co., Deb. 2M ^s .	1964	148,922.50	151,500	3,937.50
300,000	Seagram (Joseph E.) & Sons, Inc., Deb. 2#s.	1966	298,500.00	282,000	7,500.00
400,000	Shell Union Oil Corp., Deb. 2 ¹ / ₄ s.	1971	405,236.36*	388,000	10,000.00
400,000	Socony-Vacuum Oil Co., Deb. 2Ks.	1976	390,278.75	384,000	10,000.00
400,000	Standard Oil Co. of California, Deb. 2 [^] s.	1966	410,219.39*	408,000	11,000.00
300,000	Swift & Co., Deb. 2 ^H s.	1972	301,651.89*	294,000	7,875.00
400,000	Texas Corporation, Deb. 3s.	1965	420,341.89*	420,000	12,000.00
250,000	Union Oil Company of California, Deb. 2K [^] s.	1970	258,919.88*	252,500	6,875.00
100,000	United States Rubber Co., Deb. 2 ^{ffe} .	1976	100,322.09*	94,000	2,625.00
300,000	Westinghouse Electric Corporation, Deb. 2#s.	1971	302,064.29*	297,000	7,875.00
	Income from bonds called.				157.50
<u>\$5,163,000</u>	Total Industrial and Miscellaneous.		<u>\$5,225,723.79</u>	<u>\$5,164,620</u>	<u>\$142,465.00</u>
<u>\$19,576,000</u>	BONDS—Funds Invested.		<u>\$19,727,386.03</u>	<u>\$19,850,676</u>	<u>\$490,978.64</u>

* After deduction for amortization of premiums on bonds purchased subsequent to January 1, 1940.

SCHEDULE OF SECURITIES OWNED JUNE 30, 1949—Continued

Number of shares	Description	Cost, amortized cost, or value at date acquired	Approximate market value	Net income
PREFERRED STOCKS				
1,000	Atutoi Hocking Glass Corp., 54.00 Cum. Pref.	1112,750.00	\$103,000	\$4,000.00
1,500	Appalachian Kleetik Power Co., 4 2/2% Cum. Pref.	159,000.00	163,500	6,750.00
2,000	Anntiong Cork Co., \$3.75 Cum. Pref.	205,500.00	190,000	7,500.00
1,500	Bethlehem Steel Corp., 7 1/4% Cum. Pref.	183,637.50	196,500	10,500.00
1,477	Bristol Myers Co., 5 1/2% Cum. Pref.	156,300.45	149,177	5,538.76
2,000	Buffalo, Niagara Kleetric Corp., 5M/1/2 Cum. Pref.	207,990.00	178,000	7,200.00
500	Case (J. I.) Co., 7/8% Cum. Pref.	62,225.00	68,500	3,467.19
600	Cleveland Electric Illuminating Co., \$5.450 Cum. Pref.	68,112.25	66,600	2,700.00
1,900	Consolidated Kdisinn Co. of N. V., \$5.00 Cum. Pref.	202,815.50	203,300	9,500.00
1,125	Continental Can Co., Inc., \$3.75 Cum. Pref.	115,312.50	108,000	4,218.76
145	Corn Products Refining Co., 7 3/4% Cum. Pref.	27,183.25	25,955	1,015.00
1,125	duPont (K. I.) de Nemours & Co., \$4.50 Cum. Pref.	116,125.00	137,250	5,062.51
1,000	KI Paso Natural Gas Co., 4.10% Cum. Pref.	111,442.21	95,000	4,100.00
2,000	General Foods Corp., \$3.50 Cum. Pref.	201,000.00	194,000	7,000.00
1,500	General Motors Corp., \$5.00 Cum. Pref.	187,937.50	186,000	7,500.00
1,000	General Shoe Corporation, \$3.50 Cum. Pref.	102,250.00	83,000	3,500.00
1,000	Grant (W. T.) Co., 3H/10 Cum. Pref.	100,447.91	95,000	3,750.00
1,500	McKehson & Robbins, Inc., \$4.00 Cum. Pref.	144,000.00	139,500	6,000.00
400	Northern Stated Power Co., \$3.60 Cum. Pref.	41,280.00	35,600	1,440.00
695	Ohio Power Co., 4 1/4% Cum. Pref.	76,552.00	75,060	3,127.52
1,500	Pacific Telephone and Telegraph Co., 6% Cum. Pref.	235,220.75	216,000	9,000.00
1,000	Panhandle Eastern Pipe Line Co., 4% Cum. Pref.	104,166.68	98,000	4,000.00
1 (KM)	Pillsbury Mills, Inc., \$4.00 Cum. Pref.	107,722.00	103,000	4,000.00
2,0(K)	Reynold-(R. J) Tobacco Co., 3.60% Cum. Pref.	199,683.75	182,000	7,200.00
1,024	Sherwin-Williams Co., 4% Cum. Pref.	112,862.09	110,592	4,096.00
1,400	Standard Oil Co. of Ohio, 3H/10 Cum. Pref. "A"	150,743.69	137,200	5,250.00
250	United States Gypsum Co., 7% Cum. Pref.	45,187.50	44,250	1,750.00
3,100	U. S. Steel Corp., 1% Cum. Pref.	443,407.57	406,100	21,700.00
	Income from stocks called or sold			22,920.13
35,241	Total Preferred Stocks	\$3,980,855.10	\$3,790,084	\$183,785.87
COMMON STOCKS				
7,100	Abbott Laboratories	\$267,476.10	\$276,900	\$10,360.00
500	Allied Chemical & Dye Corp.	96,175.97	84,000	4,500.00
4,000	American Can Company	352,201.17	360,000	15,850.00
4,700	American Gas and Electric Company	202,075.88	202,100	4,525.00
2,700	American Telephone & Telegraph Co.	399,025.39	375,300	24,300.00
5,100	Armstrong Cork Company	251,220.36	209,100	14,700.00
4,500	Boston Edison Company	203,214.54	180,000	11,700.00
1,000	Bristol Myers Co.	39,430.33	27,000	1,600.00
3,000	C. I. T. Financial Corporation	151,369.19	141,000	7,000.00
2,600	Chase National Bank of the City of New York	92,769.35	85,800	4,360.00
8,200	Chrysler Corporation	371,763.95	385,400	36,900.00
5,400	Cleveland Electric Illuminating Company	227,773.54	210,600	7,480.00

(Continued on following page)

SCHEDULE OF SECURITIES OWNED JUNE 30, 1949—Continued

Number of shares	Description	Cost, amortized cost, or value at date acquired	Approximate market value	Net income
COMMON STOCKS—Continued				
1,600	Coca-Cola Company	\$233,577.80	\$208,000	\$7,300.00
2,000	Commercial National Bank and Trust Co. of N. Y.	86,522.22	84,000	4,000.00
6,356	Commonwealth Edison Company	196,326.62	158,900	9,375.10
2,000	Consolidated Edison Company of N. Y.	67,530.37	46,000	3,200.00
1,300	Consolidated Gas Electric Light and Power Company of Baltimore	108,423.09	79,300	4,500.00
1,200	Continental Illinois National Bank & Trust Co. of Chicago	105,810.00	86,400	4,800.00
5,490	Continental Insurance Co.	260,277.60	318,420	13,850.00
9,900	Continental Oil Co. of Delaware	388,856.82	534,600	31,900.00
5,100	Delaware Power & Light Company	105,714.47	96,900	5,610.00
12,400	duPont (E. I.) de Nemours & Co.	506,088.07	570,400	33,325.00
13,100	Eastman Kodak Co.	458,548.61	524,000	21,615.00
1,710	Fireman's Fund Insurance Co.	121,050.00	131,670	4,047.00
5,188	Food Machinery and Chemical Corporation	177,575.97	124,512	8,280.50
13,700	General Electric Co.	540,183.86	479,500	25,830.00
7,600	General Foods Corporation	317,875.40	319,200	16,650.00
8,400	General Motors Corporation	447,819.07	470,400	45,800.00
400	Guaranty Trust Co. of N. Y.	98,003.91	104,800	4,800.00
8,700	Gulf Oil Corp.	391,540.45	513,300	25,500.00
2,000	Hartford Fire Insurance Co.	179,826.06	238,000	5,000.00
8,700	Humble Oil & Refining Co.	290,411.57	600,300	38,250.00
3,1475	Insurance Company of North America	255,748.31	347,500	11,043.75
2,950	International Business Machines Corp.	219,994.84	445,450	11,800.00
4,000	Johns-Manville Corp.	148,016.12	140,000	7,350.00
7,200	Kennecott Copper Corporation	362,303.69	324,000	26,500.00
7,300	Kresge (S. S.) Company	230,158.25	284,700	13,600.00
2,100	Liggett & Myers Tobacco Co.	189,977.30	170,100	10,500.00
320	Mellon National Bank and Trust Company	67,193.07	86,400	2,880.00
6,750	Middle South Utilities, Inc.	98,683.97	101,250
3,200	Minneapolis-Honeywell Regulator Co.	147,869.94	156,800	6,850.00
9,700	Monsanto Chemical Co.	394,552.93	485,000	19,200.00
3,000	Montgomery Ward & Co.	161,894.89	144,000	9,000.00
7,700	National Cash Register Co.	267,801.00	246,400	18,250.00
1,900	National City Bank of New York	76,087.50	74,100	2,720.00
1,800	National Union Fire Insurance Co.	62,056.90	55,800	2,520.00
10,800	Newberry (J. J.) Co.	143,047.69	334,800	21,600.00
4,100	New Jersey Zinc Co.	265,862.52	221,400	15,725.00
3,800	Owens-Illinois Glass Co.	263,802.50	201,400	11,400.00
1,750	Pacific Gai & Electric Company	121,073.09	85,250	5,500.00
11,200	Penney (J. C.) Co.	397,461.99	526,400	27,500.00
1,000	Peoples Gas Light and Coke Company	106,350.00	100,000	5,875.00
3,700	Pfizer (Chat.) & Co., Inc.	215,210.63	170,200	9,625.00
3,500	Philadelphia Electric Company	100,048.39	77,000	4,200.00
7,600	Phillips Petroleum Co.	406,186.99	402,800	22,800.00
9,700	Pittsburgh Plate Glass Co.	312,977.21	300,700	16,275.00
2,200	Procter & Gamble Co.	123,793.05	134,200	6,300.00
1,800	Reynolds (R. J.) Tobacco Co. "B"	72,455.18	64,800	3,600.00
1,400	St. Paul Fire and Marine Insurance Co.	106,987.02	112,000	2,950.00
2,300	Scott Paper Co.	103,055.95	126,500	5,335.00
16,400	Seam, Roebuck & Co.	374,427.57	623,200	45,100.00
6,600	Sherwin-Williams Co.	399,644.64	363,000	18,975.00
5,000	Southern California Edison Company, Ltd.	175,996.33	153,000	3,750.00
11,300	Standard Oil Co. of Indiana	409,733.33	418,100	21,850.00

(Continued on following page)

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SCHEDULE OF SECURITIES OWNED JUNE 30, 1949—Continued

Number of shares	Description	Cost, amortized cost, or value at date acquired	Approximate market value	Net income
COMMON STOCKS—Continued				
7,600	Standard Oil Co. of New Jersey.....	\$452,480.98	1471,200	\$17,219.50
4,600	Texas Company.....	242,815.60	230,000	12,982.50
13,700	Union Carbide & Carbon Corp.....	429,474.16	493,200	25,800.00
5,000	United Fruit Company.....	141,876.60	240,000	20,000.00
9,750	United Gas Corp.....	155,016.03	156,000
4,300	United States Gypsum Co.....	377,236.97	404,200	25,200.00
12,200	Westinghouse Electric Corp.....	333,518.44	280,600	15,050.00
6,300	Woolworth (F. W.) Co.....	281,250.61	296,100	11,800.00
	Income from stocks sold.....			44,479.60
<u>395,639</u>	Total Common Stocks.....	<u>\$16,930,579.91</u>	<u>\$18,275,352</u>	<u>\$1,019,812.95</u>
<u>430,880</u>	COMMON AND PREFERRED STOCKS—Funds Invested.....	<u>\$20,911,435.01</u>	<u>\$22,065,436</u>	<u>\$1,203,598.82</u>
	AGGREGATE INVESTMENTS (BONDS AND STOCKS).....	<u>\$40,638,821.04</u>	<u>\$41,916,112</u>	<u>\$1,694,577.46*</u>

^Represents total interest and dividend income before deduction of amortization of bond premiums.

SUMMARY OF SECURITY TRANSACTIONS JULY 1, 1948 TO JUNE 30, 1949

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July 1, 1948—Cash awaiting investment..... \$191,355.60

SALES AND REDEMPTIONS

	Gain	Loss	Book value	
Bonds.....	\$122.28		\$4,669,678.84
Preferred stocks.....		\$53,397.59	659,933.76
Common stocks.....	61,802.73		1,353,670.01
Sale of stock rights.....	18,813.77		
Mortgages.....	476.92		
	<u>\$81,215.70</u>	<u>\$53,397.59</u>	<u>\$6,683,282.61</u>
Net gain.....		27,818.11	27,818.11	6,711,100.72
	<u>\$81,215.70</u>	<u>\$81,215.70</u>	
Income applied to amortization of bond premiums.....				11,858.35
Proceeds from sale of stock dividends.....				124,545.40
Total.....				<u>\$7,038,860.07</u>

PURCHASES

Bonds.....	\$4,348,712.31	*1
Common stocks.....	2,426,958.83	6,775,671.14
June 30, 1949—Cash awaiting investment.....		* \$263,188.93

REPORT OF THE PRESIDENT
OF THE
CARNEGIE INSTITUTION OF WASHINGTON
FOR THE YEAR ENDING JUNE 30, 1949

REPORT OF THE PRESIDENT
OF THE
CARNEGIE INSTITUTION OF WASHINGTON

As this annual report is presented to the Trustees in accordance with the By-Laws, the Institution continues to pursue its program of fundamental research. The past year has brought the accomplishment summarized in the annual reports of the Departments, a few of the highlights of which are mentioned below.

In recent reports the way in which scientific programs become formulated by the staffs of the Departments of the Institution has been discussed. There has also been some consideration of the relationship of the Board of Trustees to the programs of an institution of this sort. It would perhaps be well, therefore, to write briefly this year of the position of an individual investigator within the Institution, and the relationship of his program to the whole.

We do not have a rigid hierarchy in our staff. There are senior investigators, junior investigators, aids, and technical supporters of many sorts, but even the titles vary from one Department to another, and there is little of rigidity in the whole structure. This is a desirable situation, for the extraordinary informality of our internal relationships, characteristically American, is one of our great assets. Certainly we have under one title or another senior staff members who, by reason of their attainments, have earned the right to go their own way on their researches, and to explore those puzzles of our environment which most intrigue them. Indeed, the entire organization revolves about the work of these men; the Institution exists in order that they may have the rare opportunity which they

enjoy. Junior investigators in general work in collaboration with these individuals, but with a great deal of freedom of their own and certainly with the opportunity to pursue their own problems to a very considerable extent and thus demonstrate their own effectiveness. The team is completed by younger staff members who have not as yet arrived at the point of independence, together with the technical staff which is most decidedly an integral part of the structure. There are no age requirements, nor are there any seniority rules or scheduled promotions in the whole organization.

The question then naturally arises how the performance of the senior staff is measured. In the large, the answer to this question is that their performance is measured in the same manner that scientific accomplishment generally is measured under the best conditions in any scientific community, namely by the consensus of opinion of colleagues, and of investigators in the same general field who are located elsewhere. But there are certain negative aspects of this subject which are well worth mentioning. Only too often in research organizations the product is weighed, sometimes almost literally, and the staff are under pressure to produce results at intervals, which are not stated explicitly but well understood. When a man is considered for a scientific position or for election to a learned body, only too frequently the number of his published papers is counted, and it is carefully noted whether he has published regularly. The sort of artificial pressure which this tendency creates is absent from

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the Institution. The staff all recognize, of course, that the man who shirks the sometimes disagreeable job of getting his results promptly into print is neglecting an essential part of his undertaking. But the staff also recognize that one good solid article that really constitutes a milestone is worth any number of dribbles, that the article that really pins a subject down completely is far better than the one that is rushed into print while still wobbly in its reasoning. Moreover, the staff recognize that there is such a thing as luck in research, and that the best of investigators may at times miss an opportunity, and struggle long and deviously before he emerges from a temporary obscurity. In fact, the absence of pressure for periodic public performance is one of the strongest reasons why the staff of the Institution are enthusiastic scientists. We take pride in the fact that the stature of our scientists is an affair of a lifetime, and no matter of flashy performance. Yet there is no doubt that the staff regard very seriously the obligation and responsibility that are theirs, because of the rare opportunity they have to advance the sum of human knowledge without arbitrary or artificial pressures or distractions.

The world of science has a place for almost every sort of individual. At the one extreme there are those gregarious cooperative types who can operate more effectively in concert and who become important individuals in a group attack upon a particularly knotty subject. At the other extreme are those detached lonely workers who simply must pursue their own affairs by themselves, without collaboration, and sometimes in the extreme even without assistance. The Institution does not have much of the type of organized group attack which is necessary and sometimes very effective in applied research,

particularly in great commercial laboratories. Neither, on the other hand, do we seem to produce the type of genius who can operate only in isolation. Without definite planning in that regard, our staff, for all its variety, lies somewhere between these two extremes. We expect, and by this we mean that staff opinion expects, that the individual who is pursuing his own line of research will so select his problems and so conduct them that his pursuit of them will reflect favorably upon the progress of the work being conducted by his colleagues in adjacent areas. In addition we expect him to be a helpful individual, who will go out of his way and forget his own pursuits at times to help a colleague out of a difficult situation, and all of this occurs. But if a senior investigator believes he has a lead which will take him to far-reaching results along the lines of our general interests, there is nothing in our rules or in our practice to prevent him from pursuing his individual way with full intensity and with the full support of his colleagues in so doing. This is the sort of fundamental pursuit of knowledge which yields momentous results in the long run. It is only necessary to be sure that those who have the extraordinary privilege are worthy of it, and for this we must rely on their reputations among their own peers.

The Institution differs from a university in many ways. For one thing, the staff are not distracted by the manifold duties that are inevitably a part of the operation of a great university. For another, the business organization of the Institution is designed to lift from the shoulders of investigators as far as is physically possible the burdens that sometimes inevitably intrude in an organization having less singleness of purpose.

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But there is a third way in which we differ, and this is not so favorable. A professor in a university comes in contact necessarily with young minds. An investigator in an independent research organization can shield himself from such contact quite completely if he wishes to do so, or even unconsciously if he naturally shrinks from the give and take, sometimes roughly pursued, which is inherent in contact with young active minds. There is a distinct danger here, for we depend upon young and vigorous minds to keep us from getting stale, and the scientist who removes himself from direct and almost daily criticism is only too likely to get into a groove, or to cherish some personally constructed and bizarre fashion of thought. There are, however, ways in which we offset our otherwise serious isolation from teaching and from youth generally. Several of our Departments are located adjacent to great research centers in universities. Those in the city of Washington participate closely in the scientific affairs of the Federal Government. We have

summer conferences, our staff members occasionally give courses of lectures on invitation, and there are always the important scientific meetings, national and international, attendance at which is sometimes salutary. But we now have in addition a fellowship program which brings young scientists into our midst on a temporary basis, and also a plan for inviting scientists of standing to be our guests. Neither of these plans is rigid in outline. There are, for example, in connection with our fellowships, no rules which limit selections in accordance with academic status or nationality. In inviting guests we have no formalism to consider, but only the question whether a visit will be genuinely beneficial to the guest and to ourselves and thus to the advancement of science. The fellowship program in particular seems to be working well. We have had an excellent group of younger people and they have performed admirably. The program is still in its formative stages and warrants close attention and reasonable expansion.

RESEARCH ACTIVITIES

In the development of modern science it often happens that the creation of a new tool for research is as important a step in attaining the ultimate goal of new knowledge as the actual use of the tool in a research program. This is especially true in astronomy, since most astronomical investigations deal with extremely distant and faint objects. Without telescopes of great light-gathering power to collect an adequate amount of light, the astronomers would be nearly helpless.

Furthermore, the design and construction of such a tool may require as much ingenuity, effort, and expense as its later use for research. Thus the attainment of the great light-gathering power of a

modern telescope means the design and construction of very large optical parts and of huge moving structural members to support them. Moreover, combined with this size must be the accuracy of form and precision of movement of the finest small laboratory instrument. Because of the great rapidity with which flexure, temperature distortions, and errors of manufacture increase with the size of a structure, this requirement poses problems of the greatest difficulty to the designer.

Once successfully constructed, however, such a telescope provides a basic instrument for all kinds of astronomical research during the decades to follow. The instrument may be used by itself to obtain

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direct photographs, or it may be combined with spectrographs or photometers for the study of spectra or the measurement of magnitudes. As new auxiliary instruments are developed during the ensuing years, they may be attached to the telescope to utilize the light it collects, often in ways not originally contemplated when the telescope was built. Thus many of the past "largest" telescopes, constructed thirty, fifty, and even seventy-five years ago, are still active and effective instruments of research today.

The initiation of scientific observations by two notable new instruments, each of outstanding size, was without question the most important research event of the year at the Mount Wilson and Palomar Observatories. The larger of these instruments, the great Hale 200-inch telescope, was first used for taking photographs on a limited observing program during the winter of 1949. Though at the end of the report year some adjustments and minor modifications of this instrument were still necessary before a regular observing program could be scheduled, the earlier trial program provided enough tests to give assurance of the great power of this instrument in attacking many of the outstanding problems of astronomy. Last winter, the Institution, with its friends and collaborators of the California Institute of Technology, had in the Hale telescope a masterful instrument, perfect for average seeing conditions. Powerful as it then was, it was not enough, and the delicate task of further polishing, further refinement, was undertaken. Now, the great instrument is ready, not merely for average seeing conditions, but for those rare occasions when seeing conditions are extraordinary.

The smaller of the new instruments, the 48-inch schmidt camera, was given its

test exposures in the fall of 1948 and was placed in regular service in the following winter. Like the Hale telescope, this instrument is the largest of its type that has thus far been constructed. The schmidt camera is a new development of the past two decades. Because of its ability to give almost perfect definition over a very large plate (14 by 14 inches in the present instrument), it is largely displacing lens-type cameras for survey purposes. The present instrument has fully come up to expectations from both the optical and the mechanical standpoints. These two instruments will make a most powerful asset for astronomical research, the smaller one to find, the larger one to study in detail new objects in the universe about us.

The rare stars with peculiar properties are an object of search by astronomers, since study of them may give important clues to understanding of stellar atmospheres. A thirty-year hunt for several types of these rare objects, including emission-type B stars, planetary nebulae, and T Tauri stars, all of them characterized by bright hydrogen lines, was completed during the year, Dr. Bowen reports. It was a highly successful search. Some 1400 emission line stars, mostly of class B—a many-fold increase in the number of known objects of this type—have been detected in the photographic plates resulting from the study. About 200 new planetary nebulae, more than doubling the observed number of these objects, have been found. In this search, characteristic of astronomy in its patient duration, a 10-inch wide-angle camera with an objective prism and red-corrected lens made it possible to work with the red part of the spectrum, where the strongest of the hydrogen lines is located. This survey of the northern sky having been completed, the search will now turn to the southern sky. The camera

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and its mounting have been loaned to the University of Michigan for a similar survey from the University's station in South Africa.

The investigations at the Geophysical Laboratory are now focused sharply on the formation and properties of rock-forming minerals with and without the presence of mineralizers, such as water, for the general purpose of understanding the complex processes by which the earth was formed. Of the many thousands of minerals that are known to the geologist, only a dozen or so are of any great consequence in the genesis of rocks; and in this limited class two, quartz and feldspar, occupy a pre-eminent position. The Laboratory, which in its earliest days logically gave primary attention to these particular minerals, has now with vastly improved apparatus and techniques turned again to the study of some of their characteristics. Quartz has the interesting property of changing at 573° C. from one crystalline form to another. Recent measurements have shown that the transformation temperature is not a fixed point as it was formerly believed to be, but may vary by nearly 2° , which even at moderately high temperatures is an interval that is readily measurable with modern precision thermometric devices. It appears that the variation in the behavior of different quartz specimens is related to the conditions under which the quartz originally crystallized; and, therefore, that a further correlation of inversion temperature with geologic environment will furnish a valuable clue to the temperature of formation of any given sample of quartz.

Related information has been provided by the measurements on feldspars, which consist mainly of three different types depending on the predominance of lime, soda, or potash. In the work on hydro-

thermal synthesis, it has been discovered that the sodic form, albite, may exist in either of two different modifications depending on the temperature of formation. Natural albite is usually of the low-temperature form, whereas that produced synthetically hitherto has always been of the high-temperature variety. Other peculiarities in feldspars are understandable through the discovery that the solid solutions existing at elevated temperatures sometimes unmix at low temperatures, the extent of unmixing depending on the temperature. An X-ray technique permits ready determination of the composition of many feldspars. Further work is needed, yet already the new method of study appears to make it possible to determine the temperature of formation of feldspar when any two feldspars are found together in a single place. Thus there is now a strong likelihood that both quartz and feldspar will provide reliable geologic thermometers by which the temperature of past earth processes may be inferred.

The Department of Terrestrial Magnetism has brought to successful conclusion a series of experiments focused on one of the few direct and clear-cut puzzles in geophysics, that of the maintenance of the earth's electric charge. Since the voyages of the *Carnegie* during World War I, it has been known that the earth always carries a rather large negative electric charge, shown by the electric field near the surface in fair-weather areas over land and sea. This charge is maintained despite a measured total air-earth current, summed up for all fair-weather areas, of 1500 amperes, flowing in the direction which tends to dissipate the charge. The suggestion was made in England more than twenty years ago that thunderstorms might supply the necessary reverse current, but it has never been possible to prove or

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reject this idea because of the tremendous variability of the air-earth currents observed beneath thunderstorms.

At the end of 1946 the Department requested the U. S. Air Forces to collaborate in an effort to resolve this problem by making appropriate measurements in the clear air above a thunderstorm. The experiments were undertaken and during the past two years have necessitated many hours of flight, often at record heights. The resulting measurements have shown that the electric current between the upper atmosphere and the ground, in localized areas above active thunderheads, is reversed in sign to the fair-weather current and greatly increased in intensity. The activity observed over a typical storm area, resulting in a net current from the solid earth upward to the ionosphere, multiplied by the average number of storms in progress over the whole earth at any one time, is just enough to counterbalance the 1500-ampere current to the earth which was found for the total of all fair-weather areas.

When the staff of the Department of Terrestrial Magnetism resurveyed the status of knowledge of "the electric and magnetic condition of the earth and its atmosphere" in 1946, three conspicuous puzzles were selected as basic problems of primary significance, disregarding the point that each of these seemed at the time largely inaccessible to direct investigation: (a) What are the direct and indirect causes, inside the earth, of the main part of the earth's magnetic field? (b) What mechanism supports and maintains the earth's net electric charge? (c) What and where are the origins of cosmic-ray particles? One of these underlying problems, that of the earth's electric charge, is now resolved with reasonable clarity, and important information on a second

one, the origin of cosmic rays, was unexpectedly provided by the Department's observations, reported two years ago, of sudden cosmic-ray increases accompanying solar flares and radio fade-outs. Since, in general, direct contributions toward the solution of fundamental puzzles, even when the basic questions are recognized and conspicuous, are the exception and not the rule in scientific work, these unexpected successes further encourage a policy which deliberately focuses attention on the formulation and attacking of basic problems even when they seem inaccessible.

For years discussion has been aroused by the theory that the continents of the earth as we know them may have drifted from some earlier configuration to their present pattern, and, indeed, that they may still be imperceptibly moving. Studies of the direction of magnetization of rocks laid down in early epochs now show some promise of yielding evidence bearing on this question. There are rocks in the Blue Ridge Mountains near Washington which are magnetized as though they had originally been laid down in South Africa. Possible instability of magnetic north and possible large-scale local magnetic disturbances from electric current systems inside the molten earth will have to be reckoned with in any effort to explain why this is so. That effort certainly will also have to consider the hypothesis that the crust has moved with respect to geographic north since the ancient epoch when these rocks were laid down*

Studies by the Department on prehistoric changes in the direction of the earth's magnetic field—the compass direction—hitherto made at specific locations in New England and under the Pacific Ocean, by determinations of the residual magnetization of samples of clay and unconsolidated sediments, were extended this year back

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into geological periods earlier than two hundred million years ago. This was done by making observations on a long series of sedimentary rocks. The unfolding of this story of ancient magnetism in recent years at the Department has been dramatic as it progressed, first in painstaking fashion back through twenty thousand years of the last glacial period, then leaping back one million years by measurements on ocean cores. This encouraged an expedition to the west, carrying the record back through eighty million years by measurements on rock samples collected in the region extending from South Dakota through many parts of the Rocky Mountains to the Cascades. This year the story rolls time back more than two hundred million years to a period prior to the folding of the Appalachian Mountains, by studies of sandstones found in Maryland and Virginia which were laid down more than three hundred and fifty million years ago. Studies of the direction of residual magnetization of rock samples taken from different portions of a fold show that the magnetic vectors in the different samples would be parallel if the folded rock were returned to its original flat condition. These "reconstructed compass directions" are found parallel to each other for locations 50 miles apart amid the folded rocks, although the vector found differs violently from the present compass direction. The complex magnetic pattern found in the folded rocks could not be produced by any possible ancient system of localized earth currents, but the parallel vectors in the original flat rocks might well be a record of the compass direction when the rocks were laid down in that ancient Silurian epoch, just as the varved clays of New England show the compass direction of twelve thousand years ago, and clay suspensions redeposited

in the laboratory show the 1949 compass direction in Washington.

Any conclusions on the problems suggested by the findings thus far made in this study will be unjustified until they are supported by long and comprehensive studies of similar and related rocks from widely distributed sites. In support of the initial series of exploratory observations required for this purpose, the Carnegie Corporation has made a special grant to the Institution, and field studies have been started at a series of locations from Alabama to Maine.

It was hardly anticipated that studies of the prehistory of the earth's magnetic field, in search of causes internal to the earth which could produce that field, would lead to these startling questions relating to motions in the earth during early Paleozoic time. It is reasonable to expect that a highly complex but interesting picture eventually will be found in the magnetic pattern of these ancient rocks. It is reasonable also to expect this magnetic pattern in part to be related to "secular variation foci" which we believe are caused today by electric currents deep inside the earth. These special effects may have been much greater in those ancient periods when the earth was younger and presumably more fluid. In any event it will be interesting to follow the further development of these studies of such a delicate and intangible phenomenon as the compass direction and its variations, through one age after another during the long sweep of geologic time.

Steadily increasing pressure of population the world over confronts us with the need for new sources of food. Great changes are surely to be foreseen in the ways by which mankind is fed; some of them will involve new syntheses; others will be simply the improvement and elabo-

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ration of customary methods. One promising possibility is that lower organisms such as yeasts and algae can become sources of the protein and the fats that we must have. Studies of the green alga *Chlorella* which have been proceeding for some years past at the Division of Plant Biology give us a gauge of possibilities. Under controlled conditions, with generous supply of carbon dioxide, *Chlorella* cultures constitute a plant growth that doubles itself every day and that can be made to yield more heavily of protein or of fat by suitable changes in growing conditions. The proteins produced by these cultures contain all the amino acids commonly associated with nutrition. The program has shown the desirability of investigation of the practicability of producing *Chlorella* on a large scale for the possible production of foodstuffs. Much remains to be learned, of course, and the problem becomes primarily one of engineering. The Institution hence has entered into arrangements with Research Corporation providing for support to the Stanford Research Institute in an intensive investigation of the process and products on a pre-pilot plant scale. The Institution will co-operate in the undertaking.

The process of photosynthesis, by which green plants transform water and carbon dioxide into the foodstuffs on which we depend, is a primary focus of the Division's program. The first step in photosynthesis is the splitting of water molecules by the action of light absorbed by the chloroplast pigments in the plant. This has been investigated during the year in colloidal solutions of chloroplast material. In such preparations obtained by ejection of chloroplast suspensions through a needle valve under high pressure, Dr. French reports, the activity has been found to be greatly increased by the aggregation of

the colloidal particles on addition of salt solutions in the presence of dilute methyl alcohol.

From measurements of the effectiveness of different wave lengths of light on corn and bean seedlings which have been grown in darkness, it was found that only the light which is absorbed by protochlorophyll, and not that absorbed by any other pigments present, can lead to chlorophyll formation.

To further the development of improved range grasses with the ultimate aim of better pasturage for food animals, cooperative experiments on forty-six strains of range-grass hybrids have been arranged by the Division of Plant Biology with a number of widely scattered laboratories. This has been done to test the climatic adaptability of *Poa* hybrids over a wide range of climatic conditions such as are found in the state of Washington, in southern California, North Carolina, Scotland, Wales, Holland, Denmark, Norway, Sweden, and Transjordan. Fortunately, many of these improved strains breed true from seed, and thus are easy to distribute. Some of these grass hybrids of particular interest have been selected for use in a quantitative experiment on their growth response in controlled laboratory environments.

At the Department of Embryology the collection, preparation, and study of human embryos proceeded successfully during the year. A human egg in the 2-cell stage and a blastocyst of about 4¹/₄ days were added to the collection by Dr. Arthur T. Hertig, Research Associate, and Dr. John Rock, collaborator. These are stages of normal development never previously seen. At about the time of implantation the human embryo differs from those of other mammals in certain peculiar characteristics. It is therefore of interest that

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these two specimens, earlier than the implantation stage, closely resemble the embryos of other mammals of comparable age. About a dozen valuable human embryos of later stages were serially sectioned by Dr. Heuser for addition to the Carnegie Collection. The collection of specimens bearing on the problem of defective development was augmented by five embryos and fetuses that had been aborted by physicians because the mothers had German measles in early pregnancy. Such defects as these specimens may prove to have will no doubt throw light on the embryological nature of the damage done in such circumstances, which so often leads to blindness, deafness, and mental retardation of the infant.

Dr. J. R. Schlegel, guest of the laboratory on a Rockefeller Fellowship, developed a promising method for determining the time of passage of solutes from the blood vessels to the lymphatic collecting vessels. The method depends upon the use of a dyestuff which is highly specific for the endothelial lining cells of vessels. Being fluorescent, it is readily detectable in ultraviolet light.

Among the important publications of the year was a monograph in the Contributions to Embryology of the Carnegie Institution on the blood vessels of the uterus and particularly of its lining membrane in pregnancy and of the placenta, representing many years of work by Dr. Elizabeth M. Ramsey. Dr. S. R. M. Reynolds and a group of collaborators brought out a series of papers dealing with the physical form of the uterus as it changes during pregnancy to accommodate the fetus, with the adaptation of the uterine blood vessels to pregnancy, and with the physiology of the uterus during labor.

Dr. Louis B. Flexner and his associates,

including Dr. Dean B. Cowie of the Department of Terrestrial Magnetism, made an important contribution to the question of the permeability of blood capillaries, showing that the whole of the capillary wall, not merely the intercellular cement substance, as some have thought, is available as the path for the diffusion of electrolytes from the blood into the tissues outside the capillaries. A striking finding from these studies is that the rate of passage of water through the capillary walls is very high. An amount of water equal to a man's entire weight passes out of his blood capillaries, and is replaced by an approximately equal amount, every 20 minutes.

At the Department of Genetics, additional experimental evidence has been obtained that the unit of heredity, a gene locus, may control several reactions, some of which may have several biological effects. Working with maize, Dr. McClintock by a special technique is able to bring an unstable gene into proximity with a stable gene locus and is thus enabled to analyze more fully the composition and action of the normal locus. One of the normal loci studied is shown by this analysis to be compound, in that it accounts for at least two reactions associated with the appearance of a single end product.

Dr. Demerec's studies of the resistance of bacteria to antibiotics such as streptomycin give another kind of evidence of the great complexity of the gene locus. What appears to be a single gene locus, it is indicated, controls a series of reactions. One change in this locus gives a mutant which is resistant to streptomycin; another change in the same locus produces a mutant which must have streptomycin in order to survive; still other changes produce still other mutants. The im-

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portance of this locus may be great indeed. If "nature strikes back" with bacteria which withstand the antibiotics developed by man as a defense against disease, nature apparently strikes back in one of its reactions to our interference by subtle changes in this minute spot. Different changes here control reactions giving biologically different end results. A study of reverse changes in this locus indicates that they are probably not brought about through a reversal of the original chemical reaction, but through some other change in the same gene locus.

Cancer, on which the massed attack of many sciences is leveled throughout the nation, is an elusive foe. We know little about its basic origins. It takes many forms. One of these is leukemia, the condition due to disarrangement of the blood-making organs which results in the formation of an excess of white cells in the blood. Leukemia in mice has been under study for years by Dr. MacDowell at the Department of Genetics, and he continues progress toward elucidation of the problem. During the report year, for example, his investigations have indicated that among mice the resistance to leukemia previously noted in the offspring of older mothers as compared with the offspring of younger mothers is closely paralleled when the offspring of young mothers are fed by older nurses. It thus appears that in mice the age of the nurse has as certain an influence after birth upon the incidence of spontaneous leukemia and upon length of life as has the age of the mother before birth.

The rodlike bodies called chromosomes in the nucleus of the cell are of extreme importance because of the fact that they are the carriers of genes, the units of heredity. Nearly infinitesimal—typically about seven millionths of an inch in diam-

eter—the chromosome threads are truly the network of vital inheritance. Naturally they are a center of scientific interest. Cytochemical studies at the Department of Genetics by Drs. Kaufmann and McDonald continuing during the year past have utilized purified enzymes in the effort to explain the organization of the chromosome. Successive treatments with nucleases and proteases are used by the investigators to dissect the chromosome. The investigation indicates that structurally the chromosome is an integrated fabric whose proteins constitute an interrelated system and whose nucleic acids are closely linked with the proteins and perhaps with one another. No single protein or nucleic acid, it appears, may be considered the basic structural component of the chromosome.

Studies of the prehistory of Middle America have recently been developing the fact that the early pre-Classic cultures of those regions were much more advanced than once had been thought. This knowledge is important in providing a proper understanding of the amazing burst of civilization that occurred in the succeeding Classic Period, a cultural advance that at one time had seemed almost autogenic in its suddenness and its apparent lack of capable ancestry. The Division of Historical Research has taken a leading part in the study of these early cultural horizons. The work of Mr. Shook has shown that the pre-Classic, sometimes called Archaic, cultures which existed in the Guatemala highlands about the beginning of the Christian era present a considerably more complex picture than had been suspected. Rather than a single cultural phase, it is clearly demonstrated, there were at least three sequent phases. Social organization already was developing the ceremonial and hierarchic, probably theocratic, pattern that was to become so char-

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acteristic of the Classic Period. Technology no longer was primitive. These people had a generous background of accomplishment. The primitive beginnings, the proto-pottery cultures, continue to elude discovery; and so long as that condition exists, one cannot disregard the possibility of distant and very early inter-American, or even trans-Pacific, influences.

Other progress of note was made in the solution of problems concerning the lowland Maya area. Dr. G. W. Brainerd's work in eastern Campeche gives promise of solving, or in part solving, a question of chronology that long has been a bone of contention. Although materials collected are still in process of analysis, it is

believed that relative chronology can be established for the previously undated Chenes-Rio Bee remains of Campeche and Puuc remains of Yucatan. Preliminary results suggest that the Campeche ruins are of Late Classic period, and that the Puuc remains are slightly later but in part Late Classic. This cross-dating by means of pottery will place the ruins of two important archaeological areas within the general framework of the native Maya calendar and should add to the understanding of other areas and later periods in Yucatan. In addition to its bearing on chronology, Dr. Brainerd's work has interesting possibilities relative to cultural contacts and influences between areas.

STAFF

It is a pleasure to report that distinguished recognition has come during the past year from various sources to members of the Institution's staff for their contributions to the advancement of knowledge, and to refer specifically to several among the many instances. The Commander's Cross of the Royal Order of Saint Olav was conferred by the King of Norway on September 7, 1948, on Dr. John A. Fleming, retired Director of the Department of Terrestrial Magnetism and now adviser to the Institution on international scientific relations. To Dr. George W. Morey, physical chemist of the Geophysical Laboratory, on November 12, 1948, was awarded the first Arthur L. Day Medal of the Geological Society of America. The medal, named in honor of Dr. Day, retired Director of the Geophysical Laboratory, came to Dr. Morey in recognition of his achievements in the

application of physics and chemistry to the solution of geological problems.

Dr. Ira S. Bowen, Director of the Mount Wilson and Palomar Observatories, is the recipient for 1949 of the Rumford Premium of the American Academy of Arts and Sciences. In the words of the citation, Dr. Bowen is thus honored "for his many contributions in physics and astrophysics, . . . including . . . the development of numerous devices for testing the 200-inch Palomar reflector, which is under his personal charge in these critical days of installation." The Cyrus B. Comstock Prize of the National Academy of Sciences for 1948 was awarded April 26, 1949, to Dr. Merle A. Tuve, Director of the Department of Terrestrial Magnetism, for his accomplishments ranging from pioneering work in the study of the upper atmosphere and in fields of nuclear physics to the development of the proximity fuze.

FINANCES

June 30, 1949 marked the close of the first complete fiscal year since the Institu-

tion's fiscal closing was changed from October 31 to June 30 by amendment of

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the By-Laws on December 12, 1947. During the fiscal year just ended, income available for operating purposes was substantially greater, approximately \$200,000, than for comparable periods ending in 1947 and 1948. This increase in income came about partly from increased investments in common stocks and partly from higher dividend declarations. The increase was anticipated to a certain extent and was taken into consideration in the preparation of the budget for the calendar year 1949, with the result that it was possible to increase the amount budgeted for 1949 substantially over the preceding year. This action has permitted allocation of

funds for projects which had been held under restraint because of lack of funds, and also made it possible to make some adjustments in staff salaries to bring the salary scale to a level comparable with those of leading educational and research agencies.

At the close of the fiscal period a year ago, it was necessary to transfer a moderate sum from reserves to supplement income collections for that period; it is now possible to report that at the close of the past fiscal year we were able to credit reserves with an amount in excess of the sum transferred a year ago.

FREDERIC C. WALCOTT

The death, on April 27, 1949, of Frederic C. Walcott, former Senator from Connecticut, took from the Institution a counselor of wide experience in affairs, long and thorough knowledge of the Institution's activities, and generous interest in its well-being. A member of the Board of Trustees since 1931, Senator Walcott served as a member of the Executive Committee of the Trustees from 1932 until his resignation in 1948. Manufacturer, banker, conservationist, and humanitarian, Sena-

tor Walcott did distinguished work in association with Herbert Hoover in relief efforts and food administration during the First World War. In the Senate of the United States he served as a member of the Committee on Banking, and was the principal author of the bill creating the Reconstruction Finance Corporation. He drew unsparingly on this wealth of experience in his association with the Institution, and we miss him greatly as a valued counselor and a loyal friend.

JAMES FORRESTAL

One of the most tragic casualties of the Second World War was the death, on May 22, 1949, of James Forrestal, first Secretary of Defense of the nation which he had served with devotion. Of his

selflessness, his dedication to duty, no additional word is needed. It was a matter of profound gratitude to the Institution that he had accepted election to the Board of Trustees in 1948.

REPORTS OF DEPARTMENTAL ACTIVITIES
AND CO-OPERATIVE STUDIES

ASTRONOMY

Mount Wilson and Palomar Observatories

TERRESTRIAL SCIENCES

Geophysical Laboratory

Department of Terrestrial Magnetism

Special Projects

BIOLOGICAL SCIENCES

Division of Plant Biology

Department of Embryology

Department of Genetics

HISTORICAL RESEARCH

Division of Historical Research

Special Projects

MOUNT WILSON AND PALOMAR OBSERVATORIES

OPERATED BY THE CARNEGIE INSTITUTION OF WASHINGTON
AND THE CALIFORNIA INSTITUTE OF TECHNOLOGY

Pasadena, California

IRA S. BOWEN, *Director*

OBSERVATORY COMMITTEE

IRA S. BOWEN, *Chairman*
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EDWIN P. HUBBLE
MAX MASON
H. P. ROBERTSON

The current year was marked by the first actual use for scientific observations of both the 200-inch Hale telescope and the 48-inch schmidt camera. During much of the year extensive tests and adjustments of both instruments have been in progress. Though most of the features of these telescopes have performed remarkably well, some modifications, particularly of the 200-inch instrument, have been found necessary to attain the perfection of operation and performance that has been the continual aim in the design and construction.

Since the inception of the 200-inch project, it has been realized that the key to the success of the telescope lies in maintaining the exact optical shape of a mirror of this size in all orientations and often with rapidly changing temperatures. Thus, a simple dimensional analysis shows that the flexure of such a mirror under its own weight is proportional to the fourth power of the diameter of the mirror and inversely proportional to the square of its thickness. Obviously, if the standard astronomical practice of using a fixed ratio of mirror diameter to thickness (usually 8:1) is followed, the flexure under gravitational forces increases as the square of the aperture of the telescope. Because of the difficulties of carrying even the weight of such an 8 to 1 mirror on a moving telescope, it is impracticable to decrease this ratio of diameter to thickness appreciably. In order

to obtain the greatest possible stiffness within the weight limitations, a ribbed structure was adopted for the 200-inch mirror rather than the conventional solid disk. Nevertheless, actual measurements show that with a simple three-point support similar to that which is customary for very small mirrors the flexure would be 500 to 1000 times as great as the permissible value of a very few millionths of an inch.

Obviously, if distortion is to be eliminated as the telescope moves into various orientations, the support system for the mirror must be much more elaborate and precise than any hitherto devised. For this reason the mirror is supported at thirty-six points by units designed to balance the force of gravity on the section of the mirror assigned to each support by applying the correct components of force in all directions. As the orientation of the mirror changes, each of these force components must vary in such a way as to compensate the force of gravity with an accuracy of at least 0.1 to 0.2 per cent.

Similarly, distortions of a mirror caused by temperature changes incidental to actual operation increase rapidly with the dimensions of the mirror. Every effort was made to reduce these distortions by having the mirror cast of Pyrex glass, which has a coefficient of expansion only one-third as large as that of the glass used in most of the earlier astronomical mirrors. Further-

more, the ribbed structure of the 200-inch mirror has a marked advantage over a solid disk in this regard, since the rate at which thermal equilibrium is reached varies inversely as the square of the thickness of the glass. The maximum thickness of the ribs of the 200-inch is 4 inches, whereas a solid disk would have been 20 or more inches thick. Even with these advantages, thermal distortion is a serious problem for a mirror of this size, and every precaution must be taken to insure an equal flow of heat to all parts of the mirror.

Every effort was made to solve these problems by designing the mechanical parts properly; nevertheless, it has been realized that extensive tests and experiments would be required with the instrument itself before all factors could be adjusted to give optimum performance.

Last year's report outlined the results of the early tests of the mirror support system. These indicated that the friction in the original support system was so great that it would be practically impossible for the system to exert the correct supporting forces with the high precision necessary. During the summer of 1948 the lower part of each support unit was replaced with a new part especially designed to reduce the friction. Further tests of the mirror in October and November 1948 indicated a satisfactory reduction in the vertical friction from 1.3 per cent to 0.12 per cent.

During this same period studies were made of the behavior of the outer edge of the mirror as the zenith distance of the telescope was varied. This outer edge of the mirror overhangs the last row of supports from 10 to 20 inches, and concern had been felt as to the amount that this edge might droop when the mirror axis was changed from the horizontal position, in which all laboratory tests in Pasadena had been made, to the vertical. Contrary

to expectations in the early tests, this outer edge turned up more when the telescope was pointed near the zenith than when pointed near the horizon. Further studies, however, indicated that this change was accompanied by a shortening of the focus of the 200-inch mirror as the zenith distance was increased. By a careful analysis it was possible to show that the decrease in the amount of upturn of the edge with increasing zenith distance was a secondary effect on the ribbed structure of changing focal length. Furthermore, when the change of focal length was eliminated by an appropriate adjustment of the support system, the edge drooped as the telescope approached the zenith, but by an amount which did not cause a sensible deterioration of the image formed by the mirror.

After these problems had been solved, extensive tests were carried out to determine the effect on the mirror of sudden changes in temperature similar to changes that are occasionally encountered in actual observing. From these tests it was at once evident that the outer edge of the mirror was changing in response to a new temperature more rapidly than the central part of the back of the mirror. Thus when a sudden drop in temperature occurred, the edge contracted more rapidly than the back central zone of the mirror, with the result that the outer zones of the mirror were pulled back from their proper position by as much as 20 to 40 millionths of an inch. With rising temperatures the opposite effect occurred and the edge was raised. To speed up the changes in the back central zone, twelve fans were installed in the cell underneath the mirror in February 1949. These have partially corrected the situation, and it is anticipated that still further correction can be attained by properly insulating the outer edge of the mirror.

All tests of the 200-inch mirror, including those made in Pasadena prior to its transportation to the mountain, indicated that its outer edge was too high by amounts ranging up to 20 millionths of an inch. Because of the uncertainty as to how much this edge might droop when the telescope was placed in a vertical position, it was considered inadvisable to correct this outer zone until the mirror could be thoroughly studied in all orientations. This was particularly true since a turned-down edge, which would result if the correction were carried too far, would be much more difficult to rectify than a turned-up edge.

With the completion of the studies and modifications outlined above, it became possible to proceed with confidence with the removal of the proper amount of glass to correct this outer zone. The mirror was taken from the telescope in May 1949 and its original coat of aluminum removed. Polishing off the high zones was started in June with simple equipment designed for local figuring. The polishing is carried out on the observing floor of the dome with the mirror resting on the base of the aluminizing tank. After the removal of as much glass as seems advisable, the mirror is replaced in the telescope and tests of the figure are made using a star as a source. As might be expected, the survey of the whole surface of the mirror with the necessary accuracy of a very few millionths of an inch after each period of polishing is a much more time-consuming operation than the polishing itself.

Two types of tests are made. The first of these is the standard knife-edge test, which provides a qualitative survey of the deviations from a true paraboloid at all points of the surface. To provide a permanent record of the shape of the mirror at each stage of this final figuring process, a large number of photographs are taken of the knife-edge patterns. In general, eight

different angles of approach of the knife edge are studied, and at least four photographs representing different positions of the knife edge are taken for each direction of approach. The second type of test consists in quantitative surveys along twenty diameters across the mirror, made with a full-sized Hartmann screen having twenty holes per diameter.

Because of the closing of the California Institute Optical Shop, Dr. Anderson and Mr. Brown, who had so ably carried out the original grinding and figuring of the mirror, were not available for these final corrections. Mr. Hendrix, of the Mount Wilson Optical Shop, has therefore been in charge of this figuring.

Early in 1949 it seemed desirable to obtain checks on the results of the Hartmann and other tests of the mirror from a study of the performance of the telescope when in actual operation on a limited astronomical program. Consequently, during gaps in the testing program the telescope was used for a moderately extensive series of test photographs with apertures of 140 to 200 inches. Although confirming the fact that the outer 15 or 20 inches of the mirror were only partially effective, these photographs were on the whole remarkably successful. Many objects were clearly recorded that are well beyond the range of any other telescope. In fact, the theoretical gain over previous instruments was so nearly reached that there is every reason to believe that after the correction of the outer zone the telescope will fully come up to all expectations.

The optical parts of the 48-inch schmidt camera, including the very difficult non-spherical correcting plate, were completed by Mr. Hendrix, of the Mount Wilson Optical Shop, in the summer of 1948 and were installed in September of that year. After the usual adjustments and minor

modifications were made, the instrument was placed on a regular schedule of observations in January 1949.

The performance of this instrument has been remarkably fine and has in every way come up to the specifications for it. This camera normally uses plates 14 by 14 inches and therefore photographs about 40 square degrees of the sky at one exposure. The definition of the optical system is so superb that at all parts of this large plate the definition is limited by the resolving power of the photographic emulsion rather than by the optical system. Early tests have shown that a limiting magnitude of 20.3 is regularly reached. The mechanical and electrical parts of the telescope have likewise performed very satisfactorily.

In June 1949 announcement was made of an agreement providing for joint support by the National Geographic Society and the California Institute of Technology of a project for mapping, with the 48-inch schmidt camera, the whole sky visible from the Palomar Observatory. Each section of the sky will be photographed once in red light and once in blue light. Since approximately 1000 plates in each color will be required to cover the sky, it is anticipated that a period of four years will be necessary to complete the survey. This survey makes it possible for the first time to map the whole sky to a depth and in detail comparable with that reached by the large reflectors. In several decades of operation, these large reflectors with their limited fields have covered only one or two per cent of the sky; hence it is anticipated that complete coverage with the new schmidt camera will bring to light a great wealth of objects. These objects will provide material for detailed study with the 200-inch and 100-inch telescopes and with the large instruments of other observatories. In addition, the survey plates will

themselves provide important material for statistical studies of the distribution of many types of faint objects.

During the year rapid progress has been made in the design and construction of auxiliary equipment for the 200-inch Hale telescope. The base frame and many of the mountings for mirrors, plateholders, and gratings of the large coude spectrograph have been installed. This spectrograph will use a four-unit composite grating, designed to accommodate a beam from the collimator 12 inches in diameter. Schmidt cameras having focal lengths of 18, 36, 72, and 144 inches and giving dispersions of 18, 9, 4.6, and 2.3 angstroms per millimeter are to be provided.

As the construction period of the Palomar project has drawn to a close, extensive reorganization of the shops built for this project has been carried out. Since in the future only a small fraction of the capacity of the machine shop on the campus of the California Institute will be required for Observatory operations, it has been reorganized as a central machine shop for the use of all departments of the Institute. Though much of the Observatory construction will continue to be done at this shop, the reorganization will substantially decrease the fraction of the shop overhead to be carried by the Observatories. The optical shop ceased operations on May 1, 1949, with the completion of the last of the major optical parts for the Hale telescope. All future optical construction of the Mount Wilson and Palomar Observatories will be carried on at the Santa Barbara Street shop under the supervision of Mr. Don Hendrix. Mr. Melvin Johnson, of the California Institute shop, has been transferred to Santa Barbara Street to augment the original staff.

During the present year the location of the 200-inch dome has been determined

by the United States Geological Survey as follows:

Latitude: 33 degrees, 21 minutes, 22.41
seconds north
Longitude: 116 degrees, 51 minutes, 50.38
seconds west
or * 7 hours, 47 minutes, 27.359
seconds

The elevation of the observing floor of the 200-inch dome is 5598.5 feet above sea level.

For the past several years reports have been given of the progress of a search for planetary nebulae and early-type stars with emission lines, with a camera having a 10-inch red-corrected lens of the triplet type. This survey has now been completed for all parts of the Milky Way visible from Mount Wilson. During the present year this camera with its mounting and drive was loaned to the University of Michigan, which has transferred it to the Lamont-Hussey station at Bloemfontein, South Africa. At this station Mr. Karl Henize, of the University of Michigan, will use the instrument to complete the survey of planetary nebulae and emission-line stars in the southern part of the Milky Way that is invisible from northern latitudes.

Current investigations at the Mount Wilson and Palomar Observatories are continuing along the lines of the two broad programs that have characterized much of the work of the Mount Wilson Observatory for the past quarter century.

The first of these programs includes a series of investigations planned to extend our knowledge of the large-scale structures of the universe. Studies are first made of the distances, spatial arrangements, and motions of the stars in our own Milky Way system. Already it has been shown that this system is a huge disklike structure some 100,000 light-years in diameter* containing billions of stars and rotating

slowly about a central axis perpendicular to its plane. Similar investigations are then made of the structures, dimensions, distances, and internal motions of the nearer of the extragalactic nebulae, which are now known to be other stellar systems of the same general size and type as our own Milky Way system.

Finally, in another series of studies the spatial distribution and the motions of the more distant of these extragalactic nebulae are investigated. These latter studies are extended out to the greatest distance at which these huge systems can be photographed, and are concerned with such problems as whether the nebulae are uniformly distributed throughout observable space or whether a boundary is finally reached beyond which no further objects are found, thereby indicating a limit to occupied space. Earlier studies of the motions of these nebulae produced the result that all these objects appear to be receding with velocities proportional to their distances. This led to the concept of an expanding universe. The true significance of this phenomenon, however, awaits further velocity measurements on still more distant nebulae.

For the Hale telescope it is estimated that the limiting distance to which these investigations can be carried is one billion light-years, or twice as great as that reached by any previous instrument. In other words, this instrument will extend the boundaries of the observable universe to include a volume of space eight times as large as could previously be reached. Within this volume it is estimated that there are some hundreds of millions of these stellar systems.

Obviously the answers to the most fundamental problems of cosmology, including the structure and extent of the universe, the significance of the expanding universe, the possible curvature of space, and many

other similar problems, depend on further observations in this field. High hopes are held for the effectiveness of the Hale telescope in obtaining the solutions to many of these problems which depend on pushing out to the boundaries of the observable universe.

Much of the information about the structure of the Milky Way and the structure and spatial distribution of the extragalactic nebulae is obtained from a study of direct photographs of these objects taken with the telescopes used as cameras of long focal length and great light-gathering power. With the exception of a few of the closest stars, the distances of the objects are determined from a comparison of the absolute brightness or candle power of the object with its apparent brightness as estimated from a photograph or measured with a photoelectric cell. The facts necessary to assign a star to a given class of known absolute magnitude are found from a study of its spectrum or, if it is a variable star, from a determination of the period and form of its light-variation. Motions of near-by objects across the line of sight are measured by observing the change in position over a period of years; velocities toward or from the observer are readily fixed by a study of the displacement of the lines in the spectrum of the object.

The second program is concerned with the physical and chemical properties of individual stars and other single objects. Since, with the exception of our own sun, all stars appear as points even in the most powerful telescopes, such investigations are limited to a detailed analysis of the star's light by means of spectrographs. In the past century the spectra of all the standard stellar types, as well as those of most of the more unusual types such as variables and novae, have been studied and described with increasing detail as telescopes and spectrographs have become more

powerful. Thus the great light-gathering power of the 100-inch and 200-inch telescopes combined with recent advances in spectrograph design now make it possible to study the spectra of all naked-eye stars in as much detail as was possible for the sun a half century ago. Such spectroscopic studies continue to constitute a substantial part of the observational program of the Observatories.

With the development of theories of atomic structure in the past quarter century, it is rapidly becoming possible to utilize these spectroscopic observations to obtain a more quantitative understanding of stellar characteristics. Thus from measurements of both the positions and the intensities of the lines of a stellar spectrum one may obtain a quantitative chemical analysis of the stellar atmosphere. Similar measurements make it possible to fix the temperature, the pressure, and the strength of the magnetic field in the observable surface layers of the star's atmosphere. Study of the spectrum also throws light on the structure of the star's atmosphere and the circulation of gases in it. With the aid of theory it is possible to extrapolate the temperatures, pressures, and densities to the center of the star and to understand the flow of heat out from the central regions where it is generated.

Recent nuclear experiments and theories point definitely to the hypothesis that the energy which a star radiates in such tremendous quantities comes from nuclear transformations of one chemical element into another. These reactions are of the same general type that gives the atomic bomb its enormous power. Information concerning the central temperature and density of typical stars combined with a knowledge of chemical composition is necessary to determine which of the possible nuclear reactions are most effective in each stellar type. Quantitative determina-

tions of chemical composition also provide information as to the extent to which these nuclear fires have progressed in burning up the available supply of fuel. Eventually such studies may throw light on the origin of the chemical elements.

As new theoretical approaches begin to find solutions to these problems, they almost invariably raise additional problems that must be solved by further observations before the final answers are reached. In particular, recent studies of the types mentioned above are requiring quantitative measurements of the intensities of spectrum lines in addition to the measures of their positions which were provided by earlier observations. The Observatories have recently completed a new direct-intensity microphotometer which will very greatly speed up these quantitative intensity measurements.

Naturally all these spectroscopic studies of stellar atmospheres are checked where possible with detailed observations of surface features of the one star where they can be directly observed, namely, our own sun.

As in all scientific work, these broad programs are built up out of a large number of detailed investigations. It is only as the results of these highly specialized studies, carried out at many institutions, are brought together that the general broad pictures of our universe begin to emerge.

The following sections of this report outline the detailed programs that have been in progress during the current year. As-

tronomy is almost entirely an observational rather than an experimental science. For this reason, any observing program must take into account many factors over which the astronomer has no control. Thus, observations of any given object can be made only during the time of year at which the object is in the sky for a substantial part of the night. Particularly if long exposures are necessary, observations are therefore limited to a period of from two to four months each year. Several years may thus be required to accumulate the necessary observations on a particular object, and parallel studies of other objects must be planned to utilize the remainder of the observing periods during each year. Furthermore, many programs are concerned with changes in the spectra, in the brightness, or in the positions of objects. Many of these changes occur slowly and often may require years to complete a cycle. Again, occasional observations spread over long periods are necessary for these studies.

Because of these conditions it is customary and indeed necessary for effective operation that any given observer have a substantial number of programs in progress at any one time, many of which will require several years for completion. As a result, any record of the projects to which the members of the staff of the Observatories have devoted time in a given year is a long one, much longer than would be normal for a staff of the same size working on experimental problems.

OBSERVING CONDITIONS

The total precipitation for the season of 1948-1949 was 21.63 inches, which is the third lowest on record on Mount Wilson. This dry season following the record low rainfall of the preceding year has had a serious effect on the water supply of the mountain. By the application of moderate

restrictions on the use of water it has been possible, however, to maintain an adequate reserve in the reservoirs, and no serious difficulty is anticipated unless there are additional unusually dry years. The snowfall was 84 inches.

Solar observations were made on 312

days between July 1, 1948 and June 30, 1949. During this period the 60-inch telescope was used on 282 nights, the 100-inch on 263 nights.

SOLAR RESEARCH

SOLAR PHOTOGRAPHY

Solar observations were made by Hickox, Hoge, Nicholson, and Richardson. The numbers of photographs of various kinds were:

Direct photographs	612
<i>Ha</i> spectroheliograms of spot groups, 60-foot focus	972
<i>Ha</i> spectroheliograms, 18-foot focus	1,040
K2 spectroheliograms, 7-foot focus	41,680
K2 spectroheliograms, 18-foot focus	879
K prominences, 18-foot focus	1,098

SUNSPOT ACTIVITY

The magnetic classification and study of sunspots and related phenomena have been continued by Nicholson and Miss Whitney. Co-operative programs have been carried out with the United States Naval Observatory, the Observatory of Kodaikanal, the Department of Terrestrial Magnetism of the Carnegie Institution, and the Central Radio Propagation Laboratory of the National Bureau of Standards.

During the calendar year 1948, observations were made on 334 days, none of which was without spots. The total number of sunspot groups observed was 582, compared with 663 in 1947. The number of groups in the northern hemisphere decreased from 309 in 1947 to 271 in 1948; in the southern hemisphere, from 354 to 311.

The monthly means of the number of groups observed daily during the past two and one-half years are given in the accompanying table.

The number of sunspot groups observed daily has varied in characteristic short-

Month	Daily number		
	1947	1948	1949
January	9.9	10.6	8.7
February	10.5	8.1	13.6
March	11.8	8.0	14.7
April	11.5	14.4	12.1
May	16.8	12.7	10.2
June	13.4	12.2	9.6
July	12.9	11.4
August	14.1	12.4
September	13.7	10.8
October	12.4	10.7
November	10.8	9.2
December	10.1	10.2
Yearly average..	12.3	10.9

period fluctuations. Three marked maxima have occurred at intervals of 11 months, in May 1947, April 1948, and March 1949, with monthly means, respectively, of 16.8, 14.4, and 14.7 groups per day. The great activity of May 1947 and of the other months of that year marks the middle of 1947 as the maximum of the cycle.

SUNSPOT POLARITIES

Magnetic polarities in each spot group have, so far as possible, been observed at least once. The classification of groups observed between July 1, 1948 and June 30, 1949 is indicated in the table on the next page. "Regular" groups in the northern hemisphere are those in which the preceding spot has S (south-seeking) polarity and the following spot N polarity; in the southern hemisphere the polarities are reversed.

Hemisphere	Regular	Irregular	Unclassified
North	226	6	79
South	214	4	71
Whole sun—	440	10	150

FLARES

As a result of the discovery of apparent relations between solar flares and such phenomena as radio transmission, terrestrial magnetism, and cosmic rays, there has been a steadily increasing interest in data on flares and the spot groups associated with them. The automatic patrol program was amplified in March 1949 to take five times as many spectroheliograms as before. The photographs are now taken at the rate of one per minute in series of five exposures, each exposure with a different diaphragm over the collimator. These graded exposures serve as an intensity calibration, furnish a much finer time scale, and make the detection of faint flares more certain.

PROMINENCES

The eruptive prominence of June 3, 1949 was photographed by Hickox with the 13-foot spectroheliograph, and variations of its velocity with time were studied by Pettit. The prominence rose to a height of 870,000 km in 12 hours. The time-distance diagram gave successive velocities of 1.5, 7, 33, 85, 137, and 192 km/sec. Of these, the 15 and 33 km/sec velocities were very definite. The motion was somewhat erratic during the periods having average velocities of 7 and 192 km/sec and appeared to accelerate slowly during the periods having average velocities of 85 and 137 km/sec.

SOLAR GRANULATIONS

Spectra of the solar granulations have been taken at the center of the solar disk by Martin Schwarzschild, of Princeton University Observatory, and Robert S. Richardson, using the 425-mm image of the sun at the 150-foot tower. Differences in radial velocity are readily apparent from inspection on plates taken with exposures of 10 seconds during periods of fine seeing. Preliminary measures show the hotter material to be rising with respect to the cooler background with a relative velocity of about 0.2 km/sec.

THE H AND K LINES AND MAGNETIC STORMS

Richardson has remeasured the plates taken during the great magnetic storms of September 18, 1941 and March 1, 1942 to obtain improved data for a test of the suggestion by Chapman that a cloud of charged particles moving earthward during a magnetic storm might be detected by a faint absorption line shortward of the solar lines.

The intensities of 48 selected points in the continuous spectrum from A3888 to A4007 were compared on plates taken during magnetic calm and magnetic storm. For the great storm of September 18, 1941, three plates taken a few minutes apart 20 hours 30 minutes after the storm were measured. For the great storm of March 1, 1942, eight plates were measured taken from 15 hours before to 16 hours after the storm. Flares of intensity 3+ were observed about 20 hours before the beginnings of both storms.

Results were obtained in fair agreement with those published in 1944, which were based upon fewer plates and a different method of reduction. For the storm of September 18, 1941, apparent absorption bands dropping 0.06 below the normal

spectrum were observed to the violet of both H and K. For the storm of March i, 1942, apparent absorption bands dropping 0.03 below normal were observed. The bands indicate a range in velocity of inter-solar ionized calcium of from -600 to -1400 km/sec, although the uncertainties in these velocities are large.

If the absorption bands are real, the effect is barely above the limit of observation by photographic photometry. Even these improved measurements fail to furnish definite observational evidence for the assertion that magnetic storms are due to corpuscular emission from the sun.

GENERAL MAGNETIC FIELD OF THE SUN

Fifteen sets of visual measures of the sun's general magnetic field were made between October 1, 1948 and April i, 1949 by Nicholson and Hickox using the *Fe* line at 6173 Å. The individual sets gave values ranging from +43 to -28 gauss, reduced to the north pole of the sun, with a mean value of -2.3 ± 3.3 gauss. The

probable errors indicate that the range in the measures was accidental and not due to a varying field.

RELATION BETWEEN SOLAR AND TERRESTRIAL PHENOMENA

Nicholson and Dr. Oliver Wulf, of the U. S. Weather Bureau, have extended their studies of the relation of bright solar flocculi to geomagnetic activity, previously reported for 1943 and 1944, to include the years 1941 and 1942. They have compared geomagnetic activity with the declination of the moon over 294 lunar cycles (1925-1946) and have found a tendency for activity to be highest near and following the northerly and southerly maxima of declination, when the lunar air tide is asymmetric with respect to the earth's axis of rotation and to the average air circulation. These and other observations give further evidence that the circulation of the upper atmosphere plays a role in the production of recurrent geomagnetic activity.

STELLAR INVESTIGATIONS

LIGHT-CURVES OF NOVAE

The visual brightness of Nova Puppis 1942 as measured by Pettit with the wedge photometer has now fallen to mag. 11.7. A plot of all the measures since November 10, 1942 on a logarithmic time scale shows a general drop from maximum light with some long-period fluctuations of a few tenths of a magnitude. From Sanford's measures of the distance to the nova and the expansion velocity of the shell it can be estimated that the present diameter of the expanding shell is about $1V2$. Since the minimum disk detectable under ordinary circumstances is probably about 2" in diameter, the shell cannot be expected to be visible before 1952.

The visual brightness of T Coronae Borealis fell gradually from the secondary maximum of mag. 8.0 in the summer of 1946 with some small fluctuations to mag. 9.8 during the year July 1947 to July 1948. It then brightened and is now about mag. 9.6. Slow oscillations of this kind were observed in 1867 at the same interval, 1238 days after primary maximum, and in the same magnitude range. It appears that the light-curve has behaved like the 1866 curve in considerable detail.

SPECTRAL ENERGY-CURVES OF STARS

Pettit has investigated the adaptability of a panchromatic photomultiplier tube to the measurement of spectral energy-curves

of stars. For this purpose the tube in the regular nebular photometer mounting was attached to a Bausch and Lomb quartz monochromator at the Newtonian focus of the 60-inch telescope. Deflections at hundred-angstrom intervals of the spectrum were taken between A7000 and X3000 on a Leonis and ν Virginis. When properly reduced, these measures yielded spectral energy-curves outside the earth's atmosphere.

The energy-curve of ν Virginis, mag. 4.2, type Mi, shows a general drop in the visible spectrum and a rapid drop from A4500 to A3900, after which it is about constant at about one-tenth the intensity at A5500. The curve for a Leonis, mag. 1.34, type B8, rises from about one-eighth maximum intensity at A6000 to a maximum at A4800, drops rapidly to about half this value at A3700, then rises throughout the ultraviolet.

STELLAR SPECTROSCOPY

RADIAL VELOCITIES

Completion of the older Observatory radial-velocity programs was delayed, mainly by unfavorable weather conditions during the winter months, when most of the remaining observations must be made. About 65 plates of 53 stars are still needed.

During the report year, 411 plates were taken with the 60-inch telescope on these and the supplementary radial-velocity programs, 319 by R. E. Wilson. In addition, 676 plates were obtained with the 60-inch in connection with special investigations by various observers. Measures of the plates taken on the regular programs are up to date.

Radial velocities have been completed for about 2000 stars. The results together with revised spectral classifications are being prepared for publication by R. E. Wilson and A. H. Joy.

The card file for a general catalogue of radial velocities has been kept up to date; a study has been made of systematic differences between the various observatories engaged in radial-velocity determinations; and the format of the catalogue has been discussed and adopted.

E. R. Dyer, Jr., Carnegie Fellow, who is on a two-year leave from the University of Virginia, is measuring the radial velocities of dwarf M stars selected from objective-

prism plates by Vyssotsky and others at the Leander McCormick Observatory by means of spectrophotometric criteria. It is expected that the inclusion of these stars will give a solution for space velocity less affected by selection than previous material. These stars have visual magnitudes from 8.9 to 11.5 and are distributed over the sky north of -20° declination. From October 1948 to June 1949 slit spectrograms of no A/mm dispersion have been obtained for 148 stars, mostly with the 60-inch reflector, and about half of these have been measured. Eight new stars with Balmer emission have been found, and more than 40 stars with bright H and K lines of *Can*.

The spectral types and radial velocities of 90 dwarf stars of late type which have been under observation for parallax at the McCormick Observatory were published by Joy and Mitchell.

VARIABLE STARS

Spectrograms of 34 of the high-luminosity variable stars of globular clusters were obtained by Joy. By use of the spectral characteristics and the periods it is possible to determine the type of variation of the star. A tabulation indicates that with a few exceptions the cluster variables belong to the RR Lyrae, W Virginis, RV Tauri,

and semiregular (periods 60 to no days) classes. The radial velocities yielded by the spectrograms serve to identify the stars with the clusters as well as to help determine the velocities of the clusters.

Forty-four coudé spectrograms of cepheid variables and spectroscopic binary stars have been obtained by Sanford for the determination of radial velocities. The spectrum of W Virginis, a cepheid of stellar population II, was found to have double absorption lines for the first couple of days after light-maximum.

Further studies have been made by Sanford of the radial velocities of RR Lyrae from high-dispersion spectrograms. The absorption lines of hydrogen and of H and K were found to become double in each cycle for a short time near median magnitude on the rising branch of the light-curve.

Joy has obtained additional spectrograms of RR Lyrae stars, bringing the number of unpublished stars to 60.

Sanford has continued his investigation of the recurrent nova T Coronae Borealis. Spectrograms obtained in January and February 1949 are essentially similar to the one obtained in May 1948. The study of earlier plates has shown a velocity variation of the lines of the class M spectrum. This variation follows a sine-curve with a semi-amplitude of 21 km/sec and a period of 230.5 days. No velocity variation is shown by the source of the emission lines; hence the assumption that T Coronae Borealis is a binary is beset with difficulty.

Spectrograms of Nova Cygni 1948 were obtained by Sanford, Mowbray, and Joy in June 1948. Emission and absorption lines characteristic of the phases shortly following maximum were present. Velocities of the expanding shells were 560 and 1270 km/sec. A spectrogram obtained on July 1, 1949 showed an advanced nebular stage with weak continuous spectrum and

strong emission lines of H, *Hen*, Nni, [Ne m], and [Oin].

STARS WITH EMISSION LINES

The Mount Wilson survey of the northern Milky Way for the detection of stars whose spectra have bright hydrogen lines was completed during the past year. This survey, begun by Merrill in 1919, combined the advantage of (1) objective-prism photography with the wide-angle 10-inch telescope with (2) the use of the strong red *Ha* line. Recent photographs have been made with a lens especially designed for red and yellow light; they are far superior to the early plates. Many observers have taken part in the program, the principal ones having been P. W. Merrill, M. L. Humason, W. C. Miller, and A. G. Mowbray. A very large part of the assessment of plates and preparation of catalogues has been done by Miss Cora G. Burwell. The third section of the general catalogue, now ready for printing, brings the number of Be stars to 1088. Of these, about 800 have been detected at Mount Wilson. Awaiting future listing are about 600 additional bright-line objects discovered at Mount Wilson whose spectral types have not been determined; but it is reasonably certain that most of them are of class B.

The spectra of most of the brighter objects discovered in the survey have been photographed again, in more detail, with slit spectrographs attached to the 100-inch or the 60-inch telescope. Several stars have become the subjects of extensive investigation.

Shell stars offer special inducements to detailed study because many of them have extremely active outer atmospheres. About 35 are now known (not counting P Cygni stars or those with combination spectra), of which only a few have been adequately observed. In several stars (HD45910,

187399, 218393) studied during the year by Merrill, the hydrogen lines undergo striking changes in structure and displacement from plate to plate. Of special interest in certain stars is the progression in radial velocity from line to line along the Balmer series. Studies have been continued of the long-period damped oscillations in 48 Librae and in HD33232. In DM—27⁰ 11944 an outflow of gas at the rate of 300 km/sec has continued since 1921. It is hoped eventually to obtain reliable interpretations of the remarkable velocities and accelerations which occur in the extended atmospheres of these objects.

O. C. Wilson has nearly completed the observational part of the spectrographic survey of the northern Wolf-Rayet stars. Three stars with variable displacements presumably due to orbital motion are HD177230 and 197406 and BD + 40°4220. Another star, HD50896, shows large variations both in displacement and in shape of some of the emission bands, but it is not yet known whether these effects can be attributed to orbital motion.

An orbit for the Wolf-Rayet binary HD190918 has been completed and published. The velocity variation is relatively small, and if this fact is attributed to small inclination of the orbit, the results show that the observed red shift of A4686 He 11 in such stars is probably independent of the direction from which they are viewed.

A complete list of the stars whose spectra are known to have H and K (*Can*) in emission was prepared by Joy and R. E. Wilson. The intensities of the emission lines were estimated from Mount Wilson spectrograms. Of a total of 445 stars, there are 37 supergiants, 93 giants, 15 subgiants, and 277 dwarfs. Calcium emission evidently occurs frequently among stars of various kinds, but is largely limited to the cooler atmospheres of stars of spectral types later than G5. In the giants and super-

giants the emission lines are divided by an absorption reversal.

ABUNDANCES OF CHEMICAL ELEMENTS

Greenstein is continuing his investigation of abundance, pressure, temperature, and turbulence in stellar atmospheres. Additional studies have been made of F stars, and a detailed comparison of the spectra of giant, subgiant, and dwarf G stars has been started with the highest resolution available. These F and G stars are sufficiently like the sun to provide fairly direct comparisons when detailed studies are made with high dispersion. A preliminary investigation of the subdwarf Groombridge 1830 reveals large differences from a normal dwarf, due to the higher pressure and the higher opacity in the subdwarf.

Two stars with apparent peculiar abundances of certain elements are also under investigation. The carbon-rich, hydrogen-poor star R Coronae Borealis normally shows a metallic-line spectrum remarkably close to that of a late F-type supergiant, the C₂ bands, and a few as yet unidentified features. A detailed program of measurement and identification of the lines in the helium-rich, hydrogen-poor star υ Sagittarii is in progress; high-dispersion spectra are available from A3200 to A6600. In the red, weak emission of *Nai*, *Feu* is observed, besides the strong absorption of *Ne* 1, *N*1, and the ionized metals. The variation of line intensities around the radial-velocity orbit has been studied by Dr. Adams and found to be small.

GENERAL MAGNETIC FIELD OF EARLY-TYPE STARS

The search for and analysis of stars showing evidence of strong magnetic fields has been continued by H. W. Babcock. Construction and use of a new optical

phase-shifter have permitted effective extension of the observing program to stars north of the equator. This phase-shifter, or compensator, largely overcomes the limitation imposed by the oblique reflection from the coudé flat of the 100-inch telescope. One result of this extension is the discovery that the well known bright spectrum variable α^2 Canum Venaticorum has a strong magnetic field, and that the field not only varies, but reverses its apparent polarity in a manner rather similar to that previously found for HD125248. The magnetic variations are very probably synchronous with the 5.5-day period of line-intensity variations. This observation is significant for the interpretation of the spectrum of α^2 Canum Venaticorum, which has been almost intractable heretofore.

Two other stars, HD10783 and HD 153882, have been found to be spectrum variables of type A, and to possess strong magnetic fields that reverse their apparent polarity. Periods have not yet been determined. Considerable evidence on magnetic fields in other stars has been accumulated.

A number of additional spectrograms of HD125248 have been obtained; together with the plates of last year they make a valuable collection, well distributed over the 9-day period of spectral and magnetic variation. Measurement of these plates, with the assistance of Miss Burd, is now well advanced. Tentative results show that elements of the three groups characterized by the rare earths, iron, and chromium are generally consistent among themselves, but that at certain phases systematic differences appear among the three groups in radial velocity and in indicated polar magnetic field strength. Additional data are being assembled.

The elementary theory of magnetic intensification of stellar absorption lines in

the presence of a general magnetic field has been developed during the year. It is shown that the maximum intensification factor for a saturated line in the presence of a strong field is n/i , where n is the number of components in the Zeeman pattern. Conditions favoring the effect include high atomic weight, moderate temperature, and, of course, a Zeeman pattern of many widespread components. Many lines of *Eu* 11 and of *Cr* 1 are particularly sensitive to magnetic intensification. Evidence for the presence of the effect in α^2 Canum Venaticorum is given in the form of a comparison of theoretical intensification factors with variations in line intensity observed by Struve and Swings. It is apparent that many phenomena observed in spectrum variables still require explanation.

Microphotometer tracings of the spectrum of the white dwarf 40 Eridani B obtained with the analyzer show no evidence of polarization. It is concluded that the great widths of the hydrogen lines in the spectrum cannot reasonably be accounted for by Paschen-Back broadening, as suggested by Blackett.

MISCELLANEOUS

The near-by dwarf star L 726-8 was observed by Joy and Humason at the request of W. J. Luyten. At the time of the first spectroscopic observation with the 100-inch telescope, August 25, 1948, the star was found to be a visual binary. The spectra of both components were estimated as $dM5.5e$. Measurements of a direct photograph showed a separation of $1''.5$ in position angle 117° . At the time of a second spectroscopic observation, September 25, 1948, one of the components, which could not be identified because of poor seeing, increased its brightness noticeably. The spectrogram showed that the outburst pro-

duced a strong continuous spectrum which nearly blotted out the normal M-type spectrum, the emission lines of hydrogen being strengthened. The line of ionized helium at X4686 appeared in emission. This type of spectral activity had previously been found by Joy in the T Tauri variables and in the bright-line stars involved in the Taurus clouds.

Among the dwarf stars of lowest luminosity, seven were observed whose spectra were dhfy or later but without emission lines. No subdwarf characteristics were seen.

The intensity distribution of the continuous spectrum of the well known subdwarf Wolf 489 was estimated to be about that of a G₀ star. No lines could be distinguished in the spectrum.

Sanford has shown that the radial velocities derived from the interstellar Ca II lines in the stars in open clusters, as well as the stellar velocities, manifest differential galac-

tic rotation. Furthermore, there is evidence that both equivalent widths and velocities from the interstellar lines have merit as criteria of distance.

Additional spectrograms of stars of standard spectral types have been obtained. One set, dispersion at $H\gamma$ 35 Å/mm, was obtained largely by A. H. Joy.

Microphotometer tracings of the widened lines in the spectrum of the G-type star HD 17555 indicate a rotational speed at the equator of at least 75 km/sec. This observation by Merrill would indicate that hydrogen atoms thrown out from the equatorial zone may be responsible for the bright $H\alpha$ line.

Zwicky, Johnson, and A. G. Wilson have completed the coverage of the Milky Way both with direct photographs and with objective prism on the 18-inch schmidt telescope. This provides the base comparison plates for an extensive search for novae in the Milky Way.

GALACTIC NEBULAE AND STAR CLOUDS

PLANETARY NEBULAE

The search for planetary nebulae by Minkowski has led to the discovery of 34 new planetaries during the past year. The total number of planetaries discovered on the 10-inch objective-prism plates is 194. Objective-prism exposures with the 18-inch schmidt have to date led to the identification of 20 new planetaries; a considerable number of objects found with the 18-inch schmidt still remain to be investigated. Of the total of 370 planetaries now known, 114, or almost one-third, appear within 10° of the galactic center. This demonstrates the high concentration of planetaries toward the center of the Galaxy. It is hoped to obtain information on the kinematics of the central region of the Galaxy from an investigation of the radial velocities of planetaries; such an investi-

gation has been started, but no results are as yet available.

The survey of internal motions in the brighter planetary nebulae with the coude spectrograph has now been completed by O. C. Wilson. In general, relatively large velocities of expansion of the nebular shell are found. Except in the case of hydrogen, larger velocities are more normally associated with high-excitation particles than with low-excitation particles. Moreover, slitless spectrograms show that $[N\text{v}]$, which always gives a smaller than average velocity of expansion, also always gives an image slightly smaller than hydrogen, whereas lines of $[O\text{ II}]$ and $[N\text{ II}]$ tend to give slightly larger slitless images. These facts suggest that radiation pressure, arising from radiation produced within the bright rings, is responsible for the observed

effects. Such considerations have led to a somewhat different picture of the structure and dynamics of planetary nebulae from that now current.

NOVA HERCULIS

Plates taken by Baade in the summer of 1948 at the Newtonian focus of the 100-inch telescope show no marked changes in the structure of the ejected shell within the past four years. Images of A3727 [On] and A4959, 5007 [O in] are elliptical rings with weak condensations at the ends of the major axis. The image of *Ha* A6548, 6584 shows the same remarkable structure noted in 1942.

VARIABLES IN THE NUCLEAR REGION OF THE GALACTIC SYSTEM

The observations of the field around the globular cluster NGC 6522 ($A=328^\circ$, ($3=-4^0$) were concluded by Baade with extended series of plates taken from July to September 1948. Continued search for variables has brought their number to 284 for the field investigated, or to 750 variables per square degree. Magnitudes for the variables so far found have been estimated by Dr. Sergei Gaposchkin, of Harvard College Observatory. The derivation of the light-curves, the joint work of Dr. Gaposchkin and Dr. Cecilia Payne-Gaposchkin, is completed for more than half the material. The results confirm the earlier surmise of Baade that the stars of the nuclear region of our galaxy belong to population II. Roughly 50 per cent of the variables are cluster-type variables of large amplitude with very asymmetrical light-curves and periods of less than 0.4 days. The semiregular variables come next in frequency.

During the present year observations of

a new field of the nuclear region centered on CD-28°14334 ($X=332^\circ$, $\xi=-6.5^\circ$) were begun. In contrast with the field centered on NGC 6522, which shows some irregularities caused by absorption, the new field is exceedingly uniform.

STAR CLUSTER WITHIN THE ORION NEBULA

The large star cluster in the Orion nebula recently discovered by Baade, using plates sensitive to the A6900-7500 region, has been searched for variables. A comparison of two pairs of plates led to the discovery of 50 new objects of this type.

GALACTIC STRUCTURE

With the 48-inch schmidt camera the central region of our galaxy was covered by Baade with red exposures, the exposure times ranging from 1 hour to 3 hours. A search on these plates led to the discovery of a few new globular clusters. All are rather close to the galactic equator (the line of greatest obscuration) and are exceedingly faint.

Baade is making an attempt to single out the near-by spiral arms of our own galaxy, using the highly luminous B and O stars and the emission nebulosities which from studies of the Andromeda nebula are known to occur only in these arms. Because of heavy obscuration, it appears simplest to search for the emission nebulae in *Ha* light. This search will be followed by the identification of exciting stars, and the determination of their spectral types, absolute magnitudes, apparent magnitudes, and color excesses from which distances can be calculated. As a first step in this program, the Milky Way was covered along the galactic equator from $A=310^\circ$ to $A=90^\circ$ with red exposures of 1 hour's duration with the 48-inch schmidt camera.

EXTRAGALACTIC NEBULAE

NEBULAR PHOTOMETRY

The program of photometry of the extragalactic nebulae for which Humason has derived velocities has been continued by Pettit with the 1P21 photomultiplier cell, principally with the 60-inch telescope. Magnitudes on the photographic scale and color indices have been obtained for each object. Of over 500 systems, approximately 70 remain to be measured. Many of the remaining systems are large and require measurement of numerous galactic stars. Some systems will have to be measured with the 20-inch telescope.

Of the 175 nebulae measured by Stebbins and Whitford, 22 have been remeasured, one-half of which are indicated by them as being of doubtful accuracy. The average departure regardless of sign is 0.1 mag.

RADIAL VELOCITIES

Final revisions of measured red shifts and spectral types for over 500 nebulae have been completed and are now being prepared for publication by Humason.

Two spectrograms of NGC 4151 have been obtained with the coudé spectrograph (10.3 A/mm) by O. C. Wilson. The emission bands have been measured for wave length on one plate, with the result that AX/A appears to be constant to within about 1 per cent from A3400 to A6600. The second spectrogram is somewhat stronger and shows unmistakable structure in some of the emission bands; no analysis of the structure has yet been attempted.

SURVEY OF THE ANDROMEDA NEBULA

The 100-inch survey of the Andromeda nebula with ultraviolet, blue, red, and near infrared exposures, covering an area 1.75 by 5.0, was practically completed by Baade during the present season. Among the

final results will be a complete list of all emission nebulosities brighter than -3^M (some 300) and a similar number of star clusters to about the same limit.

MEMBERS OF THE LOCAL GROUP

Intercomparisons with Selected Area 68 were made by Baade for both NGC 6822 and the Andromeda nebula, since there exists some uncertainty regarding the magnitude of the brightest stars in these systems. Long exposures of the Andromeda nebula with the 48-inch Schmidt in the visual and red regions revealed what appears to be a tidal effect in its elliptical companion, NGC 205. Besides its two axes due to projection, this nebula shows a third axis which is directed toward the center of the Andromeda nebula. This third axis or bar extends in either direction beyond the projected disk of NGC 205.

DISTRIBUTION OF NEBULAE

A substantial part of the first photographs taken by Hubble with the 200-inch Hale telescope and by Hubble, Baade, and Zwicky with the 48-inch schmidt camera were devoted to exploratory investigation of the distribution of extragalactic nebulae in space. Hubble, with the assistance of A. R. Sandage, is studying on some of these plates the distribution of faint nebulae in standard apparently normal regions of the sky.

Almost a dozen distant clusters of nebulae were found in an area of 300 square degrees in Virgo by Baade, and several additional clusters were picked up by Zwicky, especially in the Corona Borealis region. A number of clusters discovered on plates taken with the 48-inch schmidt telescope were checked on plates taken by Baade with the 100-inch. It is obvious from these tests that the few

clusters of nebulae previously known represented a very meager sample indeed, and that the rich material to be expected from the 48-inch schmidt survey will greatly facilitate all investigations in which one must fall back on clusters of nebulae (velocity-distance relations, etc.).

Zwicky has used the 48-inch schmidt plates to study the luminosity function of nebulae, particularly at the low-luminosity end of the distribution-curve. An additional dwarf system was found which presumably is within the perimeter of the local group, and many dwarf systems were picked up which belong to other groups of nebulae. As a limit of dwarf systems, the suspected existence of intergalactic matter was confirmed through some preliminary investigations of the central regions of the Coma cluster, where luminous intergalactic matter seems to be recorded on the 48-inch schmidt plates over a region about one-half million light-years in diameter.

The work on the constitution of clusters of nebulae with the 18-inch schmidt was continued by Zwicky and was extended to the 48-inch schmidt for the Cancer cluster and the Coma cluster. The studies made originally with the 18-inch were corroborated; in particular, it was found that bright and faint nebulae segregate in the sense of heavy and light masses in a Boltzmann assembly.

An area 18° by 18° covering the Virgo cluster was photographed by Baade. The plate material will permit an accurate classification of all members of this cluster, since the scale of the 48-inch schmidt (1 mm = 67 seconds of arc) is large enough for study of the detailed structure of the nebulae.

Plates taken by Baade in connection with a study of the group of nebulae associated with M 81 and 101 revealed a third dwarf member associated with the physical pair NGC 5236 and 5253.

THEORETICAL STUDIES

The so-called galactic radio noise has been studied theoretically by Greenstein. Since it may originate in thermal agitation in interstellar clouds of ionized hydrogen, an attempt has been made to determine the effective optical thickness of the ionized regions in our galaxy. This appears to be small except along the spiral arms, and galactic radio noise, if produced in interstellar space, should come only from the spiral arms.

With Professor Leverett Davis, Greenstein has developed a theoretical explanation of the recently observed polarization of light transmitted through interstellar clouds of absorbing dust particles. If the particles are not spherical but elongated, as most crystalline materials are, they absorb light preferentially of a plane of

polarization perpendicular to their long axes. To produce observable polarization, the long axes should remain nearly parallel over long regions in space. This orderliness is disrupted by the bombardment of the particles by the interstellar hydrogen. At $10,000^\circ$ K, an average collision with hydrogen produces an angular velocity of the particle of 10^6 radians per second. Thus only at very low temperatures (10° K) will there be orderliness unless a decelerating mechanism is found. If the particles have some ferromagnetism, their rotation in a galactic magnetic field will dissipate energy and slow them down, by hysteresis. A field of 10^{10} gauss is required. Recent theoretical progress suggests that perhaps the particles may continue to spin and nutate about the magnetic fields, with

a statistical preference for the long axis to lie perpendicular to the field. A much smaller field, with smaller energy loss, is then required. The existence of a magnetic

field, either general or associated with the interstellar clouds, is of significance in recent theories of the production and trapping of cosmic rays.

INSTRUMENTATION

The program for ruling improved gratings for spectrographs of the 200-inch Hale telescope and other instruments has been actively pushed by Prall under the supervision of H. D. Babcock until February 1, 1949, and of H. W. Babcock since that date. Between April and December 1948, five large gratings (6 by $7\frac{1}{2}$ inches) of excellent resolving power were made, and during this period the advantages of the graphite-on-diamond end-thrust bearing for the screw were proved. In 1949, three additional 6 by $7\frac{1}{4}$ -inch gratings have been ruled, as well as one slightly smaller. There has been a progressive rise in the quality of the gratings as small residual sources of error have been reduced or eliminated. Periodic error has been reduced to such a level that Rowland ghosts have intensities of less than 0.5 per cent in the sixth order, with normal slit widths. Ghosts are not seen in useful orders of the latest gratings. The periodic error has

been practically eliminated by the use of a cam which introduces a compensating periodic displacement of the grooves having an amplitude of 3×10^{-7} inch. One remaining problem is connected with proper lubrication of the ways of the diamond carriage when long grooves are ruled; this seems to represent the one serious obstacle to the production of gratings of high quality.

The integrating exposure meter for the coude spectrograph has been rebuilt by H. W. Babcock on a new design. It is superior to the old one and has proved useful in routine work for several months.

A low-dispersion grating spectrograph for use in nebular studies at the Newtonian focus of the 100-inch telescope was designed by Nichols and Minkowski and constructed in the Observatory shop. It uses one of the gratings recently ruled by Prall and Babcock.

GUEST INVESTIGATORS

In addition to the permanent staff, a substantial number of guest investigators from other institutions have made use of the facilities of the Observatories to obtain material for their studies. Plates already in our files have been used, and additional observations have been obtained with the large telescopes.

Dr. Lyman Spitzer, Jr., of the Princeton University Observatory, continued his investigations of interstellar absorption lines during the fall of 1948. About one hundred of Adams* high-dispersion plates showing

interstellar K and H were microphotometered to obtain equivalent widths of these lines and their components. New plates of some 20 stars were taken in the yellow, in a search for components of the interstellar D lines. About half of these showed complex structure, with components at the same radial velocities found by Adams in K and H. In addition, a search was made for interstellar *U* and *Be* lines, with negative results. If the abundance of *Be* relative to *Na* is the same in interstellar matter as in the earth and in meteorites, this cl-

ment should be about at the threshold of measurement with the 32-inch camera used.

In the summer of 1948 Dr. A. E. Whitford, of Washburn Observatory, University of Wisconsin, extended the previous study of colors of remote extragalactic nebulae to include spirals in the Corona Borealis cluster. In the small sample available, late-type spirals do not show the excess reddening found in elliptical nebulae in the same cluster. This observation supports Schwarzschild's suggestion that near-by elliptical nebulae have become bluer in the past hundred million years, owing to the burning out of the red supergiants. The photoelectric calibration of magnitudes and colors of stars in Selected Areas 57 and 61, begun by Dr. Joel Stebbins in 1947, was continued, and work started on Selected Area 68. The study of interstellar reddening out to 2 microns, begun in 1947, was extended to several additional highly reddened B stars, using an improved lead sulfide photometer with refrigeration.

Dr. John C. Duncan, of Whittier Observatory, Wellesley College, continued his photographic studies of nebulae. Negatives were obtained with the 18-inch Schmidt camera and red-sensitive films which showed much more extensive nebulosities in the Cygnus region than had previously been observed.

During the summer of 1948 Dr. George Herbig, on leave from the Lick Observatory as a National Research Fellow, made a study, on coude plates, of the occurrence of the titanium isotopes in M-type stars in which the titanium oxide bands appeared in adequate strength. A precise determination of the abundance ratios was not feasible owing to the photometric and other difficulties inherent in quantitative work with such heavily lined spectra, and to the present impossibility of identifying all the

TiO lines which were confused with the weak isotope features. The results indicate that, in the stars examined, and within the rather large uncertainty of the observations, there are no large deviations from the isotopic constitution of terrestrial titanium. Certainly there is no enhancement of the relative abundance of any of the rarer titanium isotopes in the M-type stars such as is apparently observed for C^{13} in some carbon stars.

A rather strong molecular absorption band with head at $\lambda 3682$ was found by Merrill and Joy in the spectra of a number of long-period variables. Herbig identified this band with *ZrO* by comparison with a spectrogram of the zirconium arc in air taken by Dr. R. B. King. The rotational structure of the band on the laboratory plate is that to be expected of an electronic transition with $\Delta A = 0$; a $^1Z \rightarrow ^*Z$ transition seems most probable. If this is the case, this band is probably the (0,0) head of a new system of *ZrO*.

The "channeled" appearance of the *H δ* line in Omicron Ceti was shown to be due largely to absorption by rotational structure of the (2,0) and (3,1) bands of the blue-green system of titanium oxide.

In two visits to the Observatories in 1948 and 1949, Dr. Sergei Gaposchkin, of the Harvard College Observatory, carried out an extensive investigation of variables in the vicinity of the galactic nucleus around the globular cluster NGC 6522. Estimates were made of the brightness of several hundred variables, previously discovered by Baade on over 100 plates of this region. The classification of the variables on the basis of these measures was used for a general discussion of the problem of the population of the galactic nucleus. In addition, some colorimetric studies of the globular clusters NGC 6522 and 6528 and a spectroscopic investigation of a few in-

dividual stars such as SX Cassiopeiae and AE Aquarii were carried out.

During the summer of 1948 Dr. Cecilia Payne-Gaposchkin, of the Harvard College Observatory, examined and classified over 2000 spectra of cepheid variables, cluster-type variables (RR Lyrae stars), and RV Tauri stars, from the Observatories' files. For some stars (especially on spectra of higher dispersion) the intensities of selected lines were estimated by eye in lieu of classification. The material obtained from this examination is being used for the formation of mean spectral and velocity curves of these stars as a function of their period, and for an analysis of the atmospheric and physical conditions of the stars.

Dr. Daniel M. Popper and Dr. Everett C.

Yowell, of the University of California at Los Angeles, have carried out spectroscopic observations of a group of eclipsing binaries.

A study of the intensities of the lines of C, AT, O, Ne, Mg, Al, Si, and S has been undertaken for early B stars by Dr. Lawrence H. Aller, of the University of Michigan, using coudé plates from the Mount Wilson Observatory files. The survey includes nearly all objects with sharp lines between O and B5. These intensities are being used for a determination of the abundances of these light elements.

Mr. W. C. Miller and Dr. A. G. Mowbray, both of Pasadena, have made further important contributions to the Be star program by obtaining many slit spectrograms with the large telescopes.

THE LIBRARIES

During the year 1948-1949 the library on Santa Barbara Street has acquired 562 volumes, 276 by gift, 103 by purchase, and 183 by binding, making a total of 16,892 volumes. Volumes from the Hale collection still form a large proportion of the gifts, but a number have come from other sources, including a selection from Dr. Pease's library, presented by the executor of his estate. His copy of Smith's *Optics*, 1738, is an excellent addition to the collection in the Hale Room.

About 500 volumes have been selected from Dr. Hale's library to send to Palomar Mountain, for the book room of the 200-inch Hale telescope. Some of these are duplicates of astronomical books already in both the Santa Barbara Street and Robinson Laboratory libraries; others, of a more

general scientific character, will furnish good reading during inclement weather.

To the 4000 volumes in the library at Robinson Laboratory, 387 volumes have been added during the year: 141 by purchase, 22 by binding, and 224 gifts. Among the latter, 85 volumes came from Mrs. Richard C. Tolman as a memorial to Dr. Tolman, 24 from the Bateman library, and 82 from the Hale, Pease, and van Maanen collections. Although not yet complete, particularly in reference works, this "working library" began in the fall of 1948 to serve the new department of astronomy of the California Institute of Technology and those staff members of the Mount Wilson and Palomar Observatories whose offices are in Robinson Laboratory.

STAFF AND ORGANIZATION

Dr. Alfred H. Joy retired on October 1, 1948 after thirty-three years as a member of the staff of the Observatory. After first

participating in the solar program of the Observatory, Dr. Joy gradually shifted his efforts to stellar spectroscopy. He col-

laborated extensively with Dr. Adams in the study of the effect of absolute magnitude on stellar spectra and in the use of these relationships for the determination of stellar parallaxes. In recent years Dr. Joy has investigated the spectra of a large number of variable stars. These studies have very substantially advanced our knowledge of the classification and characteristics of this difficult group of objects. Radial-velocity measurements of these stars have been applied by Dr. Joy to studies of galactic rotation and similar problems.

From 1920 until his retirement Dr. Joy held the post of Secretary of the Observatory. In this position he very ably handled most of the official correspondence. He was also responsible for the public relations of the Observatory, including arrangements for visitors, press releases, and the preparation of official literature. Mr. Milton Humason was appointed Secretary of the Observatory on Dr. Joy's retirement.

Miss Cora Burwell retired on July 1, 1949 after forty-two years of service on the computing staff of the Observatory. In addition to assisting in many investigations in the field of stellar spectroscopy, she was co-author of the very extensive *Mount Wilson catalogue of B- and A-type stars having emission lines*.

Mr. Edison Hoge transferred to the Hydrodynamics Laboratory of the California Institute of Technology in March 1949. For twelve years Mr. Hoge had been in charge of the photographic laboratory of the Observatory and had assisted in the observations of the solar department. Mr. William Miller was appointed photographer and will devote his whole time to the photographic laboratory. Simultaneously much of the routine photographic finishing work of the Observatory was transferred to the photographic laboratory of the California Institute. With these

changes it is expected that the photographer will be in a position to carry out regular tests of the speed and color sensitivity of each batch of plates used by the Observatories. He will also investigate methods of hypersensitization and other procedures designed to obtain the maximum effectiveness of the plates when used for the long exposures that are necessary in most astronomical investigations.

RESEARCH DIVISION

Solar Physics: Seth B. Nicholson, Edison R. Hoge, Edison Pettit, Robert S. Richardson, Joseph O. Hickox, Irene Whitney.

Stellar Spectroscopy and Motions: Paul W. Merrill, Horace W. Babcock, Ira S. Bowen, Jesse L. Greenstein, Milton L. Humason, Alfred H. Joy, Rudolph Minkowski, Roscoe F. Sanford, Olin C. Wilson, Ralph E. Wilson, Fritz Zwicky, Sylvia Burd, Cora G. Burwell, Mary F. Coffeen, A. Louise Lowen, Barbara Olsen.

Nebular Photography, Photometry, and Spectroscopy: Edwin P. Hubble, Walter Baade, Milton L. Humason, Josef J. Johnson, Rudolph Minkowski, Edison Pettit, Albert G. Wilson, Fritz Zwicky, Alice S. Beach.

Secretary of the Observatory: Milton L. Humason

Editor: Paul W. Merrill

Assistant Editor and Librarian: Elizabeth Connor

Photographer: William C. Miller

INSTRUMENT DESIGN AND CONSTRUCTION

Design: Bruce Rule, project engineer; Edgar C. Nichols, chief designer; Harold S. Kinney, draftsman.

Optical Shop: Don O. Hendrix, superintendent; Floyd Day, Melvin Johnson, opticians.

Instrument Shop: Albert McIntire, superintendent; Elmer Prall, instrument maker; Fred Scherff, Oscar Swanson, Albert Labrow, Donald Yeager, machinists.

MAINTENANCE AND OPERATION

Mount Wilson Observatory and Offices

Office: Anne McConnell, bookkeeper; Wilma Berkebile, secretary; Dorothea Otto, stenographer and telephone operator.

Operation: Ashel N. Beebe, superintendent of construction; Hugh Couch, carpenter; Kenneth de Huff, engineer; Murdoch McKenzie, janitor and relief engineer; Thomas A. Nelson, Ralph Bennewitz, Eugene Hancock, night assistants; Emerson W. Hartong, truck driver and machinist helper; Anthony Wausnock, Margie Wausnock, Alexander Kochanski, stewards; Arnold T. Ratzlaff, Homer N. Joy, janitors.

Palomar Observatory and Robinson Laboratory

Office: Eleanor G. Crawford, secretary and librarian; Dorothea Davis, secretary.

Operation: Byron Hill, superintendent, Palomar Observatory; Fred Feryan, Gladys M. Feryan, Harley C. Marshall, George W. Pettit, Joe Stehlik, Benjamin B. Traxler, Gus Weber, Raymond L. White.¹

¹ The Palomar Observatory is still in a transition stage from construction to operation. In most cases the final positions in the operating organization have not been assigned at the time of this report

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GEOPHYSICAL LABORATORY

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Research may be likened to the exploration of a series of small fields spread in a broad pattern over the landscape and traversed by a network of paths along one of which the investigator chooses to proceed, pausing now and then to observe, to ponder, and to experiment. He seeks to understand all that he perceives; and if after a while he is satisfied with what he has learned in one particular field, he passes on to another, sometimes following along the original path and sometimes along an intersecting one. Steadily he pushes forward from that which is known into that which is unknown and, therefore, mysterious. As he acquires experience and confidence, he is encouraged to enter areas of greater complexity and to attack more formidable problems.

Usually the true scientist finds the greatest satisfaction in extending the limits of knowledge and selecting paths that lead into new fields of inquiry, but it may happen that in undertaking step by step the solution of one of nature's broad-gauge problems, he finds that the path he must follow to success will turn back toward its starting point. Two striking examples of the way in which fruitful research may curve back on itself after many years are furnished by the Geophysical Laboratory's current program in experimental petrology. Although this is a forward-looking program and advances are being made into hitherto unexplored fields, it has seemed desirable also to re-examine two old subjects because of an increasing realization of their place as key factors for the unraveling of some mysteries concerning the formation of the rocks of the earth's crust.

These two items are the minerals feldspar and quartz.

Probably the most important group of rock-forming minerals consists of the feldspars, which are aluminosilicates containing other oxides, principally lime, soda, and potash. The first paper published from the Laboratory (1905), entitled *The isomorphism and thermal properties of the feldspars*, is a monograph now generally considered to be one of the great classics of petrology and to have laid the foundation for the subsequent program of silicate research. Day and Allen gave the first proof that the interrelationships and genesis of minerals may be studied with success by quantitative laboratory methods. Their work still evokes the admiration of the most critical, and it was done the hard way. Improved experimental methods, especially the quenching techniques and the automatic control of temperatures, were developed later, but these investigators had to make use of the method of heating curves in its most difficult application, and they were obliged to regulate temperatures laboriously by hand for long periods of time.

After the quenching method was developed, several years later, it was applied to the feldspars with resulting refinement of the original work. Feldspars are found as mineral phases in many of the silicate "systems" that have been studied at the Geophysical Laboratory, and there has been a strange lack of correlation between the synthetic feldspars and the natural ones. Pure feldspars made in the laboratory show an unbroken series of solid solutions, but careful determination of the

crystal structure of natural feldspars reveals that the two end members of the plagioclase series, albite and anorthite, have certain differences of lattice structure which seem to forbid belief that they could form a joint lattice with continuous range in composition from end to end. Also, in the laboratory the soda feldspar, albite, and the potash feldspar, orthoclase, form complete solid solutions; but in deep-seated rocks, where the concentration of potent volatile fluxes may be greater by reason of greater pressure, and where the temperature of crystallization is lower, albite containing very little potash and potash feldspar with only moderate amounts of soda are found side by side. Moreover, artificial crystalline albite does not have properties corresponding to those ordinarily given for natural albite. The field evidence, then, has seemed to run counter to the laboratory evidence.

The reason for this seeming lack of desired correlation between natural and artificial feldspars is becoming evident from the new work, made possible on the one hand by improved methods of subjecting materials to the simultaneous action of high temperature and high pressure in the presence of water, and on the other hand by the commercial development of new types of X-ray spectrometers. The recent investigations have shown that the difference between natural albite, as usually found, and artificial albite is that the natural albite is a low-temperature form. This dual character is exhibited by other plagioclases as well as by albite, and is especially marked in feldspars close to albite in composition.

Furthermore, the peculiarities in the alkali feldspars are now seen to be a consequence of an unmixing at lower temperature of the continuous series of solid solutions that may form at high temperatures. The extent of this unmixing depends on

the temperature; and, by a special technique the essence of which is to measure very precisely one of the crystal spacings, it is possible to determine rapidly and easily the compositions of the feldspars formed at any temperature. The situation with respect to the plagioclase feldspars has not yet been cleared up completely, but the indications are that by means of the new methods it will be possible to determine the temperature of formation of any pair of feldspars associated in a single rock.

Thus, it may be seen that the return to one facet of an old problem, after improved apparatus and techniques became available and after further field evidence was obtained, has led to a major advance in our knowledge of the minerals of which igneous rocks are composed. Previous discrepancies are being removed and a possible method for determining the temperature of mineral formation has resulted.

A second area of early exploration to which the orderly prosecution of silicate research has impelled us to return is the fundamental nature of the mineral quartz. This and other forms of silica have been subjected to study from the early days of the Geophysical Laboratory. In 1906 Day and Shepherd described a method for making clear and bubble-free silica glass by fusing quartz crystals under pressure—a method that subsequently was adopted by industry. The use of quartz as a geologic thermometer was suggested in 1909; Fennel's classic work on the various forms of silica and their mutual relations appeared in 1913; and the effect of pressure on the high-low inversion of quartz was studied in 1928.

The transformation of quartz at 573° C. from one crystalline form to another is one of the important characteristics of this mineral. Up to the present it has been considered to be a fixed point, like the melting point of pure Ice, independent of

the origin and previous history of any particular sample. Recent work with improved apparatus, details of which are presented in a later section, has cast doubt on the immutable character of this transformation. Indeed, parts of the same crystal may show transformation temperatures that differ by a large fraction of one degree, and individual samples of quartz may vary by nearly 2° . It is not yet possible to give a complete explanation of the

variations found in natural quartz, but enough has already been learned to justify the conclusion that the variations in properties of quartz from different localities will provide a new geological thermometer for indicating the temperature and the general conditions prevailing in rocks with which quartz is associated.

The following is an account of the principal investigation carried forward during the past year.

ANHYDROUS SILICATES

METHOD OF STUDY OF COMPLEX MIXTURES

Knowledge of the melting relations of anhydrous silicate mixtures is of fundamental importance in the application of physical-chemical methods to problems of rock formation. Much of the past work of the Laboratory has been on dry melts, from which has come information of basic interest not only in connection with the Laboratory's problems, but also in connection with those of industry. This work has progressed from the simple to the complex, the complexities arising almost entirely from the difficulty of comprehending and explaining melting relations in systems of several components.

In studying these multicomponent systems, it is our practice to separate the various systems into smaller parts, each of which can be treated as a unit. One object of this is to simplify both the theoretical discussion and the practical work. For example, the ternary system nepheline—diopside—silica, discussed below, is part of the quinary system $\text{CaO—MgO—Na}_2\text{O—Al}_2\text{O}_3\text{—SiO}_2$; but it is a part which can be studied and discussed as a unit. Another reason for the separation of a complex system into simpler parts is that we are thus able to approach our objective by a series of steps each of which may be con-

sidered a research that is complete in itself. Although a full knowledge of all the interrelations of all the rock-forming constituents is a goal to be attained in the far-distant future, we advance toward the goal in such a way that our progress may be described in terms of completed projects.

This method of subdividing a complex system is illustrated by current work on the four-component system $\text{K}_2\text{O—MgO—Al}_2\text{O}_3\text{—SiO}_2$. Here the compositions are represented graphically by means of a tetrahedron, but, to minimize the necessity for trying to think in terms of three-dimensional space, the system is separated into smaller parts by treating each group of three compositions as end members of a triangular "join." This is equivalent to passing a plane through these three points in the tetrahedron. Such a join may be a true ternary system, by which is meant that, when any mixture of the three end members is melted and crystallized, all compounds and compositions remain within the triangle. An example of this situation is the join leucite—forsterite—silica, discussed in last year's report, on which additional measurements have been carried out.

This condition is not found in the join leucite—clinoenstatite—potassium tetrasilicate, also discussed last year, on which im-

portant progress has been made. During crystallization of mixtures in this join, the compounds $2\text{MgO} \cdot \text{SiO}_2$ and $\text{K}_2\text{O} \cdot 5\text{MgO} \cdot 2\text{SiO}_2$ are formed, and, since these are outside the plane under consideration, the resulting residual liquid also must be outside, and on the opposite side, of the join. In spite of such complications, the study and discussion of the quaternary system is always greatly facilitated by separating it into smaller portions. Much progress has been made on the joins mentioned in last year's report and on some additional ones.

THE JOIN JADEITE—ACMITE IN THE
QUATERNARY SYSTEM Na_2O —
 Al_2O_3 — Fe_2O_3 — SiO_2

Substantial progress has been made on the study of other anhydrous systems. Some of this has been motivated, in part at least, by the puzzling problem of the mineral jadeite ($\text{Na}_2\text{O} \cdot \text{Al}_2\text{O}_3 \cdot \text{SiCfe}$). The stability relations of jadeite are not known, and it never has been made in the laboratory. Previous studies have shown that natural jadeite on being heated begins to decompose as low as 800°C . and that at about 850° it melts to a clear glass which later crystallizes to nepheline and albite.

Since some of the natural pyroxenes are solid solutions of jadeite and its ferric iron analogue, acmite ($\text{Na}_2\text{O} \cdot \text{Fe}_2\text{O}_3 \cdot 4\text{SiO}_2$), it was believed that a study of the join jadeite—acmite would be desirable and might clarify the stability relations of jadeite. During the past year nine compositions between jadeite and acmite have been prepared, and these are now being studied. For those compositions between 100 per cent acmite and 20 per cent acmite, the primary phase is hematite or a hematite-corundum solid solution. Between 100 per cent and 80 per cent jadeite, the primary phase is a nepheline-albite solid solution. As a consequence, during crystal-

lization of all melts in the join jadeite—acmite, the composition of the liquid phase leaves the join, and its behavior may be described only by reference to the quaternary system Na_2O — Al_2O_3 — Fe_2O_3 — SiO_2 . For those compositions in the join between 100 per cent acmite and 48 per cent acmite, the primary phase (hematite or a hematite-corundum solid solution) is next joined by acmite (or an acmitic pyroxene) below liquidus temperatures. For those between 52 per cent and 80 per cent jadeite, the second solid phase to appear is a nepheline-albite solid solution; and for those between 80 per cent and 100 per cent jadeite, the second solid phase to appear is a hematite-corundum solid solution. At subliquidus temperatures, and especially where only a small amount of a liquid phase is present, equilibrium between crystals and liquid is attained only slowly, and some necessary runs of long duration are now in progress. The fine-grained crystalline phases present at the completion of a run are being identified by their X-ray diffraction patterns.

THE JOIN JADEITE—DIOPSIDE AND COMPOSITIONS
IN THE JOIN NEPHELINE—
DIOPSIDE—SILICA

Some years ago fifty separate compositions in the triangular join nepheline—diopside—silica were prepared, and from time to time as the opportunity occurred the liquidus relations in this join were determined. This triangular join is not ternary and is a plane in the quinary system CaO — MgO — Na_2O — Al_2O_3 — SiO_2 . Some of the fifty compositions prepared were chosen to lie in the joins albite—diopside and jadeite—diopside. The results (which have not yet been published) show that albite—diopside is not a binary system and that between pure albite and 90.5 per cent albite the primary phase is not pure albite, but an albite-rich plagi-

clase. The join jadeite—diopside cuts the fields of nepheline-albite solid solutions, plagioclase, and diopside. The observation that those compositions in this join which lie in the diopside field do not reach the boundary surface plagioclase-diopside at the same temperature indicates that the diopside is not pure $\text{CaO} \cdot \text{MgO} \cdot 2\text{SiO}_2$, but a more complex pyroxene.

During the past year the subliquidus relations in the join jadeite—diopside have been investigated in greater detail, and the solid phases have been identified by means of X-rays. No jadeite has been encountered at the temperatures investigated. Those compositions in the triangular join nepheline—diopside—silica near the side line nepheline-diopside have been completely crystallized, and a study of the solid phases is now in progress. These studies should throw light on the mutual stability relations between nepheline, forsterite, diopside, melilite, and plagioclase.

THE QUATERNARY SYSTEM MAGNESIA—
FERROUS OXIDE—ALUMINA—SILICA

During the past year some further data were obtained on subliquidus relations in the join FeO-Mg cordierite—Fe cordierite. Primary phases encountered in the join are only three: mullite, spinel, and wüstite. Cordierite is obtained only as a subliquidus phase in the mullite field and in the low-iron part of the spinel field in mixtures containing up to about 40 per cent FeO. At around 50 to 60 per cent FeO in the spinel field, a pyroxene is obtained as a subliquidus phase. Charges containing more than 60 per cent FeO, still in the spinel field, are opaque on quenching because of rapid growth of dendrites of fayalite and possibly wüstite below the liquidus, with the result that it is very difficult to establish the liquidus temperature.

It is planned to continue the laboratory study by completing two additional joins: Mg cordierite—clinoenstatite, and Mg cordierite— SiO_2 -FeO. Both these planes should intersect the cordierite phase volume and assist in outlining the stability relations within the tetrahedron, besides supplying valuable data on the cordierite solid solution series.

Some investigation was made of the factors that influence the attainment of equilibrium between metallic iron (of the crucible) and iron-bearing silicate melts. As a result of previous work it is known that in a neutral atmosphere an equilibrium condition is reached between the metallic iron and the ferrous and ferric iron in a melt. In the course of attaining this equilibrium (minimum of ferric iron), there is a consequent change in the total iron content of charges, the composition changing along a line that on the diagram may be drawn through the FeO corner and the point representing the initial composition. It was found that the amount of change of total iron content of a charge depends primarily upon the total available oxygen in the surrounding gas. Charges heated above the liquidus in an iron crucible sealed in evacuated silica glass tubes for periods up to 14 hours show only slight change (maximum 2 to 3 per cent) in total iron content. On the other hand, charges heated for 2 hours in an atmosphere of purified nitrogen change by an amount which varies from 5 to 15 per cent, or more, dependent upon the partial pressure of oxygen in the nitrogen. The previously used technique for purifying the nitrogen supply was improved by passing the tank nitrogen through cleaned steel wool at 700° to 800° and by reducing the resultant oxide coating on the steel wool with a slow stream of hydrogen fed through overnight between runs.

STUDIES ON THE INVERSION CHARACTERISTICS
OF QUARTZ

Preliminary studies on the inversion temperature of quartz (see last year's report) indicated that the temperature of the high-low inversion of quartz varies by almost 2° in different samples. This variation is apparently related to the geologic environment in which the quartz was found, and there are grounds for believing that with sufficient information on the behavior of a variety of samples of natural and synthetic quartz it will be possible to make a direct correlation between the inversion characteristics and the conditions of formation.

A new method has been developed for studying the inversion and specifically for measuring the magnitude of the heat effect, the temperature at which the inversion begins, and the temperature range through which the transition takes place.

The present apparatus for determining the inversion characteristics involves the use of a silver sample block with four chambers in which are placed crushed and sized samples of the quartz, cryolite (Na_3AlF_6), arkanite (K_2SO_4), and granular alumina. Cryolite and arkanite have inversions at 562° and 584° , respectively, and give reference temperatures for the quartz inversion, which is at 573° . Thermoelements of gold palladium-platinum rhodium are placed in each of the four materials and connected in such a manner that no electromotive force is generated when all four materials are at the same temperature. If, on cooling or heating, any of the materials absorbs or gives off heat, its temperature will be temporarily above or below that of the others, and an emf will be produced, which deflects a sensitive galvanometer. The galvanometer deflec-

tion is recorded on a Brown strip chart recorder by means of a light spot reflected from the galvanometer mirror and falling on a divided photocell connected so as to form a "light-follower" circuit.

Preliminary runs with the apparatus showed that the inversion temperature of a quartz sample can be bracketed between the cryolite and arkanite inversions with an error of repetition of less than 0.05° C. The preliminary and calibration runs also show that some quartz samples differ from others not only in temperature of beginning and duration of inversion, but also in the magnitude of the thermal effect accompanying inversion and in the amount of hysteresis between heating inversion and cooling inversion. All quartz samples studied also exhibit an as yet unexplained difference between the temperature of beginning of inversion in the initial heating run and the temperature of beginning of inversion in subsequent runs.

One particularly interesting quartz specimen from Minas Geraes, Brazil, is a perfectly clear and colorless crystal the basal part of which was formerly used as the reference quartz. Upon exposure to X-rays, sections of the crystal are selectively colored and exhibit three distinctly separate growth zones. The inner and outer zones of this crystal have inversion temperatures which differ by from 0.6° to 0.8° , the greater difference being obtained on cooling. It appears that a further detailed study of even this one crystal may furnish a valuable clue to the relation between inversion temperature and structure and composition, and measurements on quartz samples prepared in the laboratory under controlled conditions may provide quantitative relationships for making quartz a reliable geological thermometer.

SILICATES IN THE PRESENCE OF WATER UNDER PRESSURE

Fundamental though they are to geology, the results of studies of dry systems cannot be applied directly to many geological problems without some knowledge of the effect of volatile components, especially water, in altering the phase-equilibrium relations. Water is an essential constituent of rock magmas, and its presence in magmatic systems raises many problems both theoretical and experimental. The techniques that have been developed will carry us a long way toward the solution of such problems. The positive control of pressure obtained by the use of a pump with a pressure regulator to inject water into the pressure vessel has increased both the ease of manipulation and the certainty of results and has eliminated the doubtful calculation of pressure by means of equations of state known not to be applicable. Two successful types of pressure vessel are in daily use; both offer possibilities for almost unlimited investigation in fields that are just being opened up.

FELDSPARS

Among rock-forming minerals the feldspars are of the highest importance. In the igneous rocks of the accessible crust of the earth they far outweigh all other minerals in abundance; in the metamorphic rocks they are but little less important; in sedimentary rocks they occur not merely as detrital grains derived from other rocks, but also as new, authigenic growths. It is only natural that the feldspars have been much studied and that more is known about them than about any other rock-forming mineral group. As is ever true in scientific investigation, the knowledge gained serves to reveal and to emphasize what there is yet to learn.

Studies are now being carried out that are designed to throw further light on the

conditions under which feldspars develop. The work consists principally of laboratory experiments in which feldspars are grown under a wide range of measured pressures and temperatures, followed by determination of the properties of the feldspars and the manner of the variation of these properties with the controlled conditions of their growth, and this again by careful measurement and comparison of the properties of the synthetic feldspars with those of natural feldspars of widely variant geologic environment. By reason of the ubiquitous character of natural feldspars, it is expected that quantitative knowledge of the conditions of formation of a wide range of natural rocks will thus be greatly extended and refined. Significant progress has been made, some details of which are now presented.

There are three principal feldspars: potash feldspar (KAlSi_3O_8), soda feldspar ($\text{NaAlSi}_3\text{O}_8$), and lime feldspar ($\text{CaAl}_2\text{Si}_2\text{O}_8$). No natural feldspar ever has the composition indicated by any one of these chemical formulas. They are always mixtures of the three, not mechanical mixtures but true solid solutions, or "mixed crystals." In the language of the theory of phase equilibrium, these chemical compounds are the components of the solid solutions; or in the language of crystal-structure theory, K, Na, and Ca (also Si and Al) can proxy for each other in the crystal lattice within certain limitations which are variable and whose variation is controlled by the conditions of formation of the feldspar—and, of course, by the composition of the medium (solution or melt) from which the crystals formed.

The components of the feldspars are also conveniently referred to by mineralogists as end members; and mineral names are given to these end members^ the soda

feldspar being known as albite, the lime feldspar as anorthite, and potash feldspar as orthoclase or microcline depending on its crystal form. Pure albite, anorthite, and orthoclase can be made in the laboratory, but, as has been noted, when formed in nature each of them contains some of the others. Certain occurrences of albite are known in which there is a total of little more than 1 per cent of anorthite and orthoclase. Usually, however, significant or even notable amounts of all three components enter into the composition of the natural crystals, though the potash component is always low when the lime component is high.

We may now state in more specific terms the special problems posed by the feldspars. Igneous and other rocks contain feldspars that appear to show every gradation of composition between albite and anorthite; and these two components have, therefore, been regarded as forming an unbroken series of solid solutions known as the plagioclases. Plagioclase is the principal constituent of the most common lava flows, the basalts. Early studies at this Laboratory of the melting temperatures of pure synthetic mixtures of these components seemed to confirm the view that an unbroken series exists. On the other hand, examination of natural feldspars by the new and powerful methods of X-ray diffraction shows that anorthite and albite have differences in atomic arrangement that seem to preclude the possibility of continuous and complete substitution of one end member for the other. It is possible that at high temperatures the lattices are such that unbroken gradation of composition can occur, but that at the ordinary temperatures at which X-ray studies have been made this situation no longer prevails. The solution of this particular feldspar problem is important because, if the plagioclases represent an unbroken series,

the composition of a plagioclase occurring in any rock will depend only upon the composition of the medium from which it is formed; if, on the other hand, the series has a hiatus at some temperatures, then the composition of a plagioclase will depend as well upon the temperature at which it is formed. It thus might prove possible to determine the temperature of formation of any plagioclase.

Over against the great subgroup of lime-soda feldspars known as the plagioclases stand the other subgroup commonly referred to as the alkali feldspars. These are potash feldspar, occurring in nature in two principal forms, orthoclase and microcline, and the soda feldspar, albite. Albite is thus a member of both subgroups. The sodic end member of the plagioclases is sufficiently like potash feldspar in mode of genesis to warrant grouping them together. Not only that, but these two feldspars also form solid solutions, that is, homogeneous crystals which are intermediate between them in composition. These alkali feldspar solid solutions show much more complicated relations than the plagioclase series have usually been regarded as showing.

Crystals of alkali feldspars, when formed as early crystals in lavas and, therefore, presumably grown at high temperatures, may show a wide range of mutual solid solution of the potash and soda components. On the other hand, when grown in deep-seated rocks, probably in the presence of volatile materials held in by the greater pressure, with consequent lowering of the temperature of crystallization, the soda and potash feldspars in nearly pure form occur side by side as separate crystals. In natural rocks the indications are, therefore, strong that in this series of feldspars the extent of solid solution is a function of temperature.

In order to determine the quantitative aspects of the relation thus qualitatively

stated, studies have been made on the behavior of mixtures of various compositions between $\text{NaAlSi}_3\text{O}_8$ and KAlSi_3O_8 in the presence of water vapor, which is the principal natural volatile flux, at pressures up to 2000 atmospheres and at temperatures up to those at which the mixtures are completely liquid. In the language of phase-equilibrium theory, we have investigated the liquidus, solidus, and subsolidus relations in the system $\text{NaAlSi}_3\text{O}_8$ — KAlSi_3O_8 — H_2O at various pressures. The synthetic mixtures approach closely the composition of the natural volcanic rock trachyte and its deep-seated equivalent syenite, since these rocks consist essentially of alkali feldspars.

A necessary, or at least a most desirable, preliminary to the study of any system having water as one of the components is a study of the system without water, in this case the system $\text{NaAlSi}_3\text{O}_8$ — KAlSi_3O_8 . Fortunately this work had previously been done, and in addition we were fortunate in having many mixtures available for our studies with water, for the mere making of the mixtures is in itself no mean task. Since liquids of these compositions are extremely viscous, attainment of a homogeneous liquid requires long periods of special treatment.

The extreme viscosity of the liquids also introduces great difficulties in the attainment of equilibrium between crystals and liquid, but these have been overcome by increasing the time factor, and a satisfactory equilibrium diagram for the system without water (the dry system, as it is called for convenience) is available. This diagram gives only the liquidus relations, that is, the temperature of completion of melting of various mixtures. The solidus relations, that is, the temperatures of beginning of melting, were not determinable in the dry way. Although not altogether conclusive, the relations found in the dry

way strongly suggest that KAlSi_3O_8 and $\text{NaAlSi}_3\text{O}_8$ form a complete series of solid solutions at high temperatures, of the type with a minimum melting temperature, complicated only by the incongruent melting of compositions rich in the potash component.

With the mixtures and the information available from previous work, a study of the behavior with water was undertaken. For this purpose, the Tuttle apparatus (last year's report) has proved very convenient, and in the detailed study of the crystalline phases the Norelco X-ray spectrometer with Geiger counter attachment has been of inestimable value. As was known from previous work, the recalcitrant behavior of alkali feldspar compositions examined in the dry way largely disappears in the presence of water vapor under pressure.

The homogeneous glasses of intermediate composition crystallized readily under hydrothermal conditions, and it was quickly established that the two components do indeed form an unbroken series of solid solutions or mixed crystals at high temperatures. Examination of the crystalline products in the X-ray spectrometer established this fact, and preliminary studies of optical properties indicate that there is a continuous change in these properties with composition. The X-ray powder spectra of the crystalline end members are very similar, but close examination reveals differences, one notable difference being the position of a rather prominent peak (maximum of intensity of X-ray reflection) on the spectrometer chart. The peak has a position corresponding to a 2θ value of about 22° for the soda end member and of about 21° for the potash end member. By reference to the single-crystal studies of W. H. Taylor this peak is identified as due to the [201] reflection, and its position is, of course, determined by the [201] spacing. Careful determinations of the position of

this peak were made for the end members and for 13 intermediate compositions by measuring the intensity of reflection as determined by the total count on the Geiger counter during a 5-minute period, at successive fixed positions spaced only 0.05° apart, all measurements being referred to a constant standard having a peak close to 23° . It was thus found that, for compositions crystallized at 900° and 300 atmospheres pressure of water vapor, the position of the peak changes continuously from end to end of the series, and that the variation with composition is sensibly linear. Since it was found that measurements on the same material could be duplicated within 0.02° and the total variation is about 1° , this must be regarded as a very satisfactory demonstration of the linear variation of the [201] spacing in an unbroken series of solid solutions.

Conversely, by measuring the position of this peak, the composition of any feldspar of the series can be determined within an error of 2 per cent in proportions of the end members, which is at least as high accuracy as can be obtained in silicate chemical analysis of high grade.

Starting with a series of compositions crystallized as indicated above and proved to be homogeneous mixed crystals, we have proceeded to determine the solidus and liquidus relations at two isobars, 1000 atmospheres and 2000 atmospheres. It was established beyond question that at both isobars the series is of the type showing a minimum-melting composition, and at both pressures this composition is 70 per cent NaAlSi₃O₈. The ability to determine the composition of crystals by the position of the [201] peak on the X-ray spectrometer was here of the greatest value, for in mixtures quenched from any temperature within their melting interval, and thus consisting of crystals and glass, it was still possible to determine the composition of

the crystals. This procedure, of course, locates a point on the solidus curve for that temperature. During progressive melting of the mixture with 70 per cent NaAlSi₃O₈ there was no change in the composition of the crystals, whereas during the progressive melting of a mixture on the potash side of this minimum the crystals moved still farther toward the potash side and in mixtures on the soda side the crystals moved toward the soda side. At the composition of this minimum-melting mixture and at the minimum temperature (840°) the liquid contains 7.7 per cent H₂O at 1000 atmospheres.

It is noteworthy that the minimum temperature at the 1000 atmospheres isobar lies 220° below the minimum temperature in the dry melts, but an additional 1000 atmospheres lowers the minimum only 70° farther, bringing it down to 770° . It may therefore be stated that alkali syenites under sufficient overburden to permit a pressure of 2000 atmospheres, say at a depth of about 5 miles in the earth, and given an adequate supply of water, will crystallize from the molten state at a temperature close to 770° . At greater depths and again with adequate water available, syenitic magma would crystallize at a still lower temperature, but not much lower; for, as has been seen, the effect of increased pressure rapidly diminishes. Not all mixtures have yet been studied, but as the work progresses from the minimum out toward the end members it is increasingly evident that the results will agree very closely with the early measurements at this Laboratory by Goranson on the melting of the end members at corresponding pressures of water vapor.

Studies of subsolidus relations have also been made, and here again the X-ray method of determining the composition of the crystals proved invaluable. It was found that, when crystallized at low tern-

peratures, the intermediate compositions gave two $[\bar{2}01]$ peaks, the one corresponding to a potash-rich feldspar and the other to a soda-rich feldspar, and the composition of each is determined by the position of its peak. There is, then, as had been deduced from the evidence of natural rocks, a failure of complete solid solution of the alkali feldspars at lower temperatures, and two feldspars are formed side by side. Moreover, the compositions of the two feldspars in equilibrium with each other at any temperature can be determined experimentally. The hiatus increases as the temperature falls. Much has already been accomplished in determining the width of the hiatus, but the work is very time-consuming, and determinations for some temperatures in the critical range are yet to be made. When they are completed it should be possible to determine rather closely the temperature of growth of any associated pair of alkali feldspars in natural rocks. It will be necessary only to determine the composition of the two feldspars.

One item of outstanding importance in the results of this work has not yet been mentioned. It is that the crystalline NaAlSi₃O₈ prepared in this work does not have properties corresponding to those ordinarily given for natural albite. The reason for this is not far to seek. The best source of crystals of feldspar approaching the albite end member is in pegmatites, where the feldspar is formed at relatively low temperatures. It is optically positive and has a large optic axial angle. The soda feldspar produced in the laboratory is optically negative with a small angle, and has notably lower refractive indices than the pegmatite albites. There are also quite marked differences in their X-ray patterns. Now there are soda feldspars in natural rocks that are optically negative with a small angle and low refractive indices.

They were studied long ago by F. Fouqué and were obtained by him from lava flows in which they occurred as phenocrysts. They are, accordingly, to be regarded as a high-temperature variety. To be sure, they were described by Fouqué as "anorthose" and some of them have a high potash content, but some approach albite reasonably closely (nearly 90 per cent NaAlSi₃O₈) and render it clear that there are in nature high-temperature albites. Our synthetic albite is the high-temperature variety; and, unfortunately, no matter how low a temperature or what combination of ingredients is used, only high albite has yet been produced, though it is certain that at the lower temperatures the high albite formed metastably. It is not an unusual experience with silicates to find great difficulty in producing low-temperature modifications in the laboratory, but usually some device is hit upon which turns the trick. The secret for albite has not yet been found.

It has been possible, however, to determine approximately the temperature of inversion by finding the lowest temperature at which the low-temperature modification (ordinary pegmatite albite such as that from Amelia County Court House in Virginia) can be transformed into the high-temperature variety. The change is very sluggish at any temperature, but can be brought about simply by heating the dry powdered mineral at 1080° for one week. This temperature is far above the inversion temperature, in spite of the slowness of transformation. With the aid of a flux of Na₂Si₂O₅ and water vapor at 1000 atmospheres, the change could be induced at 725° in a week, but not at 675° in two weeks. It is concluded, therefore, that the inversion temperature is approximately 700°.

The existence of this inversion in the sodic end member of the plagioclase series

necessitates an inversion in adjacent plagioclases at least. It has been confirmed that plagioclase with up to 30 per cent anorthite has high- and low-temperature modifications distinguished by their X-ray diffraction patterns. Beyond that range of composition, plagioclases suffer changes on heating which may or may not be of the same character. This is the work on plagioclases which was mentioned earlier and which is being further prosecuted. Clearly one cannot solve the problem of solid solution in the plagioclases by selecting natural plagioclases haphazard and determining their crystal structure by X-rays, as has been attempted. The crystals studied must be selected with reference to their manner of occurrence and probable temperature of formation, and the results must be correlated with measurements on synthetic feldspars grown at controlled temperatures.

SOLUBILITY OF QUARTZ IN STEAM

The investigations with water at high temperature and pressure may be considered to open up a new field of chemistry. The usual experimental conditions are such that the water is above its critical temperature and pressure and hence will ordinarily be called a vapor or gas, but in some of the work the water is under so high a pressure that its density becomes of the same order as that of liquid water at room temperature. Owing to its high density, the "gaseous" water may have a great solvent power for solids, and the often-discussed gas transport of solid material becomes a demonstrated fact. The current work on the solubility of quartz in steam furnishes an excellent example of this phenomenon.

Water under pressure is pumped into a heated pressure vessel containing crushed quartz. The pressure is held constant by

a regulator, and on entering the heated pressure vessel the water is changed to steam. This steam is slowly passed through the pressure vessel, cooled, collected, and weighed, and the amount of dissolved silica is determined by analysis. The rate of flow is controlled by a needle valve on the exit side of the apparatus, and rates ranging from less than 0.1 to 10 grams of water per minute have been used. Temperatures have ranged from 300° to 500° and pressures from 2000 to 15,000 pounds per square inch. The lower pressures are comparable with those used with modern high-pressure steam turbines, and the temperature range includes that of superheaters. This is of practical interest because the deposition of quartz and amorphous silica on the blades of high-pressure turbines leads to a serious decrease in efficiency and requires frequent shutdown for its removal.

The experimental results are rather striking. Even at the lower pressures and temperatures, the solubility of the solid, quartz, in the gas, steam, can easily be measured. At 400°, and still more at 500°, the solubility increases rapidly with the pressure. At 1000 atmospheres pressure (15,000 psi), the gas contains 0.25 per cent silica. This corresponds to a partial pressure of silica of 2.5 atmospheres—and silica is one of the least volatile of substances. Reaction takes place rapidly. Even when the rate of passage is such that the steam is in contact with quartz for less than 10 minutes, the vapor phase becomes three-quarters saturated, and 0.8 gram of silica is carried over per hour. At this easily attainable pressure, the solubility of silica in steam is ample to account for the formation of the quartz in pegmatite deposits.

THE SYSTEM H₂O—Na₂O—SiO₂

The first studies on the silicates of sodium and potassium in the presence of

water were published in 1914, and a detailed study of the system $\text{H}_2\text{O}-\text{K}_2\text{O}-\text{SiO}_2$ in 1917. The methods and apparatus used in that study are not suitable for $\text{H}_2\text{O}-\text{Na}_2\text{O}-\text{SiO}_2$, because of the much greater pressures encountered and the resulting solubility of both Na_2O and SiO_2 in the gas. A successful technique was not developed until 1941, and progress was interrupted by the war.

In the report for 1940-1941 (Year Book No. 40) it was stated that sodium metasilicate and sodium disilicate have strongly retrograde solubilities, which fall practically to zero at the critical point of water, 374° and 212 atmospheres. This is the first intersection of the critical curve and the solubility curve; at the relatively low pressure that obtains here, the solubility in the gas is negligible. The second intersection of the critical curve must take place on the upper part of the solubility curve, a part of the curve that may be regarded as representing the lowering of the melting point by dissolved water. It is important to note that as the temperature is lowered the pressure required to hold the water in solution continuously increases. At 400° , 26° above the critical point of water, pressures on the isothermal saturation curve of sodium disilicate are greater than 1200 atmospheres, and at this pressure the solubility of solid in gas is important. Moreover, the gas does not dissolve Na_2O and SiO_2 in the same proportions that are present in the liquid; hence the gas and liquid must be regarded as ternary. The composition of gas, liquid, and, when present, crystalline phase must be determined.

In studying the system, it is found most convenient to operate at constant temperature and constant pressure. Most of the work has been at 400° , and the results for this isotherm are nearly ready for publication. Several series of isobars have been made. A silicate mixture of known com-

position is placed in a shallow platinum crucible, mounted on a pedestal in a pressure vessel (or bomb) of the metal Inconel. Stainless steel bombs were abandoned because when the Na_2O content of the gas was high it drilled holes through the 1-inch bottom overnight. The bomb with charge is placed in a furnace held at constant temperature, and water is slowly pumped in until the desired pressure is reached, the pressure then being held constant by an automatic regulator.

Usually after an overnight run the bomb is shut off from the pressure system and cooled, first in air, then in water, and opened. The contents are divided into three parts. Almost all the gas condenses to a liquid outside the crucible. This liquid is removed, weighed, and analyzed. In some experiments with high Na_2O content, it is necessary to dilute the condensed gas with water, otherwise it will partially solidify as NaOH before it can be removed from the bomb. The contents of the crucible consist of glass (representing a hardened liquid), or glass plus crystals, or crystals, plus some extra condensed gas which has reacted with the glass or crystals. This material is poured off and analyzed, and from this analysis the composition of the glass or the crystals or both, and the composition of the gas, the amount of this additional condensed gas, and the amount of material it has dissolved can be calculated.

GRAPHICAL REPRESENTATION WITH A VOLATILE COMPONENT

The method of study will be made clearer from a consideration of figure 1, which refers to a constant temperature and a constant pressure. The sides of the composition triangle $\text{H}_2\text{O}-\text{Na}^{\wedge}\text{O}-\text{SiO}_2$ are omitted, except for a part of the side $\text{Na}_2\text{O}-\text{SiO}_2$ on which is located the composition of sodium disilicate (*Di*), Na_2O^*

2SiO_2 . The lines I-I, 2-2, and *Di-Di* go from the H_2O apex to the indicated points on the side $\text{Na}_2\text{O}-\text{SiO}_2$; any mixture of *Di* and H_2O , for example, must lie on the line *Di-Di*. The dot-dash curves *Gd* and *G₂G* represent the compositions of gases in equilibrium with unsaturated liquids along the lines *LLi*, or *L₂L*, shown by solid lines. The dotted curve *GIG₂* shows the gaseous solubility of sodium disilicate. The curve *E₁L₁L₂E₂* is the solubility curve of sodium

bomb, the gross composition of the mixture must be on the line from the H_2O apex to the composition of sodium disilicate, i.e., the line *Di-Di*. If the amount put in is less than that corresponding to the solubility of sodium disilicate in steam at this temperature and pressure, the composition will be on *Di-Di* to the left of point *a*. At 700 atmospheres this is equivalent to about 0.7 gram, in the bomb used. When a little more is added, a clear glass

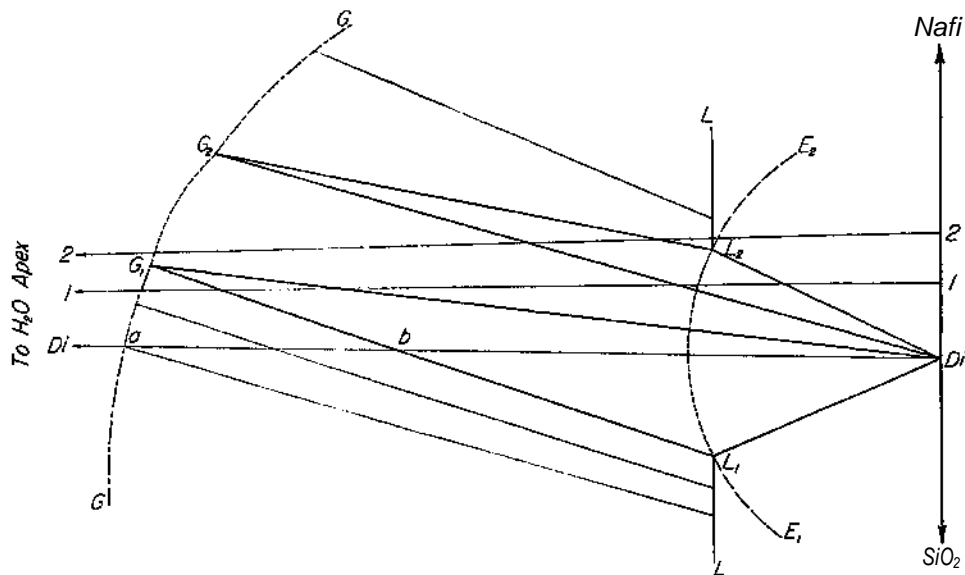


FIG. 1

disilicate in water; but, since at constant pressure and temperature there cannot be a series of solutions in equilibrium with gas and sodium disilicate, the curve is metastable except for the points *h* and L_2 . The parts *E₁L₁* and *L₂E₂* represent solutions having vapor pressures less than that for which the diagram is made, and, therefore, unstable; and the part *L₁L₂*, solutions having vapor pressures greater than that of the diagram. Only the liquids L_1 and L_2 can coexist in equilibrium with gas and solid at this pressure and temperature.

When sodium disilicate is placed in the

is found in the crucible, which is an unsaturated solution on the line *LLi*; the corresponding gas compositions are indicated by tie lines. When the amount of disilicate corresponds to point *b*, crystals of disilicate begin to form. The composition of the gas is G_1 , that of the liquid L_1 , and these remain constant as long as there are present gas, liquid, and crystals. Addition of more disilicate results only in more crystalline disilicate; the composition will remain within the 3-phase triangle *G₁-L₁-Di*, all compositions are fixed, and only the proportions change.

If the original mixture is richer in SiO_2 than Li, disilicate can be formed only at lower pressures; and as the proportion of silica is increased, the pressure gets lower and lower until the boundary of the quartz field is reached, after which quartz becomes solid phase and the pressure increases rapidly. This disilicate-quartz boundary is at a low pressure, 250 atmospheres; the liquid contains 25 per cent H_2O , and the gas 99.7 per cent H_2O .

When a mixture of the composition of sodium disilicate is put into the bomb, the gas and liquid compositions, that is, the points G_i and Li of the 3-phase triangle, depend on the pressure. At 700 atmospheres, G_i corresponds to 95 per cent H_2O , 1.8 per cent Na_2O , and 3.2 per cent SiO_2 ; and Li to 21.5 per cent H_2O , 20.5 per cent Na_2O , and 58 per cent SiO_2 . The compound $\text{Na}_2\text{O} \cdot 2\text{SiO}_2$ contains 66 per cent SiO_2 . The solid dissolved in the gas contains 64 per cent SiO_2 , and the solid dissolved in the liquid 73.9 per cent SiO_2 , so that the disilicate has been divided between a gas phase containing a lesser ratio of SiO_2 and a liquid containing a greater ratio of SiO_2 .

At 1200 atmospheres pressure the corresponding values are: gas, 93.8 per cent H_2O , 2.8 per cent Na_2O , 3.4 per cent SiO_2 , and liquid, 29 per cent H_2O , 22.7 per cent Na_2O , and 48.3 per cent SiO_2 . The solid in the gas now has 55.6 per cent SiO_2 , and the solid in the liquid 68 per cent SiO_2 . Even this higher pressure is not enough to liquefy sodium disilicate completely, although the percentage of silica in the dissolved liquid is now 68, not far from the 66 per cent of disilicate. It is a remarkable fact that at 374° the vapor pressure of the solution is about 212 atmospheres, whereas at only 26° higher it has increased to over 1200 atmospheres. If a solution of sodium disilicate in water is cooled from high

temperature, a pressure of over 1200 atmospheres will be developed as a result of the cooling and consequent crystallization. It may also be mentioned that gas and liquid are still far apart in composition, and that at a critical end point they must be identical.

Another significant fact is brought out by figure 1. The join $Di-Di$ passes from the field of unsaturated gas into the field of gas plus unsaturated liquid, then into the 3-phase region. Starting with a mixture containing more Na_2O , along the line 1-1, the sequence of fields is the same, until it emerges into a field of gas plus crystalline disilicate. The dotted line G_iG_2 represents the gaseous solubility of crystalline disilicate, and mixtures along the line 2-2 pass directly into this region.

Further work at a little lower temperature will throw light on these and other interesting phenomena in a field that as yet has scarcely been investigated either theoretically or experimentally.

FILTER AUTOCLAVE

A solubility determination with the high-pressure-steam filter autoclave comprises four distinct phases: first, heating a reaction mixture to the desired temperature in the presence of steam at a predetermined steam pressure and then stirring it under these conditions of temperature and pressure until equilibrium is reached; second, filtering the reaction mixture with maintenance of the equilibrium temperature and pressure; third, cooling the filtrate to room temperature and atmospheric pressure without a change in its over-all chemical composition; fourth, analyzing the filtrate and identifying the solid phase.

A year ago it was reported that the first complete experiment had just been performed with this apparatus. Besides indicating the need for some mechanical

improvements in the apparatus, that experiment showed that the initial procedure for the third phase of the determination (cooling) was unsatisfactory. The problem of the change in water content of the filtrate during the cooling still remains, though a great many variations in cooling schedule have been tried. Solution of this problem was the major objective of the forty-two solubility determinations and four special experiments performed since the fall of 1948. It has been learned that liquid water enters the lower chamber of the autoclave and floods the crucible holding the filtrate. The source of the pressure difference that pushes the water into the lower chamber has not yet been discovered. Absorption of water by the cooling filtrate from the atmosphere of steam that surrounds it, which at first was supposed to be the only mechanism for the introduction of extraneous water, is so far overshadowed by flooding that it is not yet possible to tell whether it has taken place at all.

The search for the source of the extraneous water may be made by performing simulated solubility determinations in which no charge of silicate is present; and this has been done in some instances. In general, however, it was considered preferable to carry out complete solubility determinations. In this way experience was gained in the manipulation of the apparatus, and the various parts were given endurance tests. The value of this policy is attested by the fact that the number of unsuccessful solubility determinations has decreased steadily, as causes of failure have been discovered and overcome. The most troublesome one, which accounted for nine of the seventeen unsuccessful determinations, was leakage of nitrogen around the flange of the filter crucible during filtration. Although this condition prevented

the obtaining of a filtrate, it did not interfere with the acquisition of information applicable to the problem of the source of the extraneous water.

The leakage of nitrogen past the filter crucible has been prevented by several successive changes in design of the holder for the filter crucible. Other improvements made in the apparatus during the past year include a thrust bearing for the stirring shaft, a centering device for the autoclave (which permits alignment of the stirring shaft with the axis of the gear and thrust bearing), a keyway for mounting the stirrer on the shaft, and a turbo-compressor for forced cooling of both the autoclave and the steam boiler.

Another advantage of making complete solubility determinations before the cause of extraneous water has been eliminated is that some information has been gained about the attainment of equilibrium. Using as a criterion the K_2O content of the glass obtained by dehydration of the filtrate, the same result was obtained when KOH and quartz or glassy $K_2Si_2O_5$ and silica gel were used as starting materials instead of crystalline $K_2Si_2O_5$ and quartz. Several experiments in which the time of stirring under equilibrium conditions was reduced to 1 hour (normally it was 4 hours) indicated that this was not long enough for equilibrium to be reached.

The variation in K_2O content of the glass obtained from determinations performed under normal conditions was so small as to suggest that the limit of accuracy of a solubility determination performed with the filter autoclave (when the extraneous water is absent) will be that imposed by the analysis of the filtrate. Furthermore, the mean value of the K_2O content at 300° and 50 atmospheres pressure agrees well with that obtained by interpolation of previous data.

EXTREME HIGH PRESSURE

An important aspect of work with high pressure is the effect of pressure, in the absence of volatile components, on the melting and transition of minerals. Apparatus has been designed and constructed capable of simultaneously producing and maintaining pressures up to 15,000 atmospheres and temperatures up to 1400°. The immediate experiments planned involve temperatures between 500° and 800° and pressures up to 10,000 atmospheres. Data on the behavior of silicates in the range of conditions that can be produced by the ap-

paratus are essentially nonexistent. For this reason, the primary program involving the apparatus will be the determination of the change of melting point of minerals with pressure. From these data, thermodynamic properties can be calculated and correlated with the results obtained by other methods now being applied at the Laboratory. Having obtained this fundamental information, we shall be in a position to make an intelligent selection of other programs for high-pressure experimentation.

STATISTICAL PETROLOGY

Further work on application of statistical methods to petrology has been carried out, and a preliminary study of the relation between grain size, area of measurement, and sample variance has been completed. It turns out that the critical problem here is an instrumental one, since it will be necessary to establish a quantitative measure of grain size, if variances for rocks of different grain size are to be compared. The problem of measurement is very simple, for by substitution of a finer thread in the mechanical stage of the point counter almost any reasonable distance between points can be obtained, and the distance traversed is given by $(n-1)^{-1}$, where n is the number of points and $\frac{1}{n}$ the distance between points. The best method of recording the measurement has not yet been determined. Several schemes are under consideration, and at present it appears that the best results would be obtained by substitution of a recording-tape adding machine for a counting cell.

Relations between ratios are often used in chemical petrology without full realization of the difficulties and ambiguities involved in passing from ratio correlations to inferences about relations between the

absolute values from which the ratios are formed. Usually it is these absolute values that are of primary concern. By application of a series of restrictions to the variables used in the Pearson general formula for index correlation, statements governing product-moment correlation between ratios in the special cases commonest in petrology are derived. Each special case is illustrated by one or more numerical examples drawn from the literature of petrology, and the general conclusion is that where interest centers on the absolute values, as is often the case, careless application of ratios is liable to be misleading and even their careful use is likely to yield ambiguous or uninterpretable results.

End-stage reactions in the crystallization of magma often take the form of pseudomorphous replacements, and the question as to whether these reactions occurred in a still homogeneous paste of solids and liquids, or after the separation of liquid residues from crystallized material, is one of recurring interest. Qualitative approaches to this problem have been largely unsatisfactory, since it is to be expected that fabric and habit will be very similar. In late magmatic and early hydrothermal crystal-

lization. Provided that the reaction is truly pseudomorphous, however, a replacement occurring at about the same time in the entire mass would lead to a positive correlation between the amount of original mineral available to the reaction and the amount of replacement product formed, for the extent of replacement would be largely a function of the amount of original mineral available. If, on the other hand, the replacement occurs only after the magmatic residue has been strained away from some parts of the rock and concentrated in others, there will be no correlation between original mineral and replacement product. By means of the conventional part-whole correlation formula, it may be shown that under such circumstances there would be negative correlation between the amount of replacement product and the amount of original mineral surviving the reaction. On this basis it has been concluded that muscovite pseudomorphously replacing plagioclase in the Barre, Vermont, granite is late-magmatic rather than post-magmatic or hydrothermal. The argument is subject to certain

limitations which do not seriously hinder its practical application.

A detailed test has been made of the homogeneity of the well known granites from Westerly and Bradford, Rhode Island. Hand specimens were taken at each of the larger quarries in each granite; and from each hand specimen three thin sections were cut in such a fashion that variance analyses would provide information on variance attributable to differences between the two granites, between hand specimens of each granite, and within each hand specimen. This latter source of variation could be further analyzed into a location and an orientation factor. The end result is that differences between the two granites, though small, are readily detectable, that orientation and location effects within hand specimens of either granite are negligible, that small differences between hand specimens of the Westerly are detectable, but that the existence of such differences between hand specimens of the Bradford granite may not be inferred from the data.

STUDIES ON VOLCANIC PRODUCTS

In last year's report on the study of the rocks extruded at Santiaguito, the new volcanic cone halfway up the side of Santa María in Guatemala, it was stated that a study of the acid constituents of the emanations from the fumaroles would next be undertaken. The detailed studies have been concluded. In addition, considerable work was done on the incrustations that were found at and near the vents of the fumaroles.

Studies in the field revealed the fact that the fumaroles could be divided into two types which for the purposes of this report may be called A and B. At type A, steam at about 300° issued freely from open vents

and no free sulfur was visible. These fumaroles were on the eastern slope of Santiaguito and were situated about 300 feet above the floor of the crater developed on Santa María in 1902. At type B, about 700 feet above the crater floor, clouds of suffocating steam vapors issued from fine cracks. At a depth of about 12 inches below the surface, a layer of sulfur approximately 3 inches thick was found. The sulfur varied from solid crystalline form near the surface to liquid form increasing in degree of fluidity with depth. This mantle of sulfur, shot through with fine cracks, helped to maintain the rather surprisingly steep slope of nearly 50°.

ACID CONSTITUENTS OF THE EMANATIONS

Samples of condensed steam were collected from type A fumaroles in 1932 and 1939. Samples were collected from types A and B in 1940. Analysis showed that all contained appreciable amounts of the following constituents: hydrochloric acid, sulfuric acid, and sulfur dioxide. The concentrations of the condensate seem to vary from year to year. From 4 to 15 grams per liter of hydrochloric acid were found. In some cases the content of sulfuric acid was about the same as that of hydrochloric, but more frequently the latter dominated. In addition, the condensates were almost saturated with respect to sulfur dioxide. In type B the condensates were led into a solution of cadmium acetate, but, as was expected, no cadmium sulfide was precipitated, a fact which indicated the absence of hydrogen sulfide. The same procedure was followed at the fumarolic area type B, where the great deposits of free sulfur were found. A copious yellowish precipitate was formed, but analysis in the laboratory showed that it was free sulfur mixed with extremely minute amounts of selenium and tellurium. It is possible for H₂S and SO₂ to coexist at temperatures above about 300° (the steam temperature at both areas), but on cooling, they promptly react to form sulfur. This circumstance is considered to provide an explanation of the presence of sulfur at type B fumaroles. At the type A area the conditions are completely oxidizing, owing, we now believe, to aspiration of air into the edifice.

In addition to the main acid constituents mentioned above, hydriodic, hydrobromic, hydrofluoric, and boric acids were found in small but readily determinable amounts in the acid emanations from type A and type B. Even though the amounts of hydriodic and hydrobromic acid were small relative to the main acid constituents,

the amounts, about 3 milligrams of the former and from 2 to 20 milligrams per liter of the latter halogen acid, are not only significant but also surprising. It seemed desirable to ascertain whether or not other fumarolic areas exhibited a similar concentration of these acids. Water samples had been collected from the crater lake of Santa Ana in El Salvador and from the crater lake of Kawah Idjen in Java. The two lakes receive the volatile products from intensely active fumaroles located below and also above the lake level. Iodides and bromides in easily determinable amounts were found in the samples from both lakes. It appears that these halogen acids are probably more abundant in volcanic emanations than has heretofore been supposed.

INCRUSTATIONS

In general the particle size of many mineral constituents in the incrustations is so small that their identification has been exceedingly difficult and in some cases, up to the present, impossible. As many of the mineral species as possible were identified by means of the microscope and X-ray analysis. Chemical analysis yielded considerable knowledge concerning the chemical environment. Additional mineral species were identified by the combination technique of breaking down each incrustation into groups by chemical methods and examining these by microscopic and X-ray methods.

As a result of study of the incrustations from fumaroles of type A by the technique just referred to, it appears that the chemical environment is so definitely an oxidizing one that only salts of the various bases contained in the andesitic rock are found in the stalactitic incrustations. Where these had been subjected to temperatures above 100°, only sulfates and partially decom-

posed rock minerals were found. In products subjected to lower temperature, chlorides of the various bases were abundant. It is interesting to note that where sulfates predominate they belong to the relatively water-insoluble group of complex aluminum sulfates; the more soluble alkaline salts are present only in subordinate amounts.

At fumaroles of type B, the mantle of sulfur, referred to above, had an orange color. Chemical analysis showed that the deep color was due to the presence of arsenic sulfide (As_2S_3), selenium, and tellurium. Microscopic examination showed that the sulfide was amorphous orpiment. Below the sulfur, a grayish-black indurated material was found. The microscope revealed the presence of opal (a hydrated form of SiO_2), sassolite (boric acid), and anhydrite ($CaSO_4$). It seemed obvious that the induration was due to the formation of opal and anhydrite. It is of interest to note that boric acid had previously been found in the condensates of the steam issuing from both fumarolic areas. The evidence seems clear that these products, which are readily volatile in this chemical environment at temperatures above 300° , have been brought up from the hotter lava below. As a matter of fact, they have an appreciable vapor pressure even at 300° , but have been held in place by the mantle of sulfur, the upper surface of which was at a much lower temperature. This gray-

black incrustation was further studied by the combination technique referred to above. It was shown that the dark color was due to about 1 per cent of exceedingly finely divided pyrite (FeS_2). In addition, between 5 and 10 per cent of the low-temperature form cristobolite (SiO_2) and a lesser amount of anatase, one of the crystalline forms of titanium oxide, were found. These minerals were formed by the interaction of the mineral constituents in the lava and the acid aqueous vapors given off by the hot lava below. The presence of the pyrite shows that at fumarole type B the chemical environment is definitely of a reducing nature. In view of the fact that the steam 5 inches below the surface has a temperature of about 300° , it is not likely that the chlorides could form; as a matter of fact, only an insignificant amount of the chloride ion was found in this incrustation.

Examination of a thin section of the indurated gray-black incrustations revealed the fact that the rock minerals had been completely altered, but the "ghosts" of the original minerals were plainly visible.

The information so far obtained from these chemical studies has an important bearing on rock alteration by means of the acid emanations and fluids that must exist on a large scale in many regions exposed to igneous activity. There is reason to believe that the results throw light on the earliest stages of such alteration.

THERMAL PROPERTIES OF MINERAL SUBSTANCES

Investigations of the thermal properties of mineral substances have been part of the recognized program of the Laboratory from the beginning, and upon the resumption of research in petrology at the close of the last war, greater emphasis was placed on this subject. Phase-equilibrium studies

show what crystalline substances are formed from silicate mixtures, the compositions of the liquids which can be in equilibrium with them, and the pertinent temperatures. But for the complete thermodynamic description of any system, it is necessary to know also the changes in

volume, and especially the heats of fusion and solution at all points on the solubility curves. Not until such knowledge is available can our results be applied to petrologic problems with full effectiveness.

In view of the scarcity of reliable thermal data on silicates, the diversity of silicate compounds, and the much greater geological importance of some than of others, a careful selection of the substances for study at this Laboratory has been made. Among the more important series of silicate minerals are the feldspars, pyroxenes, hornblendes, feldspathoids, micas, aluminum silicates both hydrous and anhydrous, garnets, melilites, and olivines, together with a galaxy of hydrous silicates and aluminosilicates. In order not to scatter efforts too widely over the field, it has been decided to concentrate at first on the feldspars and the chemically adjacent substances jadeite, nepheline-carnegieite, leucite, and kaliophilite. At present carefully "purified" natural minerals are being used, but since these are almost never pure chemical compounds, it will be necessary to study synthetic preparations as well.

Work on this project during the past year has consisted of purifying mineral samples, making heat-of-solution measurements, preparing for the measurement of specific heats, and synthesizing artificial preparations.

PREPARATION OF SAMPLES FOR HEATS OF SOLUTION

The Laboratory now has a series of excellent samples of feldspars, of some feldspathoids, and of the aluminosilicates sillimanite, andalusite, and kyanite, which have been purified by mineral separation methods and prepared in a very finely divided form suitable for solution in the hydrofluoric acid calorimeter. Analyses have been obtained for most of the samples.

THE SOLUTION CALORIMETER AND MEASUREMENTS OF THE HEATS OF SOLUTION IN HYDROFLUORIC ACID

Certain modifications have been made in the methods of measurement of the temperature rise in the gold calorimeter described in last year's report. In place of the Mueller bridge and type K potentiometer combination, a White double potentiometer has been installed as the measuring instrument, and the potential-drop method has been replaced by a method using a Wheatstone bridge with three fixed arms. The unbalance of the bridge is read on the potentiometer, the arrangement being in principle like that used in reading the pressure in the compressibility apparatus of this Laboratory. In order to utilize the sensitivity of the galvanometer to the fullest extent, the bridge has equal ratio arms for dividing the current of 2000 ohms each, and the resistance of the third arm and of the thermometer is of the order of 100 ohms. An important consideration in choosing the constants of the bridge is the current carried by the thermometer, which should be as small as possible in order that the heating effect of the thermometer be small. The third arm of the bridge is always kept at a lower resistance than the thermometer, so that the unbalanced emf will always be positive and will increase with the temperature of the calorimeter. When the temperature rise is less than 1° , the unbalanced emf is linear to better than one part in 10,000, with respect to the resistance change of the thermometer. With the setup as ordinarily used, the unbalanced emf is more than 500 microvolts per degree change in temperature, and the emf can be read to better than 0.1 microvolt, so that the error in temperature measurement does not need to be much greater than 0.0001° .

The electrical energy for calibration is

supplied to the calorimeter at a rate somewhat comparable with the rate of generation of heat by the dissolving sample. Heat-exchange correction is made using a computational procedure based on the Regnault-Pfaundler method. The calorimeter assembly is completely immersed in an oil bath kept at a constant temperature near 74.5°. The actual temperature variation is within 0.01° at present; and it is planned to improve this further, if it turns out that the accuracy of the results can be augmented by doing so. For the solution experiments, the calorimeter is filled with 819.1 grams of 20 per cent hydrofluoric acid, prepared by mixing distilled water with 48 per cent acid, of reagent grade.

The apparatus and methods were first tested by dissolving certain salts in water. These experiments gave results which were in good agreement with data found in the literature. A few orienting experiments with selected samples showed that the natural feldspars may be expected to dis-

solve in the acid without complications. Synthetic nepheline, on the other hand, though it dissolved rapidly in the initial decomposition, gave a post-precipitation of chiolite ($\text{Na}_5\text{Al}_3\text{F}_4$), the heating effect of which complicates the attainment of a constant heat-exchange rate after the solution is finished. There are still troublesome items of experimental procedure to be worked out.

Results have already been obtained for the heats of solution of quartz, albite, bytownite, anorthite, and adularia, which gave, respectively, 553, 564, 624, 646, and 527 calories per gram (rounded off to the nearest calorie). The rounded value obtained for quartz (99.95 per cent SiO_2 , 0.05 per cent impurities—mainly iron oxides) uncorrected for impurities, 553 calories per gram in 20 per cent acid, compares well with the final corrected value of Sahama and Torgeson, 549 in 20 per cent acid. Other values obtained in 20 per cent acid are: Mullert (1913) 498, Roth (1928) 517, and Troitsch (1932) 548.

DEEP SEISMIC PROSPECTING

Jointly with the Department of Terrestrial Magnetism, the Laboratory carried forward the investigation of the earth's crust by obtaining accurately timed records of the arrival of vibrations from charges of explosives set off at distances up to several

hundred kilometers. Further information concerning the thickness and nature of the crustal layers has been obtained. Details of the results are to be found in the report from that Department.

The following is a list of the papers published during the report year in technical journals. In addition there are several papers that have been prepared and are awaiting publication. These are: F. Chayes, "On a distinction between late-magmatic and postanagmatic replacement reactions*"; G. L. Davis and H. H. Hess, "Radium content of ultramafic igneous rocks. II. Geological and chemical impli-

cations"; G. L. Davis, "Radium content of ultramafic igneous rocks. III. Meteorites"; R. C. Kracek, "Phase transformations in one-component silicate systems"; J. F. Schairer, "Phase transformations in poly-component silicate systems"; A. H. Stone, "On supersonic flow past a slightly yawing cone. II"; and O. F. Tuttle, "The variable inversion temperature of quartz as a possible geologic thermometer."¹

SUMMARY OF PUBLISHED WORK

- (1104) A simple point counter for thin-section analysis. F. Chayes. *Amer. Mineralogist*, vol. 34, pp. i-n (1949).

A manually operated point counter for thin-section analysis is described. The machine is sturdy, inexpensive, and easily operated. Its precision has been tested by analyzing in duplicate 47 thin sections of rocks and computing the analytical error, or standard deviation of a single analysis, from the observed variance of the differences. The error distribution is effectively binomial, and the precision of the instrument is somewhat better than that of the Wentworth-Hunt and Hurlbut integrators. Average operating speed is about four times that of the Wentworth-Hunt and twice that of the Hurlbut machine.

- (1105) A new hydrothermal quenching apparatus. O. F. Tuttle. *Amer. Jour. Sci.*, vol. 246, pp. 628-635 (1948).

A simple apparatus has been developed for the study of equilibrium relations at high temperatures and pressures in mineral systems including volatile components. Investigations have been carried to pressures of 30,000 pounds per square inch (approximately 4.5 miles depth) at temperatures up to 900° C. Results on the system $K_2O-Al_2O_3-SiO_2-H_2O$ show that a water pressure of 15,000 psi lowers the liquidus about 100° in the orthoclase field. In compositions approaching the quartz-orthoclase join, a pressure of 30,000 psi gave relatively insignificant additional lowering.

- (1106) The radium content of varved clay and a possible age of the Hartford, Connecticut, deposits. William D. Urry. *Amer. Jour. Sci.*, vol. 246, pp. 689-700 (1948).

The radium content of the summer and winter portions of the varves in the clay deposits at Hartford, Connecticut, varies rhythmically. When the radium contents of the summer and of the winter portions are plotted against time as measured by the varve count, the curves exhibit slopes of opposite sign. The total radium content of any varve, how-

ever, is practically constant. These phenomena may be due to a disturbance of the radioactive equilibrium, but this hypothesis, though plausible, is far from proved. A greater concentration of uranium relative to ionium in the winter clay than in the summer clay would explain these phenomena. Such a disturbance of the equilibrium provides a means of determining the age of the deposits. On this basis, there is derived a tentative figure for the age of the Hartford clay (varve 3700) of 18,000 years. The hypothesis of a disturbance of radioactive equilibrium is supported by the fact that the analyses of the summer and winter curves, which are completely independent, give very nearly the same age.

- (1107) Radioactivity of ocean sediments. VI. Concentrations of the radioelements in marine sediments of the southern hemisphere. William D. Urry. *Amer. Jour. Sci.*, vol. 247, pp. 257-275 (1949).

It has been reported in previous publications of this series that the mode of variation of the radium concentration below the ocean bottom affords a method of determining time in ocean sediments. Hitherto, these researches were confined to the northern hemisphere. Similar studies in the southern hemisphere, combined with the necessary geological and biological investigations, should provide an answer to the question of the contemporaneity of glaciation in the northern and southern hemispheres. Measurements of the radium content as a function of depth in the sediment are presented here for ocean-bottom cores obtained by the U. S. Navy Antarctic Expedition of 1946-1947.

- (1109) Radioactivity of ocean sediments. VII. Rate of deposition of deep-sea sediments. William D. Urry. *Jour. Marine Research, Sverdrup Sixtieth Anniversary*, vol. 7, no. 3, pp. 618-634 (194B).

A study of the variation of the radium content during the period of re-establishment of radioactive equilibrium in the buried deposits of deep-sea sediments provides a method of

dating the record of past events in the ocean bottom. The results of such studies can be readily applied to determinations of the rate of deposition provided that knowledge of the distortion involved in obtaining core samples of the deep-sea sediments is available.

Application of the method of discerning rates of deposition is not limited, as was the application of earlier methods, to sediments deposited during postglacial time; it is possible to study the variation of the rate of deposition in the past as far back as the method of dating is applicable, i.e., for about half a million years. Rates of deposition as a function of time are reported here for red clay, globigerina ooze, foraminiferal marl, glacial marine deposit, and calcareous blue mud from areas extending from the Antarctic Ocean to the North Atlantic.

Outstanding features of these determinations are as follows: Deposition of practically all the sediments is more rapid at present than during the past half million years. The repeated climatological changes of the ice age did not have a particularly noticeable effect on the rate of deposition in general. Only during the long middle interglacial stage did the rates tend to be somewhat higher than the low rates generally prevailing in the past half million years. The lowest rates of deposition are associated with the last glacial stage and the early period of the middle interglacial stage or possibly the end of the second glacial stage. Locally, there are often interesting short-period changes in the rate of deposition which appear to be caused by climatological changes.

However, the amount of detail in an analysis of the rates of deposition varies greatly: in cores of equal length, far more detail can be obtained *in one* where the sediment was deposited in a hundred thousand years than in one where the sediment required a million years for deposition.

Average rates of deposition, in so far as they are comparable, are in good agreement with previous estimates by the *stratigraphic* and *supply* methods.

(mo) On ratio correlation in petrography. F. Chayes. Jour. Geol., vol. 57, no. 3, pp. 239-254 (1949)-

The same ratio correlation may be generated by many different combinations of relations between absolute measures, but a single set of absolute-measure statistics leads to one, and only one, correlation between any particular set of ratios formed from these absolute measures. The passage from ratio correlation to inference about relations between absolute measures is ambiguous at best and often misleading.

Algebraic statements exhibiting ratio correlation as a function of absolute-measure statistics are offered for types of ratios commonly used in petrography. These statements are all derived from Pearson's general formula for index correlation. They yield good approximations only if the fraction s/\bar{x} for each absolute measure is suitably small.

Several practical examples drawn from petrographic literature are described. In most of these cases the ratios seem to have been used either to order the data or *in* the hope that they would throw some light on relations between absolute measures. The results are shown to be on the whole indecisive and ambiguous and in a few cases decidedly misleading.

The formation of ratios should be confined to those problems in which hypotheses being tested deal with ratios. Absolute measures are always preferable when large numbers of observations must be recorded without benefit of satisfactory hypothesis. Ratios can always be drawn from tables of absolute measures; frequently, absolute measures cannot be reclaimed from tables of ratios.

(mi) The system MgO—SiO₂—H₂O. N. L. Bowen and O. F. Tuttle. Bull. Geol. Soc. Amer., vol. 60, pp. 439-460 (1949).

Equilibrium in the system MgO—SiO₂—H₂O has been determined at temperatures up to 1000° C. and at maximum pressures of water vapor varying from 15,000 pounds per square inch at this maximum temperature to 30,000 psi in the range 900°—600° and 40,000

psi in the range 600°—300°. Thus were fixed the univariant pressure-temperature curves of the following five reactions: I, serpentine + brucite \rightleftharpoons forsterite + vapor; II, serpentine ± 5 forsterite + talc -j- vapor; III, forsterite + talc ± 5 enstatite + vapor; IV, talc \rightleftharpoons enstatite -h quartz + vapor; and V, brucite \pm periclase +• vapor. Pure magnesian serpentine has a maximum temperature of existence at approximately 500°, varying only about 10° in the whole range of pressure, 2000 to 40,000 psi. Forsterite is stable in contact with water vapor down to a temperature of about 430° (at 15,000 psi). Only below that temperature is it transformed into serpentine and brucite. Iron-bearing olivines are stable in contact with water vapor down to still lower temperatures.

No liquid is formed in any composition of the system throughout the range of temperatures and pressures at which experiments were conducted, a condition which remains unchanged when the mixtures have upwards of 7 per cent FeO.

There is consequently no likelihood that any magma can exist that can be called a serpentine magma and certainly no possibility of its existence below 1000°. There seems no escape from the conclusion that ultramafics can be intruded only in the solid state.

(1112) Some examples of the application of thermochemistry to petrology. Th. G. Sahama and D. R. Torgeson. *Jour. Geol.*, vol. 57, no. 3, pp. 255-262 (1949).

A brief summary is presented of measurements of heats of solution of minerals belonging to the forsterite-fayalite and to the enstatite-orthoferrosilite series and of artificial ilmenite and geikielite.

In the olivine and orthopyroxene series, the heat of solution of HF is found to be a linear function of the Mg : Fe ratio, indicating perfect isomorphism between the corresponding end members. The importance and possibilities of applying calorimetry to the study of isomorphism are emphasized. On the basis of the calorimetric data available for the minerals in question, the heat and free energy

are given for the reaction: olivine + quartz \rightarrow 2 pyroxene. The difference in the stabilities of MgSiO₃ and FeSiO₃ is illustrated.

The stability reaction of ilmenite and geikielite in the presence of olivine or orthopyroxene is calculated from the calorimetric data. The influence of the entropy of mixing isomorphous minerals upon the stability relation is emphasized.

(1113) Significance of radioactivity in geophysics—thermal history of the earth. William D. Urry. *Trans. Amer. Geophys. Union*, vol. 30, pp. 171-180 (1949).

It appears that the effects on the earth's thermal history of the exponential decay of the sources of atomic (radioactive) heat within the earth are such that the upper crust was heating in its early history and that subsequent cooling has been more nearly linear than had been supposed. In the deeper parts of the crust and below, the thermal history has been complex, with simultaneous heating at one depth and cooling at another depth. Temperatures in the past beneath a Pacific-type ocean have not varied in the same manner.

(iri4) Melting relations of chalcocite. Einar Jensen. *Norske Videnskaps-Akademi*, Oslo, *Mat.-Naturv. Klasse*, No. 6 (1947).

A study of the melting relations of chalcocite by the method of differential thermal analysis shows a maximum melting temperature of 1129° C. for the composition Cu_{1.99}Ag_{0.01}S, which melts sharply. Mattes containing more or less sulfur than this, including pure Cu₂S, melt lower and over a temperature interval—pure Cu₂S from 1107° to 1x27°. The limit of solubility of liquid copper in liquid chalcocite is found at a total composition of 80.21 per cent copper at 1105°. The solubility of copper in solid chalcocite is too small to be observed at 1105° and 1127°. The limit of solubility of sulfur in chalcocite could not be observed. No transformations in the solid state could be observed between 404° and melting temperatures in the composition range 77 to 82 per cent copper.

(1115) The system silver sulfide—antimony trisulfide. Einar Jensen. *Norske Videnskaps-Akademi, Oslo, Mat.-Naturv. Klasse*, No. 2 (1947).

Nine minerals are known with a composition within this binary system. Previous thermal studies of the phase relations by Pelabon, Jaeger and van Klooster, and Konno indicate the existence at the melting point of the intermediate compounds $\text{Ag}_2\text{S} \cdot \text{Sb}_2\text{S}_3$ and $3\text{Ag}_2\text{S} \cdot \text{Sb}_2\text{S}_3$ only, corresponding to the minerals miargyrite and pyrargyrite, respectively. The present study of the phase relations was performed by differential thermal analysis on pure samples of exact composition, heated in sealed Pyrex tubes provided with thermocouple wells. The results indicate that the compound $\text{Ag}_2\text{S} \cdot \text{Sb}_2\text{S}_3$ has a polymorphous transition at some temperature below 380°C . and melts congruently at 518.7° . The compound $3\text{Ag}_2\text{S} \cdot \text{Sb}_2\text{S}_3$ melts at 485.8° . As the proportion of Ag_2S in the preparations increases, the liquidus curve first falls from 554°C , the melting point of Sb_2S_3 , to a eutectic with $\text{Ag}_2\text{S} \cdot \text{Sb}_2\text{S}_3$ at 462° , 22 per cent Ag_2S , then rises to the melting point of this compound, and again falls to a second eutectic, between $\text{Ag}_2\text{S} \cdot \text{Sb}_2\text{S}_3$ and $3\text{Ag}_2\text{S} \cdot \text{Sb}_2\text{S}_3$, at 464° , 59 per cent Ag_2S . The melting curve of $3\text{Ag}_2\text{S} \cdot \text{Sb}_2\text{S}_3$ rises from this eutectic and after passing through its maximum descends to a third eutectic at 470° , 77 per cent Ag_2S . From this point the curve remains almost horizontal to the neighborhood of 80 per cent Ag_2S , from which point it finally rises to the melting point of Ag_2S at 837.0° . The author suggests that the nearly horizontal portion of the melting curve may be the result of intrusion into the binary system of the liquidus surface of some ternary compound in the system $\text{Ag}-\text{Sb}-\text{S}$. The transition in $\text{Ag}_2\text{S} \cdot \text{Sb}_2\text{S}_3$, mentioned above, was found to begin, on heating, at temperatures as low as 344° , and to end at $370-380^\circ$. On cooling there was no reverse transition; on reheating after some weeks at room temperature a negative heat effect shows up near 207° . This leads to the conclusion

that the equilibrium transition temperature lies between 207° and 344° (359° in the pure compound).

(1116) Pressure-volume-temperature relations in solutions. VIII. The behavior of some solutions of electrolytes in water. R. E. Gibson and O. H. Loeffler. *Ann. New York Acad. Sci.*, vol. 51, pp. 727-752 (1949).

This paper presents the results of measurements of specific volumes of solutions of sodium bromide and sodium chloride over a wide range of pressure, temperature, and concentration. From these data various thermodynamic properties of the solutions, namely, the volume change on mixing, the pressure-temperature coefficient, the energy-volume coefficient, and the change in specific heat with volume at constant temperature, are calculated; the data are presented in detail in the form of tables and curves.

The effects of pressure, temperature, and concentration on the various thermodynamic properties of solutions discussed in this paper are all in qualitative agreement with the current theories of the molecular distribution in water. A comparison of the thermodynamic properties of sodium bromide in water and in glycol indicates that the contractions on mixing, the thermal expansions, and the energy-volume coefficients of the aqueous solutions at the lower temperatures are largely determined by the effects of short-range forces, and that the effects of ion-molecule interactions are only secondary. At higher temperatures the effects of short-range forces have a decreasing influence on these volumetric properties. Indeed, there is good reason to believe that above 150°C , the ion-molecule interactions and other long-range forces will play the significant part in determining the volumetric behavior of aqueous solutions and that simple regularities will be revealed that are masked at the temperatures where most of our information is now available.

(1117) Annual report for 1948-1949.

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DEPARTMENT OF TERRESTRIAL MAGNETISM

Washington, District of Columbia

MERLE A. TUVE, *Director*

Three years ago, beginning afresh on "significant research toward philosophical goals" after the interruptions of World War II, the staff of the Department stated three simple problems in geophysics which remained conspicuously unsolved, despite two generations of study on "the magnetic and electric condition of the earth and its atmosphere." These were as follows: The causes, inside the earth, of the earth's magnetic field are unknown; the mechanism which replenishes the earth's measured electric charge is not identified; mechanisms which give rise to particles of cosmic-ray energies are not yet recognized. The staff considered that these three basic puzzles might well serve as landmarks for orienting the geophysics program.

It is surprising to be able to report that the second of these questions, relating to the maintenance of the earth's electric charge, has been resolved by measurements in the clear air high above thunderstorms. One must also recall that unexpected evidence for a close relation of cosmic rays to our sun and to other stars was found in 1946, as reported two years ago, when cosmic-ray increases were discovered to occur at the times when large solar flares were in progress. All three of these problems in 1946 seemed basic in our thinking, yet probably beyond the reach of direct

investigation. It is encouraging indeed to find that formulation of a central problem in the simplest and most direct fashion can, with good fortune, be followed by significant new knowledge. The measurements above thunderstorms, carried out with the help of the U. S. Air Forces, are described below.

It remains to be seen whether direct knowledge can be obtained as to the causes, inside the earth, of the earth's magnetic field. No direct approach to this puzzle has yet presented itself, but new evidence, brought out in the course of a study of prehistoric changes in the compass direction, has instead opened up surprising questions relating to the possibility of great movements of the earth's crust with respect to the core and geographic poles during ancient geological epochs. This unexpected finding has been brought to light through studies of the residual magnetization of rocks.

In the laboratory phase of the Department's work, a steady development of the biophysics program is a strong indication that men trained in the physical sciences find ample challenge in the organized behavior of living matter, can formulate problems, and can obtain answers of interest and significance in this field.

EXPERIMENTAL GEOPHYSICS

THE EARTH'S CRUST

PALEOMAGNETISM

In the last annual report, there was extensive discussion of a program for investigation of the residual magnetism of

the sedimentary rocks in the earth's crust. This program was initiated with the hope that the results might provide a detailed history of the changes of the earth's magnetic field extending back millions of years in geologic time.

Our investigations have demonstrated that when sedimentary rocks are laid down in tranquil water they may acquire a direction of magnetization as the result of the statistical alignment of magnetized grains parallel to the earth's field. Therefore, a record of the past directions of the earth's magnetic field is found preserved in some sedimentary rocks. Recent tests here have demonstrated that sediments may retain a direction of magnetization without change for as long as 200 million years, and it now appears that it should be possible to trace far back in time the history of the changes in direction of the earth's magnetic field.

Rock magnetism reconnaissance expedition of 1948. The Department's studies of the magnetization of crustal materials have heretofore been confined to relatively young unconsolidated sediments from New England glacial deposits and from ocean cores. The results of these studies indicated that for the past million years the earth's magnetic field has had much the same orientation that it has today, and that its strength has remained approximately constant. In an effort to extend our knowledge of the changes of the earth's magnetic field farther back in geologic time, a three-month expedition to the flat-lying sedimentary deposits of the western United States was carried out during the summer of 1948. Samples were taken at eight sites scattered from Colorado north-westward to Washington and eastward into Wyoming and South Dakota. The age of the rocks measured ranged from approximately 10 to 100 million years. Because many rocks are not perceptibly magnetized, and because of difficulties with the techniques of preparing samples, only 96 separate observations of magnetic polarizations could be made. The results are summarized in figures 1 and 2, in which the directions of the horizontal com-

ponent and of the inclination of the rock magnetizations are indicated. The limited number of sites and samplings makes it unwise to draw extensive conclusions from the data, but the pronounced maxima in the two graphs suggest that for the past 100 million years, roughly, the earth's magnetic axis has remained centered, on the average, on the geographic axis. This interesting possibility is consistent with various different theories of the origin of the earth's magnetic field, and therefore cannot be advanced as an argument favoring any one of them.

Stability of magnetization. Workers in the field of rock magnetism have long recognized the necessity for demonstrating that rocks are capable of retaining their initial directions of magnetization from the time of their origin to the present. Definitive results on this question were obtained in 1948 from experiments which satisfactorily take into account long periods of time as a factor in the behavior of magnetization. This factor was treated by utilizing in the magnetism studies changes in geologic structure which took place millions of years ago. It is possible in many localities to locate rock exposures where sedimentary beds were long ago squeezed into contorted arches by mountain-building forces. Observations in these squeezed and folded beds have yielded knowledge of the permanence of the directions of magnetization.

One particular series of observations on Silurian rocks (350 million years old) that are well exposed at Hancock and Pinto, Maryland, is of special interest. In one of the folded beds, shown in plate 1, it was possible to obtain 20 suitably spaced measurements of the directions of magnetization. The directions were found to vary systematically throughout the fold in a manner that has an intimate relation to the attitude, or position in space, of each

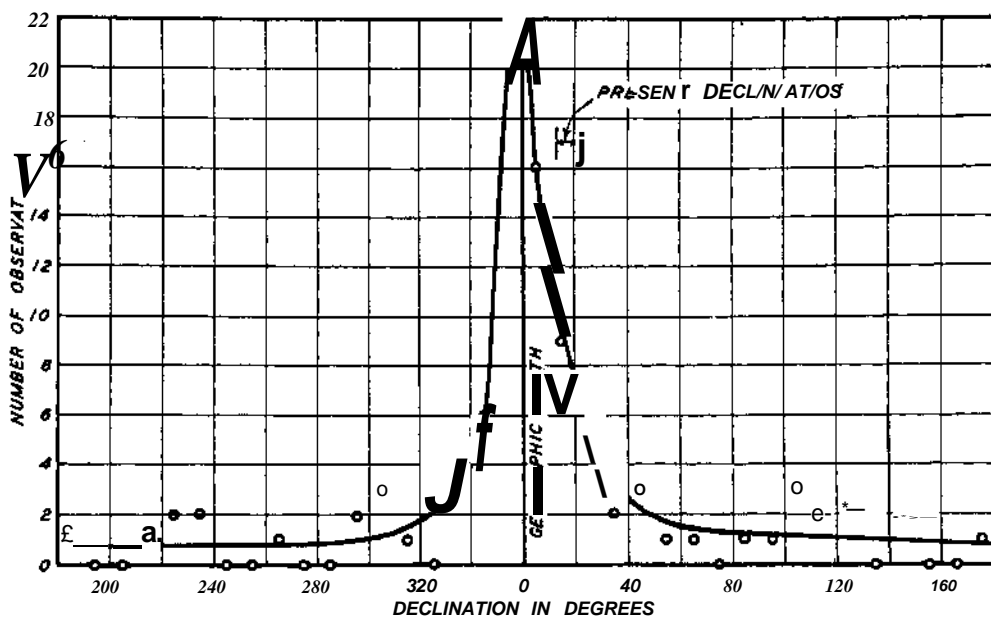


FIG. 1. Frequency distribution of declination measurements on rock samples

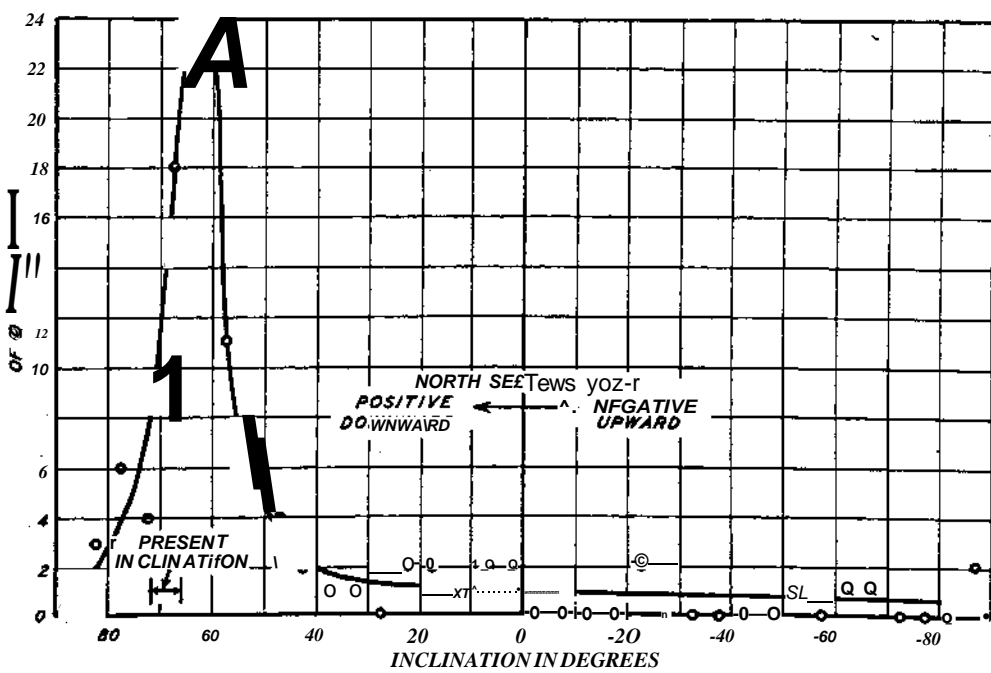


FIG. 2. Frequency distribution of inclination measurements on rock samples

part of the bed where an observation was made. The closeness of this relation is clearly brought out when a graphic reconstruction is made to restore the bed to its initial flat condition. Although the directions of magnetization in the fold are fanned out, and show differences in direction of as much as 127° , when the bed is made flat by a graphical transformation the magnetizations are all brought into general alignment so that these differences are usually less than 15° . This intimate relation of the directions of magnetization to the attitude of the bed is evidence that the bed was uniformly magnetized initially, that as it was folded it was merely flexed into its present configuration without yielding to shearing forces, and that since the time of the folding, 200 million years ago, the magnetizations at each point throughout the fold have not changed their initial directions.

There is still some question whether the reconstructed direction of magnetization of the fold at Pinto can be taken as a measure of the direction of the earth's field in Silurian time. The beds were laid down perhaps 150 million years before they were folded* and in that interval of time, long after deposition, they could have acquired their present reconstructed direction of magnetization. Further studies are needed to settle this point. There is evidence, however, that these beds acquired their magnetization, not in a field of a very local nature such as might surround a bolt of lightning or an intensely magnetized ore body, but in a field that was uniform over a large area, because beds of the same age at Hancock, 50 miles away, show the same reconstructed direction of magnetization. The surprising thing about the reconstructed direction of polarization is that the south-seeking pole is downward, contrary to the polarization of the present-day magnetic field, in which the north-seeking

pole is downward in the earth's northern hemisphere.

Along with this demonstration of stability, it has been possible to throw some light on the instability of samples stored in the laboratory. Some workers have considered that if a sample changes its direction of magnetization in the laboratory, it is inherently unstable and hence cannot be relied upon for deducing past directions of the earth's field. In the present work it has been observed that samples from the Silurian, as well as some of the younger samples from the western United States, also change when stored in the laboratory, and it has been possible to show that these changes are greatly accelerated by almost insignificant alternating magnetic fields. Stray fields of this kind exist in every laboratory building, but not in primitive geological areas. The mechanism by which such changes take place is not yet clear and requires further study.

New program. A new phase of the program of investigation now centers on determining in detail the regional character of the magnetization of Silurian rocks. It has been an astonishing experience to find that at two sites in the neighborhood of Washington there are rocks having their south-seeking poles directed downward. If it can be demonstrated that this anomalous magnetization prevails over large areas in a particular span of geologic time, a potent new tool will be at hand for studying some of the problems of earth physics.

Work on this phase of the program was initiated, under a special grant from the Carnegie Corporation of New York, by a reconnaissance study of Silurian rocks at Birmingham, Alabama, and the same anomalous magnetizations that were found at the Maryland sites were noted in a restricted series of beds. Below these beds two other major changes in magnetization of the Silurian rocks at Birmingham were



Folded Rose Hill formation, Pinto, Maryland

noted. Adequate fossils for precise dating were lacking at this site, and thus there is some question whether the rocks are strictly contemporaneous with those from Maryland.

Also, as part of the new work, an extensive sampling of the Hancock Silurian beds was made in the last few days of the report year. The data obtained indicate that in the 500 feet of deposition exposed at this site the anomalously magnetized beds occupy a middle section of approximately 200 feet, there being 100 feet above and 200 below which are magnetized much more nearly in the direction of the earth's present field.

Instrumentation. Experience with many different sediments has made clear the necessity for two basic improvements in instrumentation: greater sensitivity in the measuring apparatus, and a versatile core-drilling rig that will make it possible to sample exposures where the rocks are so massive that small oriented slabs cannot be removed easily by hand. A new remanent magnetometer has been built and shows promise of giving greater sensitivity and ease in making measurements. Since it contains many standard electronic parts, construction is relatively simple. A light-weight drilling rig is also ready for field use and, it is hoped, will make possible the study of many exposures that heretofore have had to be abandoned.

SEISMIC INVESTIGATIONS

As reported last year, a series of seismic observations from Solomons, Maryland, northwest to a distance of 300 km was carried out. When the times of the first arrived pulses were plotted as a function of their distance, it was seen that these times do not fall on a straight line, but that, beyond 130 km, each succeeding group of points appears to represent a wave

of greater velocity than that of the preceding set of points. This indicates an increase of velocity with depth, and it was possible to deduce (with the auxiliary confirmation of some arrivals later than the first or second ones) several different velocities and a multiple-layered structure down to the Mohorovičić discontinuity at a depth of some 40 km. The velocity of compressional waves in the material near the surface appeared to be 6.1 km/sec, while that below the Mohorovičić discontinuity was 8.1 km/sec.

One of the more interesting aspects of the data was a group of strong second arrivals, observed at distances of from 90 to 120 km, which came in about 2 seconds after the first arrivals. The best explanation at hand, based on the earth model deduced from all the other data, was that this arrival could be a shear-type wave (S) generated from the partial conversion of a compressional-type wave (P) at the interface between the bottom of the upper layer of rock and the top of the first intermediate layer at a depth of 10 km, the wave being called P10S. It was necessary to collect more observations in order to make sure that this peculiar wave was not a chance interference; observations were obtained at intervals of 5 km in this intensity region. The arrivals were as clearly discernible as those previously observed and seemed to furnish convincing evidence that the phase was real and due rather to the earth structure than to a fortuitous interference. This wave, if it could be proved a converted P-S wave as interpreted, might be an exceedingly useful one, because a generated S wave must rise at a steep angle from the interface, and from the difference in the arrival between it and the first arrival, the depth of the layer could be deduced with considerable precision. Thus the desired type of exploring probe would have been attained. The validity of this Interpret,!

tion was tested, however, using a new method of recording the seismic waves, and these tests showed during the past report year that this conspicuous arrival is in reality a compressional wave (P wave), not a shear wave (S wave), and hence is not a P₁ and will not serve as the desired "probe."

It thus became necessary to reconsider the data. In the meantime investigations had been made on the amplitude of the seismic waves due to the routine calibration of the amplifiers at each observation point, with a single electric pulse generator. From these data it was found that the amplitude of this second arrival rose somewhat abruptly and then dropped *off* with distance. The observations of the abrupt rise were somewhat hampered by the existence of unconsolidated sediments ending at about 80 km from the source of explosion and by the presence of a city (Washington, D. C.)—a locality of vigorous seismic unrest—at the distance of interest. The rather abrupt appearance of this wave and its P-wave characteristics seemed to indicate that it was a critical reflection as first predicted by Knott in 1899. Trial solutions showed that if the velocity below the Mohorovičić discontinuity was taken as 8.1 km/sec (as measured), and a depth of some 33 km and a velocity of 7.1 km/sec just above the discontinuity were assumed, good agreement was obtained with the data, which, though consistent in themselves, need further checking with shots so placed that the seismic waves may travel in directions other than the ones normally observed. It is hoped that, with the aid of the Navy, the Department may establish its own "quarry." For this a water-filled unused stone quarry, far from any habitation, is needed. A typical geophysical problem is here presented: the interpretation of a set of data, with the open question of how

closely this agrees with the actual properties of the earth. More measurements are necessary.

In the latter part of July 1948 the Department was notified that the Tennessee Valley Authority was about to set off 500,000 pounds of explosive for the purpose of obtaining crushed rock for a large dam on the South Holston River at a site 5 miles from Bristol, Tennessee. This turned out to be the first of three large shots. The other two were of 1,500,000 pounds and 800,000 pounds, fired in October 1948 and February 1949, respectively. They furnished an opportunity to obtain precision seismographic observations out to 1500 km. Excellent seismograms were obtained, roughly in two lines radiating northeast to Maine and northwest to Wisconsin from the dam: to the northeast, 15 seismograms from 250 to 1200 km, and to the northwest, 9 seismograms from 90 to 800 km. One other seismogram was obtained by the New Mexico School of Mines at a distance of 1500 km west. The success of these measurements was due in large part to the co-operation of the Tennessee Valley Authority. Some of the observations were made by colleagues at Columbia, and several stations were occupied by the Office of Naval Research.

The first result obtained from these observations was the measurement of the travel of the compressional (P) wave through the ultrabasic rock just under the Mohorovičić discontinuity. This ray of the wave first arriving at distant observation points takes a downward path from the explosion through the intermediate layers. It is then refracted at the Mohorovičić discontinuity and extends almost parallel to this level and not far below it, and near the observation point it is refracted once more into the intermediate layer and proceeds to the surface. If the crust were uniform and the rock just under it homogene-

ous, then the arrival times at the various stations would lie along a straight line in a time-distance plot. But the actual observations, accurate to 0.02 second, diverged as much as ± 1 second from the straight line of the mean travel time. This indicated a nonuniformity which could be due to differences in crustal velocities, in crustal structure, in total crustal thickness, or in the propagation velocity under the Mohorovičić discontinuity. For the final shot the stations were distributed from 90 km out to 900 km, and they gave evidence of a critical reflection similar to that which was observed from the shots from Solomons. The indicated depth is greater, however, under the region northwest from South Holston. The tentative conclusion is that the data indicate among other things a greater depth of the Mohorovičić discontinuity under the Appalachian Mountains, particularly northeast and northwest of South Holston at distances out to 500 km. This cannot be regarded as a definite result, however, until more work has been done on the upper crustal layers near South Holston.

THE EARTH'S ATMOSPHERE

THE EARTH'S ELECTRIC FIELD

Thunderstorm Investigation

During the thunderstorm season of 1947, four airplane flights were made over thunderstorms, but records from only three of these were satisfactory for analysis. These results, as given in last year's report, were regarded as provisional. After the close of the 1947 season, instruments were improved and the airplane was stripped of armament, so that higher altitudes could be reached and maintained.

During the 1948 thunderstorm season, again with the co-operation of the U. S. Air Forces, successful flights were made

during July, August, and September from the Clinton County Air Base, Wilmington, Ohio, guided by the V-beam radar installation at near-by Jamestown. During October, additional storms were surveyed utilizing Tinker Field Air Base, Oklahoma City, Oklahoma, as the center of operation. During the season 21 thunderstorms were surveyed and 65 traverses were made over the centers of the storms. Results of the season's operations may be summarized as follows:

The electrical conductivity of the atmosphere increases with altitude up to the limit surveyed (48,000 feet) in a manner consistent with that found on the balloon flight of *TL Explorer II*. This variation may be expressed closely by an equation of the general form $A = A_0 - f^2 A^2$, where Xh and A_0 are the positive conductivities (at height h and at ground level, respectively). A is a constant with a mean value of 2.0×10^{-8} , if h is expressed in feet, but varied on 6 flights from 1.6×10^{-8} to 2.4×10^{-8} . The mean value of A_0 is 0.7×10^{-4} esu, but it varied on 6 flights from 0.4×10^{-4} to 1.4×10^{-4} esu.

The conductivity over the thunderheads surveyed appeared to be similar to that found at the same altitude away from the strong fields of the thunderhead.

The vertical current passing through the storms surveyed varied from a small fraction of an ampere to more than 6 amperes.

The arithmetical mean of the total current taken over the 21 storms is 1 ampere. The arithmetical mean, regarding each of the 65 traverses as independent, also amounts to 1 ampere.

The direction of the current on all 65 traverses was such as to return negative charge to ground. This is opposite to the sign of the air-earth current over fair-weather areas.

The sign and magnitude of the current, if the storms surveyed on this project can

be regarded as typical, are such as just to maintain, by thunderstorm activity, the negative charge of the earth, which is demonstrated on a world-wide basis by an air-earth current of the same sign over all fair-weather areas. These studies provide the experimental basis for a satisfactory explanation of this long-standing puzzle of the maintenance of the earth's negative electrical charge.

Atmospheric Conductivity in Peru

The atmospheric-electric data recorded at Huancayo during the winter of 1947 have been thoroughly studied with the object of determining the causes of the large diurnal variation in atmospheric conductivity which has been recorded at Huancayo for some years. There is a variation by a factor of about 3 between the maximum and minimum values.

The negative conductivity shows a greater variation than the positive. This has been found to be due to a diurnal variation in negative small ion mobility, which varies in such a way as to augment the effect of the variation in negative small ion content. The mobility of the positive small ions remains constant, so that the diurnal variation of positive conductivity is due entirely to variation of positive small ion content, which, in fact, is greater than the variation of negative small ion content. Average values of mobility from all data are: $\mu_+ = 2.3$ cm²/volt/sec, $\mu_- = 2.9$ cm²/volt/sec. Reduced to sea-level pressure, these values are: $\mu_+ = 1.5$ cm²/volt/sec, $\mu_- = 2.0$ cm²/volt/sec.

Values of positive small ion content were found to fit the formula of ion balance: $q = \alpha n_1 n_2 + \beta n_1 n_3 + \gamma n_1 n_4 + \delta n_1 n_5$, where q is ionization, n_1 positive small ion content, n_2 negative small ion content, n_3 negative intermediate ion content, n_4 negative large ion content, n_5

neutral condensation nuclei content, and $\alpha, \beta, \gamma, \delta$ are the appropriate combination coefficients. These data furnish the following values of the combination coefficients: $\alpha = 0.9 \times 10^{-6}$ cc/sec, $\beta = 4.6 \times 10^{-6}$, $\gamma = 0.4 \times 10^{-6}$, and $\delta = 3.3 \times 10^{-6}$ in the period 5^h to 10^h and 10.5×10^{-6} in the period 14^h to 18^h, with intermediate values from 10^h to 14^h.

These figures show that the variation in small ion content can be attributed to the following causes in the proportion shown: variation in ionization 25 per cent, variation in intermediate ion content 12 per cent, variation in large ion content 63 per cent.

There is strong evidence that the change in μ_2 is due to the presence of more than one type of intermediate ion and an increase in the proportion of the less mobile types from morning to afternoon. On the other hand, values of μ_2 derived from day- and night-time data are surprisingly consistent.

These results are calculated without correcting the measured values of q for the fact that sources of alpha rays are excluded from the inside of the chamber. This correction will increase them by a factor of about 5/3.

If a correction is made for the exclusion of alpha-ray sources, both the total ionization figures and the percentage due to alpha rays are found to be unusually high. The table on the following page summarizes the results.

UPPER ATMOSPHERIC RESEARCH

Work directed toward exploratory geophysics of the upper atmosphere has progressed substantially along the lines indicated in the report for 1947-1948. Major effort has been devoted to the design, assembly, and testing of electronic devices and components required for the conduct

	DAY		NIGHT	
	Ionpairs/ cc/sec	Per cent	Ionpairs/ cc/sec	Per cent
Alpha rays in air...	30.0	76	38.4	80
Gamma rays in air	1.4	4	1.8	4
Gamma rays from ground and in- herent ionization of chamber.	2.8	7	2.8	6
Cosmic rays	5.0	13	5.0	10
Total	39.2	100	48.0	100

of special experiments utilizing electromagnetic waves for exploration of the upper atmosphere. An experiment to determine the precise time of certain sunrise characteristics of the ionosphere has revealed new facts concerning the structure of the upper atmosphere. Improved laboratory facilities at the Derwood Experimental Laboratory have materially raised the efficiency of operation.

Panoramic Recording of the Ionosphere

Development of a prototype high-speed ionospheric recorder has continued with the following objectives: (1) ability to explore the ionosphere at intervals of a few seconds in order to record rapidly changing phenomena; (2) maximum flexibility of operation over the entire frequency range, 1-20 Me, or any portion thereof; and (3) completely self-contained standards of time and frequency assuring dependable performance under a wide range of operating conditions.

Although technical details of instrumental improvements are included in other reports, it is of interest to note that a technique has been developed which makes it possible to approach the ultimate theoretical limit of resolution. When an ionospheric recorder is swept over a wide band

of frequencies in a few seconds, the difference in frequency between a given transmitted pulse (and the corresponding receiver tuning) and the ionospheric echo from an earlier pulse becomes a limiting factor. This "detuning effect" is dependent on rate of change of frequency between pulses, velocity of propagation of radio waves, and maximum height range of the instrument. The new development automatically compensates for the frequency change between pulses, with the result that scanning speeds can be increased and receiver performance improved through better ratios of signal to noise.

Construction of two ionospheric recorders for operation in arctic and equatorial regions has continued. Many of the components have been completed and others are well advanced. All important instrumental design problems have been solved, although some mechanical problems such as antenna structures for field installations are still being studied.

The sunrise experiment of May 5 to June 10, 1949 has revealed new facts concerning properties of the outer atmosphere. It has also demonstrated the adaptability of a high-speed recorder for the precise timing of events within the ionosphere. Recordings were made daily from 04^h 30^{min} to 05^h 45^{min} at intervals of 5 or 10 seconds. Analyses were performed to determine the precise time at which a characteristic "sunrise effect" was observable in the F region. Although the time of sunrise in the F region was at least 1% hours before ground sunrise, no increase of F-region ionization or other characteristic "sunrise effect" was observed until approximately 20 minutes after ground sunrise. This corresponds to a grazing angle of 5° or a solar zenith angle of 85°, representing a limiting angle at which the F region must be inclined to the sun's rays before a sunrise effect is observed from the ground. Nearer to the

time of ground sunrise it is inferred that the ionizing energy of the sun's rays is completely absorbed in the already illuminated atmosphere.

Several additional occurrences of ionospheric "clouds" and rapid fluctuations in ionization were recorded during the above-mentioned observing period. The dates of occurrences corresponded to magnetically quiet periods, in contrast with the magnetically disturbed interval of the original report on this phenomenon. The cause of such ionospheric "clouds" is still open to conjecture, although some evidence appears to be accumulating in favor of horizontal wind motions rather than clouds of extraterrestrial origin.

Duplex-Channel Differential Recorder

The basic principles and fundamental objective of the duplex-channel differential recorder were described in the report for 1947-1948. It will be recalled that the basic idea underlying the new development involves simultaneous transmission of radio waves on two channels separated by a small increment of wave frequency. The instrument is adjusted to record a selected characteristic of the ionosphere. The device "locks on and follows" the time variations of this characteristic and records the data on a pen-and-ink chart for immediate and greatly simplified analysis.

The computer section of this instrument, which incorporates complicated electronic counting and timing devices, has been finished and tested. Detailed circuit diagrams and descriptions of operating sequence have been prepared. Other basic circuits for all except the radio frequency components have been tested in preliminary assemblies. Operation of this instrument as an integral unit is anticipated early in the ensuing report period.

Experimental Cosmic-Ray Research

Large ionization chamber. The large ionization chamber installed at the Derwood Experimental Laboratory has been operating for several months, providing sufficient statistical data to determine the level of statistical fluctuations with the upper half of the chamber unshielded. As anticipated, large variations were found; these are doubtless due to changes in the local radioactive content of the air. This confirms the expected necessity of completely shielding the instrument with lead to eliminate these variations, which are large enough to obscure the solar-flare effects that the meter was designed to detect. Lead shielding is now being installed on the upper half of the meter.

Neutron chamber for cosmic rays. It is planned to use two available Compton-Bennett ionization chambers to record variations in cosmic-ray neutron flux. One chamber will contain $B^{10}F_3$ with enriched boron 10, and the other $B^{11}F_3$ with enriched boron 11, both isotopes now being available from Oak Ridge. Neutrons, slowed down by hydrogenous shields, are captured in boron 10 with emission of alpha particles, the ionization of which is recorded. No neutron capture occurs in the boron 11; thus by recording the difference in ionization between the two chambers, all ionization except that due to neutrons is canceled out. It is expected that such a neutron intensity recorder, especially at a high-altitude station, may be much more sensitive to changes in cosmic-ray intensity associated with solar flares, magnetic storms, and other causes than are the Compton-Bennett meters, which mainly measure meson intensity.

Worldwide network of Compton-Bennett meters. Continuous recording of cosmic-ray ionization in Compton-Bennett meters was effected at Godhavn (Green-

land), Cheltenham (Maryland, U. S. A.), Huancayo (Peru), and Christchurch (New Zealand).

A new control box for Compton-Bennett meter no. C-4 was completed. This meter, badly damaged in transit from Teoloyucan (Mexico), has been thoroughly overhauled, assembled, and filled with argon. Comparisons between meter C-4 and meter C-i are now in progress at Cheltenham to insure that quantitative changes in intensity at Climax and at Cheltenham due to solar

flares may be reliably compared. Since there is little difference in latitude and about 11,000 feet difference in elevation, quantitative comparison of solar-flare effects at these two stations should provide some indication of the nature of cosmic-radiation changes associated with solar flares. Dr. Walter O. Roberts, Director of the High Altitude Observatory at Climax, Colorado, has kindly co-operated in providing space for meter C-4, and will endeavor to insure its continuous operation.

THEORETICAL AND STATISTICAL STUDIES

COSMIC-RAY RESEARCH

Mechanism for the Solar-Flare Effect on Cosmic Rays

Work was continued in collaboration with Dr. M. S. Vallarta on a mechanism to explain the marked increase in cosmic-ray intensity observed during a few solar flares. As mentioned in previous annual reports, the rate of change of the magnetic moments of sunspot pairs appears sufficient to provide protons with enough energy, by a process analogous to that of the betatron, to reach the earth at latitudes where the cosmic-ray increases during the solar flares were observed. Except in unusual circumstances, protons of this energy, about 7 Bev, would escape from the sun only at regions very near the poles, and not at regions nearer the sun's equator, where flares are observed. The magnetic moment, however, of a pair or group of sunspots seems to provide the necessary and unusual circumstance permitting the particles to escape. To be certain whether the magnetic moment of sunspot pairs makes it possible for protons of the energy involved to escape through the sun's general magnetic field requires the integration of the equations of motion of a charged particle in the combined magnetic field of the sun

and of the sunspot. The equations of motion were set up in Cartesian co-ordinates and several points along a trajectory were obtained by numerical integration using two different numerical schedules, one of which was kindly furnished by Dr. J. von Neumann to insure that the numerical schedule could be handled by the Eniac electronic computer at Aberdeen, Maryland.

From results of a few numerical integrations, obtained with ordinary mechanical computing machines, it was found that only a very short interval of integration (arc length along a trajectory) could be used if the errors of integration were to be kept sufficiently small. This was due to the fact that the trajectory of the particle is roughly helical, with radius small compared with the solar radius. Consequently, to compute a trajectory long enough to determine whether the particle escapes from the sun would have taken, even for the Eniac, a prohibitively large number of integrations. Since the motion of the particle is approximately helical, the equations of motion have been transformed into helical co-ordinates. Parameters in the equations allow for the expected changes in the pitch and radius of the helix along the trajectory. In this system of co-ordinates it is expected

that, for a given accuracy, a much greater interval of integrations can be used than was the case for the integrations carried out in a Cartesian system. Work on the numerical schedule for the integration is now being started. By the fall of 1949 the results of the preliminary integrations should determine whether the problem can be solved in a reasonable time on the Eniac. The possibility of investigating the tunnel problem on the magnetized model of Malmfors and Alfvén was examined also. The model, because of its limited magnetic moment and the lower limit of useful electron energy for the beam, could only simulate the actual problem for a sun with magnetic moment much less than 10^{34} gauss cm^3 , the figure indicated by the cosmic-ray latitude variation, or for protons of energy much greater than the 7 Bev indicated by the latitudes at which solar-flare effects on cosmic-ray intensity were observed.

*Search for Systematic Variations in
Cosmic-Ray Intensity*

It has been pointed out by M. S. Vallarta and O. Godart that periodic variations of small amplitude are to be expected if the sun has a total steady magnetic moment of 10^{34} gauss cm^3 , as required to explain the knee of the latitude variation in cosmic-ray intensity. They have also indicated that a periodic 27-day variation in intensity would occur if the solar magnetic moment were sufficiently inclined to the axis of rotation, since in that case the solar cut-off energy would vary with the period of the sun's rotation. As a consequence of the inclination of the sun's axis of rotation to the ecliptic, a 6-month periodic variation was predicted whether or not the rotational and magnetic axes coincide. Data from several Compton-Bennett meters, obtained over a period of ten years or more,

were subjected to analyses to determine whether any of the predicted variations could be regarded as statistically significant. The 27-day variation was found to be quasi-periodic like the 27-day recurrence phenomenon in terrestrial magnetic activity. The amplitude of the periodic 27-day wave was too small to be regarded as statistically significant. The 6-month variation had maxima in reasonable agreement with the predicted times of maxima, although the amplitude was too small to be regarded as statistically significant. The results obtained therefore do not confirm, although they clearly do not deny, the existence of the periodic variations predicted on the basis of a permanent solar magnetic field.

Cosmic-ray data covering a period of about ten years were analyzed for a sidereal diurnal variation. Some indication of a sidereal wave was obtained, but its statistical significance has yet to be tested. The influence of the earth's magnetic field, however, on the interpretation of an apparent sidereal variation has only been worked out (Vallarta and others) for cosmic rays arriving vertically at the geomagnetic equator. This work shows that coincidence telescopes would be much better suited for interpretation than are Compton nondirectional meters.

GEOMAGNETIC STUDIES

An examination was made of the worldwide pattern of abrupt magnetic-field changes during the simultaneous initial phases, or "sudden commencements," of intense magnetic storms. According to the Chapman-Ferraro theory of magnetic storms, the electric currents causing the sudden commencement flow well beyond the atmosphere. These currents, which produce a field at the earth like that of an external dipole, and the effects of the

electromagnetic shielding of the ionosphere have been estimated by Price. Examination of the two sudden commencements which occurred during the Polar Year 1932-1933 has yielded results indicating agreement with theory, and the fields of two more sudden commencements are now being examined in an effort to draw more certain conclusions from the study. This work was begun with the co-operation of Sr. Mateo Casaverde, of the Instituto Geofísico de Huancayo, while he was a guest of the Department.

The westward motion of the magnetic dipole best fitting the earth's main field was investigated. This dipole is displaced from the center of the earth about 300 km in the direction of the East Indies. Since 1840, its motion, as shown from spherical harmonic analyses, has been westward at the rapid rate of about 0.25° of longitude per year. From the indications of declination charts, this motion seems to have been continuing at about this rate since AD 1600. This westward shift and the accompanying shifts of the irregularities in the earth's field have an important bearing on theories of the earth's main field; an internal structure deep in the earth is retarded, in its rotation about the axis, more than the crust is retarded. This gives us clues to dynamical properties of the core. This work is being continued with special reference to the results of varve measurements, which provide magnetic data over a longer time scale.

The weekly staff discussions on geophysical problems mentioned in last year's report were continued with active participation of other colleagues in the Washington area. During the fall of 1948, Professor V. C. A. Ferraro, of the University of the South West, Exeter, England, gave a series of lectures, followed by discussion, on theories of magnetic storms and aurora and of the ionosphere. The focus of attention

was the formulation of new problems susceptible of theoretical or experimental approach with particular reference to the electrical state of the upper atmosphere.

Hourly Frequency of "Sudden Commencements"

A statistical examination of the hourly frequency of geomagnetic "sudden commencements" was initiated in order to check the rather striking results obtained by Newton from his analysis of the Greenwich magnetograms. All available traces from several observatories in various geomagnetic latitudes and longitudes were examined, and it was found that only stations of latitude above about 30° produced curves which showed a systematic diurnal variation. When only sudden commencements which were followed by the larger magnetic storms were considered, this diurnal variation was more pronounced. A tabulation was also made of the ratio of those sudden commencements having a small preliminary impulse (Newton's sudden commencements) to all sudden commencements, and the results from seven observatories appeared to show that this ratio varies with geomagnetic longitude. Further data are being sought from the magnetograms of other observatories in strategic positions in order to reach a definite conclusion on this point.

Portable Magnetic Observatory

Active experimental study is being given to the development of an automatic, long-run, portable magnetograph capable of being set up in an isolated place and left to record the three geomagnetic elements without attention for several months. The basis of this proposed new instrument is the portable magnetograph developed during the war, with such modifications as appear necessary for the desired prototype

of a group of general-purpose recording instruments which can provide the information ordinarily provided only by a permanent magnetic observatory and staff.

LABORATORY PHYSICS

NUCLEAR PHYSICS

The major emphasis during the past year has been on the problem of proton-proton scattering in the relatively low-energy region of 200 to 500 kilovolts, using the one-million-volt electrostatic generator. This is the region which will be most sensitive to the exact shape of the potential well which is assumed in each analysis of proton-proton scattering; it will respond particularly to a possible long-range "tail" on the steep-walled potential well. This is a difficult region of energy for measuring proton scattering because of the difficulty in observing protons of low energy and in measuring accurately the number of protons in the incident beam during operation, with hydrogen gas necessarily present *in* the scattering chamber. A considerable measure of progress has been made during the year on these problems. Proportional counters have been built and operated which will reliably count protons having as low as 90,000 electron volts energy. It has been found that the proton beam current can be monitored by allowing the beam, after passing through the scattering volume, to bombard a lithium target; observing the number of alpha particles emitted in the disintegration of lithium of mass 7, the number of such particles will be proportional to the proton beam current. Preliminary scattering data have been taken in the region 300,000 volts. Efforts are now being directed toward reducing possible systematic errors in the data.

The high-resistance column used for measuring voltages on the one-million-volt generator has been overhauled during the

year. New Western Electric precision resistors were installed, and the assembled unit was carefully calibrated against the sharp gamma-ray resonances from boron, lithium, and fluorine bombarded by protons.

During the early part of the year, in cooperation with Dr. D. R. Inglis, of Johns Hopkins University, and Dr. E. M. Hafner, of Brookhaven National Laboratory, experiments were completed on the study of the proton groups emitted in the disintegration of carbon of mass 12, bombarded by deuterons. The yield and angular distribution curves of the protons were obtained for deuteron energies from 1 to 3.5 million electron volts. This work illustrated the complexity of the nuclear energy-level system in this region of the atomic table. As many as 21 levels of the intermediate nucleus (nitrogen 14) were observed.

BIOPHYSICS

The biophysics group continues to approach biological problems from the viewpoint of physics. The group is more convinced than ever of the benefits to be derived from cross-fertilization between the various disciplines. In addition to the three staff members of the group who are physicists by training, there are present among visiting investigators and fellows a cell physiologist, two biochemists, and an organic chemist.

To gain a more solid background for the biophysics program* a considerable effort has been made and is continuing in group study. In one series of seminars the book entitled *Dynamic aspects of biochemistry*, by Baldwin, was thoroughly covered. In

a second series, *Radiation effects*, by Lea, was presented. In addition, a number of outside visitors and lecturers presented other facets. Among the guests were Margaret Murray, B. A. d'Houssay, David Pressman, Alan A. Boyden, A. Szent Györgyi, William Libby, L. H. Grey, J. W. Boag, Britton Chance, and Robert Briggs.

The biophysics program is continuing to evolve. One of the principal preoccupations is the formulation of questions and modes of approach to central problems in biology in terms appropriate to men trained in physical sciences. The nature of some of the basic puzzles encountered by physical scientists in living matter has been examined. This educative process has given rise to focusing of attention on a number of points generally related to the problem of "early life." The role of metals which have the capacity for change of valence as active centers of enzymes and coenzymes, and the importance of trace elements as clues to biological processes, both currently and in the very distant past, are examples of the type of problem which has attracted interest and roused extended discussion. These studies of biological processes have brought the biophysics group into close contact with the men concerned with geochemistry, and the resulting discussions have outlined a new field of research relating to the early history of the earth.

While examining and formulating larger problems, the group has continued to make a considerable number of specific research contributions. The work during the report year has largely been concerned with metabolic and physiological studies using radioactive isotopes, and with studies of biological effects of radiation. Among the results of projects under way or reaching completion are the following:

Studies employing proteins labeled with radioactive iron (in collaboration with

Louis B. Flexner, of the Institution's Department of Embryology, and Gilbert J. Vosburgh, of the Department of Obstetrics, Johns Hopkins University and Hospital) have thrown new light on the turnover rate from the plasma of the guinea pig to the extravascular fluid. The observed rate was slow (0.3 to 1 per cent per minute) in comparison with that previously found for electrolytes. The substance employed was ferric betai-globulinate, produced by biological synthesis from inorganic radioactive iron. Another investigation was a determination of the turnover rate of chloride ion from plasma to extravascular fluid space. The value found was a rate of 60 per cent per minute, which, perhaps significantly, is the same as that observed earlier for sodium. The two results when taken together clearly cast doubt on the "pore theory" of capillary permeability.

A further study was a comparison of the permeability of *Escherichia coli* to sodium and potassium ions. It was found that the cellular membrane is completely permeable to both sodium and potassium and that the sodium ions within the water space in the cell are in equilibrium with those in the medium. This water space constitutes 75 per cent of the volume of the cells. There is no fixation of sodium in resting, growing, or highly metabolizing cells. Potassium ions can also diffuse freely across the membrane, but the picture of pure diffusion is complicated by metabolic processes which fix the potassium in a nondiffusible form.

The importance of potassium in biological processes has long been recognized. Potassium, sulphur, and phosphorus are the most important chemical constituents of the ash of most cells, each one contributing roughly 30 per cent of the total* Potassium is also a major constituent of fertilizers, since it is known to be necessary to plant nutrition. Whereas, however, the

roles of phosphorus and sulphur are well recognized, the actual biochemical function of potassium is not at all clear.

It is known, for example, that potassium, unlike sodium (which is chemically similar), is concentrated by cells, and that the potassium once taken up by the cell is not readily removed. Consequently, it has been inferred that the potassium is bound in some way to some component of the cell. Compounds or complexes with proteins, carbohydrates, and fats have been postulated, but none of these has ever been isolated or identified.

Experiments at the Department using radioactive potassium produced by the cyclotron have given some new light on this problem. It was found that the incorporation of one molecule of glucose by cells of *E. coli* caused the binding of two atoms of potassium. As the glucose was broken down further, the potassium was released. Other experiments indicated that the potassium was bound in the cell as potassium salts of the hexose phosphates which are the immediate products of glucose metabolism. This hypothesis was further supported by the observation that during the period when two potassium atoms were bound, one phosphate group was transferred to the glucose molecule. Thus, potassium apparently participates in the major process by which the cell obtains energy from its food supply.

Furthermore, it was possible to obtain new information concerning the metabolism of glucose by observing the uptake and loss of potassium. Some of the results could be interpreted to show that a large fraction of the glucose is consumed by direct oxidation rather than by the more familiar and better-understood phosphorylative mechanism. In a system as delicate and complex as a cell, any new method of observation is highly valuable, particularly when it does not interfere in any

way with the normal metabolism of the cell. Further work along these lines is in progress using potassium and rubidium, together with phosphorus and sulphur.

Another project under way is a study of the rate of uptake of radioactive fluoride in normal enamel. The work (in collaboration with Dr. F. A. Arnold, Jr., and Dr. R. C. Lichens, of the Dental Research Institute, National Institutes of Health) includes a study of the chemistry of fixation of topically applied fluorine. The biophysics group is especially in a position to be helpful in this project. The tracer fluorine employed cannot be produced in the nuclear reactor; a cyclotron is required.

Further study of P^{32} uptake in *Arbacia* eggs has provided additional knowledge regarding the chemical form of the phosphorus immediately after it enters the cell. Fertilized eggs were immersed in sea water containing very small amounts of phosphorus in the form of inorganic phosphate. On chemical analysis after a few minutes' exposure, the P^{32} in the *tggs* was found in at least three fractions: inorganic phosphorus, adenosine triphosphate, and hexose monophosphate.

Studies on the influence of the thyroid on calcium metabolism were continued by Dr. H. H. Darby, Research Associate of the Institution, who worked at the Department throughout the year and participated in many of the activities and discussions in biophysics. The influence of vitamin D on the damage done to the thyroid by thiouracil was checked. The work reported a year ago was completely confirmed.

An attempt was made to determine the part of the thyroid on which vitamin D does its work, and whether the parathyroid is involved. Many researchers believe that the parathyroid is involved in calcium metabolism. In none of the animals to which the thiouracil was fed was there any

histological change in the parathyroid, but an immense change in the thyroid was found. When the vitamin D was fed, no histological change was observable in the parathyroid, whereas large changes were seen in the thyroid itself. The central areas of the thyroid seem to respond better to the vitamin D than do the peripheral areas, and there appears to be a difference between the anterior and the posterior part of the thyroid. Co-operative research with Dr. Pauline Beery Mack, of Pennsylvania State College, on X-ray density measurements of the epiphysis has led to interest in this type of work at the National Institutes of Health, and, to a lesser degree, at the Naval Medical Research Institute.

The essential mineral elements for the proper functioning of a biological system have in the past been thought to be the more abundant substances, such as calcium, sodium, potassium, iron. At the same time, the literature has many statements that a particular element has been found necessary in extremely small amounts for some particular animal. Animal husbandry and research have traced certain disease conditions to the shortage of such trace elements and have cured these conditions by the addition of extremely small amounts of the element to the animal's food. Many years ago in New Zealand, Australia, and Scotland a disease of sheep was shown definitely to occur when there was a shortage of cobalt. It was cured by the addition of cobalt sulphate to the pastures on which these sheep were fed. No reason was given for the necessity for this cobalt.

Recently in the continuation of the search for more B vitamins (the water-soluble vitamins), vitamin B13 was discovered and in the chemical breakdown was shown to contain cobalt. It was separately found that cobalt was required by the bacteria in the sheep's rumen, not

primarily by the sheep. Another significant finding at this time was that this active biological material B12, the pernicious anemia factor which had previously been thought to be found only in liver, was also found in the excreta of cows and fowls. A combination of the work in Scotland and Australia on cobalt and the B12 work in the United States and England now was possible, especially since radioactive cobalt was available from the Department's cyclotron. With this in mind, sheep were fed radioactive cobalt, and a collection was made of the excreta. This was then analyzed both for the cobalt and for B₁₂ using microbiological tests. The cobalt was found in the excreta in more than one chemically bound form, but one of the forms was that of B12.

The B12 molecule was then traced to its synthesis in the bacteria which occur in the rumen or first stomach of the sheep. It is interesting here to note that the cow is also a ruminant and that the fowl has a double type of stomach in the gizzard and the crop. Any two-compartmented stomach like this will give a much longer period for food to be acted upon by bacteria than will a single stomach. In these animals, therefore, the bacteria are playing a significant role in the production of necessary nutrients for the host animal. Other bacteria, including *E. coli*, were shown to take up cobalt and synthesize it into B12.

The fundamental characteristic that first attracted investigators to this substance was that it seemed to be necessary for the building of proteins. In fact, the first name given to it by the English workers was "animal protein factor." Since there is necessarily an immense amount of protein building of all types in the developing embryo, it was of interest to follow a B12 molecule from the digestive tract of a hen into the egg. Radioactive cobalt was given hens by mouth and was found to be dc-

posited in the yolk, in the albumen, and to a very much less extent in the shell and its inner lining. In this manner it was possible to show that such a trace element as cobalt, which was fed at the level of a few micrograms per day, appears in the egg and plays a significant role at a level of a hundredth of a microgram per gram. The whole field of biological significance of trace elements is of challenging interest. Especially important are those elements that are bivalent and can help in forming intermediary compounds, even if these are highly transitory and have so far escaped discovery in the chemistry of the test tube, which differs markedly from the chemistry of the body.

A series of studies of biological effects of radiation has been carried out by Dr. W. R. Duryee, of the National Cancer Institute, working at this laboratory. Through observations on transparent living amphibian ovarian eggs, he has been able to develop new and objective criteria of radiation damage. These involve chromosomal fragmentation, nuclear damage, and changes in nature of colloidal suspensions within the nuclei. Using these criteria, he has been able to show that by subjecting amphibia to temperatures of 6° C, appearance of radiation damage can be postponed for as much as two weeks. Using a micro-injection technique, he could render normal cells abnormal by injection of radiated cytoplasm, whereas injection of nonirradiated cytoplasm did not produce nuclear damage.

The lethal effect of ultraviolet radiation on bacteria is well known, but the mechanism is not at all clear. During the past year it was found that after irradiation the

bacteria (*E. coli*) were highly sensitive to many factors in the environment which had no effect on normal bacteria. Under certain conditions the bacteria would recover from the effects of the radiation and remain viable. A study of these conditions indicated that the enzyme systems had been thrown out of balance. It is probable that this unbalance was caused by cellular poisons produced by photochemical reactions within the cell.

In December 1948 it became clear that a number of young nuclear physicists had suffered radiation injuries leading to lens cataracts. In view of the widespread interest in this tragic occurrence, the Department felt it desirable to foster a survey and assessment of the situation. It seemed particularly worth while to conduct the investigation in such a way as to avoid duplicating efforts and thus to prevent unnecessary trouble for the men involved.

Acting in collaboration with Professor P. Gerald Kruger, of the University of Illinois, and with the encouragement and support of the Division of Medical Sciences of the National Research Council, survey questionnaires were sent to the various high-voltage laboratories of the country. A three-day session was held in Washington, in January 1949, which was attended by all individuals known to be afflicted. This was followed by ophthalmological examination at Johns Hopkins. The Department subsequently prepared a report of the findings of the meeting for the National Research Council, which was distributed to all high-voltage laboratories. It is hoped that this effort may aid in the prevention of future injuries.

OPERATIONS AND STAFF

CO-OPERATIVE WORK OF THE DEPARTMENT

Co-operation has been continued with individuals and organizations in this country and abroad in accordance with the Institution's policy.

Research work on the earth's crust and cosmic rays has continued under Navy contracts N7onr-290 and Nyonr-^o,, respectively, with advantage to the Government as well as to the Institution. These contracts provide for co-operative activity and the loan of equipment, but conform to the policy of no direct cash subsidy to the Institution for added or existing staff or facilities. A new task order under the first contract provides for one member of the staff to engage in co-operative research with the Navy for a limited period on atmospheric-electric problems. Some members of the staff have continued to assist various offices of the Government as consultants; one has been on full-time leave of absence for the entire year, and another since April 1, 1949.

Although the observatories at Huancayo, Peru, and Watheroo, Western Australia, have been owned and operated by the Peruvian and Australian governments since July 1, 1947, the Department retains a lively interest in their activities and has been privileged to co-operate in matters of program policy and operational detail. Messrs. Casaverde and Fernandez of the Huancayo staff were in residence in Washington as Fellows of the Institution for six months each, to receive training at our laboratory and others in the Washington area.

Sounding balloons were supplied to Professor G. Bernardini in Italy for research work there.

The Department has continued to have the advantage of collaboration with Dr. Louis B. Flexner, of the Department of

Embryology, in the biophysics program, and with representatives of the National Institutes of Health, National Bureau of Standards, Applied Physics Laboratory of the Johns Hopkins University, Brookhaven National Laboratory, Wilmer Institute, Department of Obstetrics of the Johns Hopkins University, and Catholic, Georgetown, George Washington, Howard, and Tulane universities.

The biophysics group has continued its responsibility for the operation of the cyclotron and the production of radioactive isotopes, which have been distributed without charge to some forty different groups in this country and abroad.

Dr. M. S. Vallarta, of Mexico, has continued to collaborate with the Department in the investigation of sudden increases in cosmic radiation associated with solar flares. Dr. John R. von⁴ Neumann, of the Institute for Advanced Study, has also assisted in this work. In addition, four observatories, previously mentioned, have continued to operate the Compton-Bennett cosmic-ray meters.

Joint experiments in the seismic program of the Department and the Geophysical Laboratory were carried out in close collaboration with Columbia University and New Mexico School of Mines, as well as with Navy and Army groups. Again grateful acknowledgment is made to the Navy (Office of Naval Research and Bureau of Ordnance) for under-water explosions scheduled for the convenience of our observers, and to the Tennessee Valley Authority for the unusual opportunity offered by the three large blasts at the South Holston Dam site.

We are greatly indebted to the U. S. Geological Survey for valuable assistance in selection of localities and sites for the rock magnetism studies.

PUBLICATIONS

In addition to the scientific papers appearing in current literature, two volumes of the Researches of the Department have been published, containing results of cosmic-ray and earth-current observations; they are listed under "Major publications" in the bibliography.

It is intended to publish, as Publications of the Carnegie Institution of Washington, the magnetic results from the Watheroo and Huancayo observatories (which were transferred to the respective local government agencies on July 1, 1947) for the years 1945 to 1947, the results through 1945 having already been published. The preparation of these two final volumes is now under way.

ADMINISTRATION AND OPERATION

A special experimental license W3XAU was obtained for observations on meteors.

Five panel trucks are now on loan from the Navy for use in the seismic investigations.

A modest quantity of surplus electrical material was obtained through the District of Columbia Educational Agency for Surplus Property.

The *Journal of Geophysical Research*, edited by the Director, with the help of Mr. Walter E. Scott, received support from the Institution. This journal is the continuation of the *Journal of Terrestrial Magnetism and Atmospheric Electricity*, edited for many years by Dr. John A. Fleming.

The Department continues to have the advantage of visits from guest investigators for varying periods. These are included in the appended list, which also shows the regular members of the staff, most of whom were employed for the entire report year.

SEMINARS

The recent work of Urey, Brown, and Libby has clearly demonstrated the rich discoveries that can be made by applying modern physical techniques to geophysics. Several staff members of the Department visualized the possibility that a survey of geological problems on the basis of familiarity with new techniques of physics and chemistry would bring to light new and significant problems and opportunities. It was particularly obvious that a great gap in man's knowledge is the period between the origin of the earth and the Cambrian. Accordingly, with the collaboration of the Geological Survey and the Geophysical Laboratory, a seminar was organized which had for its principal theme "Milestones in the Pre-Cambrian." The subjects discussed, during March 4 to May 13, 1949, were: Harrison Brown, Isotopic analysis of the universe; R. B. Roberts, Origin of the earth; G. Gamow, Origin of the universe; W. D. Urry, Radioactivity and the age of the earth; W. D. Urry, The radioactivity of potassium; H. E. Tatel, Radioactivity and the earth's cooling; M. A. Tuve, The mass spectrometer; H. C. Urey, Separation of isotopes by chemical processes; P. H. Abelson, Separation of isotopes by physicochemical methods; H. C. Urey, Temperature measurements on fossil shells by use of isotopic analysis; D. B. Cowie, Dating in the earth's immediate past by use of C^{14} ; E. Ingerson, The Pre-Cambrian; E. Ingerson, Isotopic separation by geologic processes; R. B. Roberts, Creation of the earth's atmosphere; W. W. Rubcy, Origin of the ocean; H. H. Darby, The origin of life (as formulated by Gparin); M. A. Tuve, An alternative mode for the origin of life; L. H. Adams, The nature of the earth and its origin; N. L. Bowen, Formation of the earth's crust; W. W. Rubcy, Mountain building; W. H.

Bradley, The problem of the ocean basins.

Some of the interesting potential research problems arising out of the talks and discussions were: (1) The possibility of detecting evidence of life earlier than the fossils by studies of trace-element patterns in sedimentary rocks or Pre-Cambrian graphite. (2) The possibility of determining a geological level corresponding to the time of appearance of living matter on earth. Since the oxygen of the atmosphere is derived from biological activity, a drastic change in the earth's atmosphere must have occurred at that time. Simultaneously, isotopic abundances must have shifted slightly. (3) The possibility of determining whether the granites are of sedimentary or of magma origin by observations of the trace-element patterns in such rocks. (4) Fractionation of hydrogen isotopes as a measure of hydrogen escape from the atmosphere.

During the period September 24 to De-

cember 20, 1948, Dr. V. C. A. Ferraro, of England, gave a series of lectures on "Geomagnetic storms and the upper atmosphere."

LECTURES GIVEN BY VISITORS

September 3, 1948, "The cause of the earth's magnetism," by E. C. Bullard.

September 24, 1948, "Gravity and the isostatic structure of the earth," by W. Heiskanen.

October 20, 1948, "A mechanism to explain increases in cosmic-ray intensity during some solar flares," by M. S. Vallarta.

December 21, 1948, "Cosmic-ray investigations," by Marcel Schein.

February 23, 1949, "Cosmic-ray investigations in Italy," by G. Bernardini.

March 4, 1949, "Isotopic constitution of meteors," by Harrison Brown.

May 19, 1949, "Work with C¹⁴," by William F. Libby.

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STAFF AND ORGANIZATION

SCIENTIFIC STAFF

Staff Members:

- Geophysics:* L. V. Berkner, S. E. Forbush, O. H. Gish (retired September 30, 1948), E. A. Johnson (on leave of absence for governmental research), W. J. Rooney, H. E. Tatel, O. W. Torreson, M. A. Tuve, E. H. Vestine, G. R. Wait, H. W. Wells.
- Laboratory and Biophysics:* P. H. Abelson, D. B. Cowie, N. P. Heydenburg, R. B. Roberts, M. A. Tuve.
- Guests, Associates, Fellows, and Visiting Investigators:* P. Bhattacharya, India; E. O. Bowles, American University; M. Casaverde, Geophysical Institute of Huancayo, Huancayo, Peru; S.K. Chakrabarty, India; H. H. Darby; Miss E. Dollman, Brookhaven National Laboratory; W. R. Duryee, National Institutes of Health; G. Fernandez, Geophysical Institute of Huancayo, Huancayo, Peru; V. C. A. Ferraro, University of the South West, Exeter, England; P. S. Gill, Tata Institute of Fundamental Research, Bombay, India; J. W. Graham, Johns Hopkins University; E. J. Habib, Catholic University; Miss

E. Hill, National Cancer Institute; R. Hossfeld, Catholic University; D. R. Inglis, Johns Hopkins University; F. Irreverre, Experimental Biology and Medicine Institute, U. S. Public Health Service; W. C. Knox, National Institutes of Health; T. Murphy, University College, Dublin, Eire; J. N. Nanda, India; A. T. Ness, U. S. Public Health Service; R. Nieset, Tulane University; W. D. Parkinson; Mrs. I. Z. Roberts, National Cancer Institute; F. L. Talbott, Catholic University; M. S. Vallarta, Instituto de Fisica, Universidad de México, D. F., and Comisión Impulsora y Coordinadora de la Investigación Científica, México, D. F.; P. Wang, National Bureau of Standards; W. D. Whitehead, Jr., University of Virginia; F. W. Wood, Australia.

OPERATING STAFF

- Administrative:* M. B. Smithy, W. F. Steiner.
- Office and Clerical:* Mrs. J. H. Campbell, J. J. Capello, W. N. Dove, H. D. Harradon, W. C. Hendrix, Miss H. E. Russell, A. D. Singer.

Instrument Shop: B. J. Haase, L. A. Horton,
J. G. Lorz.

*Research Assistants, Laboratory Assistants, and
Technicians:* Miss E. Aldous, S. J. Buynitzky,
J. B. Doak, P. A. Johnson, C. J. Ksanda, C. A.
Little, Jr., P. F. Michelsen, P. L. Moats, A. E.
Moebs,* W. C. Parkinson, R. W. Reuschlein,
Miss M. Sands, W. E. Scott.

* Resigned.

Computers: Miss E. Balsam, Miss I. Lange.

Maintenance: C. Balsam, C. W. Burger,* C.
Domton, S. W. Malvin (retired December 31,
1948), E. Quade, M. A. Quade.

Part-Time and Temporary Employees: There
were 18 part-time and temporary employees
engaged during the year, usually for short
periods, to assist in the office and laboratory
work.

SPECIAL PROJECTS: TERRESTRIAL SCIENCES

FRANK T. GUCKER, JR., Indiana University, Bloomington, Indiana. *Studies of specific heats of aqueous solutions up to high temperatures.* (For previous report see Year Book No. 46.)

This is a continuation of the study of specific heats of salt solutions up to 85° C, carried out in collaboration with Dr. Frank W. Lamb, during which there was found a maximum in the apparent molal heat capacities of three typical uni-univalent electrolytes at about 60° C., and a decrease at higher temperatures. This is exactly opposite to the increase above 65° predicted by F. Zwicky, on the basis of the theory of electrostriction and of Bridgman's P-V-T data for water, and shows that either the theory or the data require revision. An extension of the experiments up to the critical point (218 atmospheres and 375° C. for pure water) seemed desirable in order to supply data in a region where none are available, and where a theoretical interpretation may be simpler, since most of the anomalous properties of water become less pronounced at higher temperatures. The experiments require a new type of calorimeter, suitable for work at high temperatures and pressures. This year, with the collaboration of Mr. Jean M. Christens, a suitable bomb calorimetric apparatus has been designed and nearly completed, and the extensive auxiliary equipment necessary for these measurements has been assembled.

A differential system was chosen, with water as the reference substance in one of the twin calorimeters, and solution in the other. The heat capacity of the solution is measured relative to that of water, which has been studied carefully up to the critical point by Osborne, Stimson, and Ginnings at the National Bureau of Standards. Each calorimeter is provided with a single small

tube connected to the bottom, through which it can be evacuated and water or solution can be introduced. In order to avoid the formation of any vapor during an experiment, with consequent absorption of a large amount of heat, a constant hydrostatic pressure of about 220 atmospheres is maintained, measured by means of an accurate dead-weight gauge. A strong, thin calorimeter is obtained by constructing the shell of Inconel, in two nearly hemispherical halves, held together by means of a threaded ring at the center. A platinum lining prevents corrosion by the salt solutions under the severe experimental conditions. An electrical immersion heater with metal fins distributes the heat inside the calorimeter without the use of a stirrer, and a thick plating of silver on the outside of each calorimeter helps to equalize the surface temperature and to reduce radiation. The electrical heating is regulated to raise the temperature of the two calorimeters at practically the same rate, and the difference in temperature is measured by means of a 16-junction thermel connected to points distributed over the surface of each calorimeter.

The calorimeters are held within a steel case which can be evacuated to about 10^{-4} mm. of mercury to prevent thermal conduction. Within the case is a heavy copper adiabatic jacket surrounding the calorimeters, provided with electrical heaters to maintain the desired uniform temperature, measured by multiple thermels between it and the calorimeters and also by a platinum resistance thermometer. A series of thin, highly polished aluminum shields re-

duces the radiation between the calorimeters and the copper jacket, and a second series of these shields reduces the radiation to the outer case, which is near room temperature. The large evacuated space reduces the hazard from failure of the calorimeters, and a second steel case surrounds the whole apparatus as a further precaution.

The apparatus, with its bombs of known volume, dead-weight pressure gauge, and platinum resistance thermometer, may be used to measure the compressibility, ther-

mal expansibility, and vapor pressure of solutions, as well as their specific heats. The auxiliary apparatus also would be useful in the measurement of the dielectric constant of water over a wide range of pressure and temperature. It is hoped that such measurements can be carried out, since the pressure and temperature coefficients of the dielectric constant are needed to calculate the limiting slopes of the apparent molal heat capacities, volumes, compressibilities, and expansibilities of solutions.

DIVISION OF PLANT BIOLOGY

Stanford, California

C. STACY FRENCH, *Director*

The attack on photosynthesis has been concentrated primarily upon those parts of the initial steps in the photosynthetic process which involve the photochemical splitting of water molecules, rather than upon the ensuing reactions by which carbon dioxide is reduced. Thus the general program of one group of investigators has centered about the absorption of light by plant pigments, the possible transfer of the absorbed energy from one pigment to another, and the correlation of these events with the ultimate chemical changes produced in living plants.

A broad survey, extending over a period of years, of the specific chloroplast pigments which are found in the various groups of photosynthetic plants has just been completed. From this work it has become evident that the pigment complexes characteristic of the main plant families were developed early in the course of evolution, and have remained constant in spite of the great changes in the external form of plants.

Experiments with seedlings initially grown in the dark have shown that during their subsequent exposure to light the formation of chlorophyll is much more rapid than had previously been realized. Even with moderately weak light an appreciable fraction of the protochlorophyll is converted to chlorophyll *a* in a few seconds. From measurements of the effectiveness of different wave lengths of light on this transformation in corn and bean seedlings, it was found that only the light which is absorbed by protochlorophyll, and not that absorbed by any other pigments present, can lead to chlorophyll formation.

It is now considered likely that chlorophyll *b*, which develops later than chlorophyll *a*, does not originate from chlorophyll *a*. A study of the correlation of pigment formation with the development of other parts of the photosynthetic apparatus has been started.

The first detectable chemical effect resulting from absorption of light by the pigments of the photosynthetic apparatus of plants is the splitting of water, which results in oxygen evolution and the reduction of some unidentified intermediate. For the experimental investigation of the reaction, it is possible to replace the unknown natural intermediate by certain common chemicals, such as a reducible dye. By ejecting a suspension of chloroplasts at high pressure through a fine needle valve, one obtains colloidal suspensions of material the particle size of which is much smaller than that of the so-called grana. This material is in a form suitable for chemical studies of the water-splitting step of photosynthesis. The activity of such a preparation is about one-third that of the original chloroplast suspension. Nearly all the original activity can be restored, however, by treatments which cause a partial reaggregation of the individual colloidal particles. Thus there are at hand some means for correlating morphology and function at a size level between the molecular and the microscopically visible ranges.

The suggestion that controlled cultures of algae may become a valuable source of foodstuffs has received rather surprising and widespread attention. This interest in a process deviating from traditional meth-

ods of food production is no doubt due to the compelling nature of the present problems of world food supply and the concern which has been aroused by popular discussion of the social factors involved in these problems.

Some of the more important constituents of the products formed by *Chlorella* have been established: of the carbohydrates, starch and cane sugar have been isolated; of the lipids, the fatty acids have been identified; and it has been found that all the ten essential amino acids are present. From an industrial viewpoint, however, a great deal remains to be learned regarding the practicability of producing *Chlorella* on a large scale and the use that may be made of its products. These problems are essentially of an engineering nature. For their further investigation an arrangement has been effected between the Carnegie Institution of Washington and the Research Corporation. The latter has, in turn, given financial support to the Stanford Research Institute to make an intensive study of the process and the products on a pre-pilot plant scale, with the cooperation of the Carnegie Institution of Washington.

The records of the transplant and crossing experiments on *Potentilla glandulosa* discussed in last year's report have been further analyzed. The genetic linkage between various morphological characters and certain physiological variations upon which the natural distribution depends has been thus established. Species which are widely distributed in various environments are able to fit these diverse conditions because such species consist of many physiologically and genetically distinct races. Each of these races fits a different set of environmental conditions. A better understanding of evolution should be attained by the study of the **genic-physiological** relationships of suitable plant groups.

The effective prosecution of such experiments is dependent upon the utilization of species which show a wide range of form and adaptation to different environments and which grow rapidly. The suitability of the genus *Mimulus* for such work is being tested by transplanting, by crossing, and by morphological observation.

The success of the Edinburgh plantings of a number of the strains of range-grass hybrids developed by the Division has led to an increase in the number of strains tested there by the Scottish Society for Research in Plant Breeding and to the undertaking of many similar plantings to supplement those made by the Soil Conservation Service of the U. S. Department of Agriculture. Thus co-operative experiments on forty-six strains have been arranged with a number of widely scattered laboratories. This has been done to test the climatic adaptability of *Poa* hybrids over a wide range of climatic conditions such as are found in the state of Washington, southern California, North Carolina, Scotland, Wales, Holland, Denmark, Norway, and Sweden. No universally outstanding strain has been produced, but several of the selected strains show excellent performance in a number of environments. Under different environments different strains are observed to give superior growth. Fortunately, many of these improved strains breed true from seed, thus being easy to distribute. A detailed cytological investigation is being conducted on the mechanism of seed and of pollen formation and on the way in which these processes are influenced by the environment. Some of these grass hybrids of particular interest have been selected and are being grown in preparation for a quantitative experiment on their growth response in controlled laboratory environments.

The taxonomic treatment of western

North American Poas rounds out the comprehensive gathering of knowledge of the distribution, cytology, genetics, physiology, and evolution of this economically important genus of plants.

In close co-operation with the staff, a number of visiting scientists have been

carrying out related investigations with different species of plants. By drawing upon their experiences and new materials, the Division's program, which traces the complex evolutionary patterns by which plants evolve, has been both broadened and strengthened.

PERSONNEL

BIOCHEMICAL INVESTIGATIONS

Staff: C. Stacy French, *Director*, Harold W. Milner, James H. C. Smith, Herman A. Spoehr, Harold H. Strain
Fellows: Violet M. Koski, Fergus D. H. Macdowall
Research Assistants: Marie L. G. Koenig, Nancy S. Lawrence, George H. Towner
Mechanic: Frank Schuster

Systematics and Genetics, Royal Agricultural College, Uppsala, Sweden, National Swedish Research Fellow
Stanford graduate students associated with the Division: Robert K. Vickery, Jr., George H. Ward
Research Assistants: Robert W. Ayres, Helen K. Sharsmith, Mary H. Wagner
Gardener: Wesley B. Justice

EXPERIMENTAL TAXONOMY

Staff: Jens C. Clausen, Paul Grun, William M. Hiesey, David D. Keck
Fellow: Herbert G. Baker, Lecturer in Botany, Department of Botany, University of Leeds, England
Guest Investigators: Pierre Dansereau, Directeur du Service de Biogéographie, University of Montreal, Guggenheim Fellow; Hedda Nordenskiöld, Institute of Plant

RESEARCH ASSOCIATE

Ralph W. Chaney, Professor of Paleontology, University of California, Berkeley, California

SECRETARY

Wilbur A. Pestell

CUSTODIANS

William E. Larson, Jr., James W. Groshong

BIOCHEMICAL INVESTIGATIONS

CHLOROPLAST PIGMENTS

HAROLD H. STRAIN

Pigments and evolution. For millions of years, life on the earth has required a continuous supply of organic matter. Throughout the long geologic ages, most of this organic material has been produced by the photosynthetic activity of green plants. In all plants, this remarkable photochemical action hinges upon the utilization of sunlight by the pigments contained within the microscopic chloroplasts of the plant cells. Through this reaction, the chloroplast pigments harness the atomic

fires of the sun for the production of oxygen and organic matter on the earth. In this way, the chloroplast pigments form an indispensable part of the complex organizational machinery required for the maintenance of virtually all living things.

In the search for clues to the evolution of the photosynthetic apparatus and to the evolutionary development of plants and of animals, much time has been devoted to determination of the pigments of present-day plants. Plants belonging to most of the major botanical families have now been obtained from various geographical habi-

tats, and their chloroplast pigments have been isolated and compared by the sensitive chromatographic adsorption method. These plants were collected from such diverse environments as Hawaii, the high Sierra Nevada of California, the western North American desert, the coastal regions of California, mountain lakes and streams, and the sea near California, Hawaii, and North Carolina. Some of the plants were native to arctic regions, others to tropic and to temperate regions. Some of the cultivated plants were native to such remote places as Japan, China, Malaya, India, the Mediterranean region, Africa, Madagascar, the Pacific islands, and North and South America. Most of the marine plants were obtained from the intertidal zone, but some of them were dredged up from depths of 50 to 100 feet.

The pigments of all these plants showed no significant variation with habitat. There was, however, a unique correlation between the occurrence of certain pigments and the taxonomic families to which the plants belong. In conformity with earlier results, all the higher plants yielded the same principal pigments, usually in about the same proportions. In a few organisms, the proportions of the several xanthophylls varied significantly. Whereas lutein was usually the principal xanthophyll, violaxanthin occasionally predominated, and in one plant (*Fremontia californica*) zeaxanthin was at least equal in amount to lutein.

Among a large number of algae, the presence of certain chlorophyll and carotenoid pigments was also found to be characteristic of the principal taxonomic groups. As there is some question concerning the classification of certain algae, knowledge of the pigments provides another guide for establishment of their relationship. For example, the fresh-water *Vaucheria* contains pigments also found in

the Heterokontae, the yellow-green algae, of which some five unicellular species grown in pure cultures have now been examined. The similar fresh-water *Dichotomosiphon* and the marine *Derbesia* contain pigments characteristic of the siphonalean green algae of the order Chlorophyceae. Such observations on the functional pigments of the plant cells provide significant links between the fields of chemistry, physiology, taxonomy, and phylogeny.

There has been wide acceptance of the view that the so-called complementary pigments of algae have developed in response to the spectral quality of the incident light. Algae growing in deep water and receiving a preponderance of blue-green light are presumed to have developed pigments which complement the weak absorption of the chlorophyll for green light. But in the algae dredged up from deep water, the pigments were found to be characteristic of the plant group; they were not determined by the conditions under which the plants grew. If there has been any effect of light on the development of the pigment systems, the change must have occurred before evolution of the plants known today.

All these diverse facts indicate that the pigment systems characteristic of the major botanical families were established early in the evolution of the plant world. Although plants have varied enormously in form and in adaptation to different environments, the pigment system shows no corresponding variation. Even though the pigment system is remarkably unstable in killed plant material, in living organisms it is one of the most constant physiological systems ever developed.

As man and animals depend upon organic substances of particular molecular structure that stem from photosynthesis,

the pigments can be regarded as the mold in which the molecular building bricks of the organic world are formed. At this molecular level, pigments may possibly establish the pattern of asymmetry that permeates the entire organic world, and thus they may set a limit to the variability of plants as well as of animals.

One may well ask the question, Why is the system of chloroplast pigments subject to so little variation? A partial answer lies in the fact that variations of the pigment system induced by injury or by mutation usually block the photosynthetic process. Consequently, plants thus changed will be eliminated rapidly from the native populations, because they will have lost their capacity for self-nourishment and independent existence. If this intimate connection between the pigment system and the photosynthetic process is the true explanation for the constancy of the pigments, then this dependence, too, must have been established millions of years ago, before the establishment of the plant groups that are known today. But there may be other causes for the constancy of the pigment system, and these are being sought in further studies of the pigments and their reactions.

Enzymatic oxidation of the chlorophylls. Through the use of adsorption methods, a rapid enzymatic oxidation of the chlorophylls to other green pigments has been discovered. This oxidation reaction varies with the conditions under which the leaves are killed, and with the plant material. Of the plants tested, it was most rapid in the young leaves of barley and of potatoes. It occurs rapidly when leaves of these plants are permitted to stand with organic solvents in the presence of oxygen. It does not occur in the absence of oxygen or in leaves that have been exposed to heat.

The products of the enzymatic oxidation of the chlorophylls are spectrally similar to the unaltered pigments, but are somewhat more adsorbed than the chlorophylls in columns of powdered sugar, so that, with experience, they are easily isolated. Each chlorophyll yields a single oxidation product, and in this respect the enzymatic oxidation differs from the spontaneous oxidation of the chlorophylls in methanol. This oxidation in methanol (known as allomerization) yields several oxidation products, one of which is identical with the product of the enzymatic oxidation. The enzymatic oxidation takes place in the presence of small amounts of water; the allomerization in methanol, by contrast, is inhibited by small amounts of water.

Chlorophylls themselves yield interconvertible isomers when the pigments are heated. The oxidation products do not yield the analogous isomers. Apparently the oxidation of the chlorophylls alters that portion of the pigment molecule involved in the isomerization reaction.

Improvements of the chromatographic adsorption method. In spite of its remarkable sensitivity and its wide applicability to chemical separations, the chromatographic adsorption method has not found wide application in industry. One of the limitations to separations by this method on an industrial scale is the discontinuous nature of the procedure. Now, however, a modification of the column has been devised so that certain adsorbed substances can be forced to migrate horizontally as well as downward. As a result, the flow of liquid through the column can be made continuous while the components of the mixture are collected in the respective portions of the percolate. Experiments to test applications of this continuous procedure are under way.

THE PHOTOCHEMICAL ACTIVITY OF
DISINTEGRATED CHLOROPLASTSH. W. MILNER, N. S. LAWRENCE,
M. L. G. KOENIG, AND C. S. FRENCH

The studies on the photochemical splitting of water by chloroplast material have been continued with much the same ultimate objectives as were described in the report last year. Progress in this investigation has been made in several lines, one of the most interesting of which has been the finding of certain large increases in the activity of the material upon reaggregation of very finely divided colloidal solutions of chloroplast material.

The dispersion of chloroplasts into extremely small particles is now accomplished much more simply and effectively with a new device. In last year's report the use of supersonic vibration for this purpose was described. It now appears that more effective results may be obtained by extruding the suspension of chloroplasts in water containing 15 per cent methanol through a fine needle valve under high pressure. The equipment for this consists simply of a round steel bar 3 inches in diameter with a x -inch round hole 4 inches long in its center. Into this is fitted a steel plunger with a rubber and a leather washer. The bottom of the hole in the steel bar is tapped to receive a steel needle valve. In use, the whole assembly is placed in a hydraulic press. When the pressure on the liquid rises to 20,000 pounds per square inch, the needle valve is opened slightly and, with the pressure held at this level, is adjusted to maintain a flow of about 5 cc. of liquid per minute. Under these conditions it is possible to obtain about three-fourths of the chloroplast material in a state of fine dispersion which will withstand centrifuging for one hour at 12,000 times the force of gravity without

sedimenting. By this treatment the activity of the chloroplast material is reduced to about one-third of its initial value. These preparations appreciably exceed those obtained by any other method so far investigated in fineness of particle size and in total yield, and they are prepared with greater ease.

The stabilization of such preparations, which has long been the greatest technical difficulty, has been considerably improved through the discovery of the stabilizing effect of 15 per cent methanol in the aqueous suspension medium. By the use of this concentration of methanol it is possible to retain half of the original activity of these preparations for a week by storing the material at -5° C. Thus a single preparation can be used for experiments extending over several days. Equimolar concentrations of ethanol are equally satisfactory for stabilization at very low temperatures, but are inferior to methanol at higher temperatures. The stabilizing influence of methanol was found during an attempt to use it as a means of fractionally precipitating the proteins in this mixture. Even in concentrations as high as 95 per cent and in the absence of salts, methanol or ethanol does not cause the formation of a precipitate except after standing for several hours at room temperature.

In solutions with 15 per cent methanol, however, the addition of small amounts of salts causes precipitation of chloroplast material. In water solutions without methanol a higher salt concentration is required to produce a precipitate. The appearance of this precipitate at salt concentrations of 0.1 M or lower is accompanied by a great increase in activity under illumination, sometimes by as much as two or three times, above that of a similar suspension without the salt. This activity is never

greater, however, than that of the intact chloroplasts. It appears that the aggregation caused by the salt takes place in such a way that several active centers catalyzing the reaction are brought closer together.

This activation differs from the chloride effect described in the work of other laboratories in that the new effect depends upon a reaggregation of the particles. Furthermore, other salts such as sulfates give even greater effects than do chlorides. This activation connected with precipitate formation is produced by a treatment of the material with salt at relatively high concentrations in aqueous methanol, and is not found when traces of salts are added to the reaction mixture, as in the case of the chloride effect.

This activation phenomenon, which has caused considerable difficulty in the assay of fractions obtained by salt precipitation, has, however, proved to be of considerable interest for its own sake. This effect may be related to the arrangement of the lipids present in the material. The participation of the lipids became evident when it was found that extracting the dispersion of chloroplast material with petroleum ether abolished or greatly reduced the salt activation effect. This extraction removes about 2 or 3 per cent of the total lipid which is present. After the petroleum ether extraction, the capacity of the dispersions for activation by salt may be re-established by treating the material with a small amount of ethyl ether, then evacuating to remove the ethyl ether completely.

One difficulty in separating the active components from the inactive substances by fractional precipitation has been inability to obtain complete redispersion of the precipitates. This also prevents comparable activity measurements on the precipitated and dispersed material. The use

of detergents in combination with adsorption techniques offers some hope of overcoming these difficulties.

THE OXIDATION-REDUCTION PROPERTIES OF CHLOROPLASTS

F. D. H. MACDOWALL

The photosynthetic reaction is now considered to be composed of two main steps: the splitting of water, which results in the evolution of oxygen and the formation of a reducing substance; and the reduction of carbon dioxide by the substance formed in the first step. Illuminated chloroplasts, even though removed from living cells, cause the reduction of a number of substances but not the reduction of carbon dioxide. A greater negative electric potential is required for the reduction of carbon dioxide than for that of the substances which are reduced by isolated chloroplasts. It was therefore of interest to find out what reducing power, as expressed in terms of electric potential, may be reached in suspensions of illuminated chloroplasts.

Measurements were made of the potentials of bright platinum electrodes immersed in suspensions of chloroplast material containing various added reducible dyes. Illuminated suspensions always showed a lower potential than nonilluminated ones. The lowest observed potential, referred to the standard hydrogen electrode, was -0.25 volt. It was found that the potential of such suspensions was lower in freshly prepared material than in that which had stood for a short time. It thus appears that chloroplasts in intact leaves may be able to produce an appreciably greater negative potential than that attained by isolated chloroplasts, perhaps even a potential effective for the reduction of carbon dioxide.

DEVELOPMENT OF THE MECHANISM FOR THE
EVOLUTION OF OXYGEN

JAMES H. C. SMITH

When leaves grown in the dark are illuminated, they develop the ability to liberate oxygen from carbon dioxide. We do not now know at which stage during the course of illumination the ability to liberate oxygen originates, nor at which stages other parts of the photosynthetic processes have their beginnings.

One of the first observable changes connected with the development of the photosynthetic apparatus during the illumination of dark-grown seedlings is the conversion of protochlorophyll to chlorophyll *a*. According to current concepts of the structures of these two compounds, this conversion is a hydrogenation of protochlorophyll. It seemed possible that the hydrogenation of protochlorophyll might be involved in the splitting of water, which at the same time would result in the evolution of oxygen. Therefore, the first experiment performed to correlate the events that lead to the development of the complete mechanism for photosynthesis was a determination of whether oxygen is evolved during the transformation of protochlorophyll to chlorophyll *a*.

Two very sensitive methods were used to test for the evolution of oxygen during this photochemical transformation, namely, the emission of light by luminescent bacteria, and the quenching of the phosphorescence of tryptaflavine adsorbed on silica gel. Under the conditions of the tests, the evolution of oxygen was not detected by either method. From these experiments there is no evidence that the transformation of protochlorophyll to chlorophyll *a* produces oxygen. Furthermore, etiolated leaves seem to be incapable of evolving even traces of oxygen immediately upon being illuminated.

Green leaves and etiolated leaves have also been compared with respect to their ability to liberate oxygen after being placed in a vacuum in the dark and then illuminated. Under these conditions, in the absence of carbon dioxide, green leaves quickly evolve a limited quantity of oxygen but etiolated leaves evolve no oxygen. These facts suggest that during greening the leaves develop a potential reservoir for photosynthesis from which they can liberate oxygen by photochemical action.

The oxygen liberated by illumination of the evacuated leaves was estimated by measuring the quenching of the phosphorescence of tryptaflavine adsorbed on silica gel. A phosphorometer of simple design was constructed for this purpose. This instrument was capable of detecting oxygen pressures of the order of 10^{-5} mm. of mercury. Since the quenching is proportional to the logarithm of the oxygen pressure, the method is more sensitive in the lower ranges of oxygen pressure.

THE NATURE OF THE TRANSFORMATION OF
PROTOCHLOROPHYLL TO CHLOROPHYLL

V. M. KOSKI AND J. H. C. SMITH

It has been known for some time that most higher plants do not form chlorophyll in the dark and that the rate of chlorophyll formation which takes place in the light is affected by temperature. Therefore, the biosynthesis of chlorophyll must involve both thermochemical and photochemical reactions. A more detailed analysis of the effect of light and temperature on the greening of leaves has demonstrated that the formation of protochlorophyll is a thermochemical reaction and the transformation of protochlorophyll to chlorophyll is strictly a photochemical reaction.

Protochlorophyll accumulates in plants grown in the dark at room temperature,

but it is not formed either in the light or in the dark at 0° C. These observations make it clear that protochlorophyll is formed by a thermochemical process.

Since light is involved in the transformation of protochlorophyll to chlorophyll *a*, the question arises as to whether this transformation is a mixture of thermochemical and photochemical reactions or whether it is a strictly photochemical reaction. This was tested by measuring the rate of the reaction at different temperatures and different light intensities.

The rate of transformation of protochlorophyll in dark-grown corn leaves was measured at two temperatures, 5 and 18° C, and at three light intensities, 30, 120, and 240 foot-candles. The rates at the two temperatures were identical for each of the three light intensities and were roughly proportional to the light intensity. Because the rate of the transformation is independent of the temperature and proportional to the light intensity, it may be concluded that the rate-determining step is photochemical.

The rate of transformation is very rapid. Light energy of 240 foot-candles from a 40-watt 4500 White Westinghouse fluorescent lamp converts 56 per cent of the protochlorophyll in 10 seconds.

The transformation reaction was also measured with monochromatic light as the source of illumination. Light of wave lengths 640 and 650 m μ , each having a band width of 5 m μ , was used. An analysis of the rates showed the transformation to obey strictly the mathematical expression for second-order reactions.

As a result of these experiments it may be concluded that in etiolated corn leaves the transformation of protochlorophyll to chlorophyll *a* is limited by photochemical action, and that the rate of transformation is determined by the amount of light absorbed by the protochlorophyll.

In the previous Year Book it was reported that in continuous light, transformation of the protochlorophyll present in etiolated seedlings produces chlorophyll *a*. As greening progresses, chlorophyll *h* appears. Thereafter, chlorophylls *a* and *b* increase in constant proportion to each other. Similar results have now been obtained from experiments with intermittent light. These relations make it highly improbable that chlorophyll *h* is derived from chlorophyll *a*.

THE ACTION SPECTRUM FOR THE FORMATION OF CHLOROPHYLL

J. H. C. SMITH, V. M. KOSKI, AND C. S. FRENCH

The possible transfer of energy between pigments and the chemical utilization of light energy absorbed by plant pigments can be investigated in a system similar in many respects to photosynthesis. This system is the photochemical mechanism by which protochlorophyll is changed into chlorophyll by the action of light. This reaction is, in a limited way, analogous to the photochemical steps of photosynthesis in that it takes place in the leaf and the active pigment is very closely related to chlorophyll. It is simpler than photosynthesis in that protochlorophyll, the pigment concerned in the absorption of light, is also the substance which is transformed by the action of the light, and in that the product of the reaction, chlorophyll, is a known chemical substance. The process of greening of dark-grown leaves involves a system of better-known components and fewer chemical steps than does photosynthesis, and yet it is a system closely associated with photosynthesis and one in which the utilization of light energy can be more easily investigated. Under certain conditions it is possible in this system to measure a photochemical transformation which is not complicated by a number of thermochemical steps.

The specific question which this analogous reaction can help to answer is one relating to the possible participation of several different pigments in causing a single type of photochemical change to take place. It has been well established that photosynthesis may be powered by light energy absorbed by a number of different pigments. In all these cases chlorophyll *a* is always present, and it is considered likely that the energy absorbed by the other pigments is in some way transferred to chlorophyll *a*, which then initiates the first steps of photosynthesis.

In the greening of etiolated plants there is also the possibility that nonchlorophyllous pigments such as carotenoids may participate by the absorption of light and the transfer of energy to protochlorophyll. A series of experiments carried out a few years ago in another laboratory can be interpreted in this way. Whether or not light absorbed by chlorophyll can be used to cause further chlorophyll formation has long been of interest. The older methods are not adequate to settle this question.

The development in this laboratory of procedures for the extraction and analysis of chlorophyll and protochlorophyll, as well as the construction of a device for the production of high enough intensities of monochromatic light throughout the visible spectrum, has made possible a greatly improved reinvestigation of these long-discussed questions.

By means of these new techniques the effectiveness of various wave lengths of light in producing given effects in biological material can be accurately measured. If the absorption spectra of the pigments contained in the biological material are known, it is then possible to compare the action spectrum with the absorption spectra of the pigments and thus to see clearly which pigments are photochemically active.

Precise measurements have now been made of the action spectrum for the transformation of protochlorophyll to chlorophyll in normal and albino corn and in normal bean seedlings. The results make it evident that the carotenoid pigments not only are ineffective, but reduce the action of the blue light by absorbing it. Thus they act as a light filter at short wave lengths. The results with normal corn and bean leaves are in distinct contrast with the older work on oats, in which it was found that the carotenoids, even though present in abundance, did not cause internal screening. In albino corn, which contains very small quantities of carotenoids, blue light was found to be the most effective part of the spectrum.

Another experiment was performed in which about one-half the protochlorophyll present in the leaf was converted into chlorophyll, and the leaf was then illuminated with light having a wave length of 6800 Å, which is strongly absorbed by chlorophyll, but very weakly absorbed by protochlorophyll. This illumination at 6800 Å gave no significant increase in chlorophyll content. It is therefore evident that red light absorbed by chlorophyll is not involved in the transformation of protochlorophyll.

It may be said in conclusion that only light which is absorbed by protochlorophyll is active in the transformation of this pigment to chlorophyll. The action spectrum obtained for this transformation in albino corn seedlings, which contain little other pigment than protochlorophyll, gives therefore a measure of the absorption spectrum of protochlorophyll in living etiolated leaves, a result which cannot be obtained directly because of the low concentration of this pigment in such leaves.

SPECTROSCOPIC EQUIPMENT

C. S. FRENCH, J. H. C. SMITH, AND
GEORGE H. TOWNER

The recording spectrophotometer mentioned in last year's report has been considerably improved, but is not yet ready for routine use. The major change has been the incorporation of a cam which is run by the wave-length drive mechanism to take care of most of the necessary corrections. The remaining small corrections are now handled by the curve follower, an arrangement which provides for considerably greater accuracy. A means has been devised for the automatic plotting of this secondary correction curve. Comparison of the spectral absorption curve for a pure sample of chlorophyll *a* as recorded by this machine with that measured manually by another instrument indicates that the maximum error in measuring the per cent transmission is now within 3 per cent. The stability and reproducibility are somewhat better than this figure. The apparatus is now being rebuilt for greater precision, increased stability, and freedom from scattered light. From the experimental work to date, it appears that its eventual performance should be well within 1 per cent for the measurement of absorption spectra, and within a few per cent for the spectroradiometry of very weak light beams, such as the fluorescent light from leaves or algae.

A large-aperture grating monochromator has been built for the measurement of the effectiveness of different parts of the spectrum in the formation of chlorophyll in dark-grown leaves. The results of these experiments are discussed in a separate section of this report. This monochromator is based on a diffraction grating kindly loaned by Mr. Harold Babcock, of the Mount Wilson Observatory. This 4 by 4 inch grating, with 15,240 lines per inch,

is used with two large camera lenses designed for aerial photography. The lenses have an aperture ratio of $f/2.5$ and 12 inches focal length. The angle between the two optical axes is 30° , the grating being placed at the intersection of the two axes and rotated about this point by a screw-drive mechanism. This screw drive is provided with a double thread and a positioning pin moved by a cylindrical correcting cam so that for each revolution of the drive wheel, 100 angstroms of the first-order spectrum is swept across the exit slit. The wave lengths of light in the spectra of both the first and second orders are thus strictly proportional to the amount of rotation of the drive wheel and can be read continuously by means of separate counters for the two orders. Readings of these counters are correct within 5 angstroms. The first-order dispersion is 50 angstroms per millimeter. The maximum transmission of the instrument in the first-order spectrum is in the red, and in the second order it is in the blue. The light source used in conjunction with this instrument is a high-pressure capillary mercury lamp. This combination produces adequate intensity in a 50-angstrom band width for studying the formation of chlorophyll in leaves at all points throughout the visible spectrum.

Another monochromator for use in the recording spectrophotometer has been made. This instrument has a similar collimator lens and a 4 by 4 inch transmission replica grating obtained through the courtesy of Professor R. W. Wood, of Johns Hopkins University. This grating, with 14,000 lines per inch, has a high transmission in the first-order blue part of the spectrum. After passing through the grating, the light is reflected from a movable plane mirror which controls the wave length conning out of the instrument.

From this plane mirror the light goes to a spherical mirror of 63 cm. focal length. The image of the spectrum on the exit slit is thus about twice the size of the entrance slit. The linear dispersion in the plane of the exit slit is therefore 27 angstroms per millimeter. The drive wheel of this instrument is also arranged so that one rotation corresponds to 100 angstroms. These two instruments may be coupled together with selsyn motors, thus acting as a double monochromator to sweep the spectrum at a constant rate. Either of them can also be used independently for photochemical purposes.

THE URONIDES OF LEAVES

H. A. SPOEHR

Higher plants are composed primarily of carbohydrates. Cellulose, probably the most abundant organic substance occurring in nature, is the chief component of the structural and supporting parts of higher plants. Starch, one of the most common plant constituents, serves the plant as an important storage food material. Both cellulose and starch are formed by the linking together of a large number of sugar units of one type, namely D-glucose. Also of very wide distribution in all plants are the uronides. This group of carbohydrates is more complex than cellulose or starch in that its members are composed of more than one type of sugar unit, combined with either glucuronic or galacturonic acid. This greater complexity in composition of the uronides greatly increases the difficulty of gaining an understanding of the structure and properties of this class of compounds. Further difficulties arise from the colloidal character of the uronides and their occurrence in the plant in association with other carbohydrates, which makes clean separations extraordinarily difficult.

The phytochemical relations of the uron-

ides and their physiological function in the plant are still largely unknown. Yet their very complexity of structure and of chemical reactivity invites speculation concerning their role in the physiology and chemistry of the plant. It has been generally assumed that the uronides are primarily cementing elements in the structural fabric of plant tissue. But it is possible that in leaves they play a more dynamic role in the metabolism of the plant, as intermediates in the formation of other compounds, and even in the photosynthetic process. Before any such hypotheses can be tested with leaf material, considerably more exact information is required concerning the isolation, purification, and chemical composition of the leaf uronides.

Uronides have been isolated from several species of leaves, including sunflower, spinach, plane tree, and more particularly flax. It has been found advantageous to use leaves which are free of starch. The uronide content has been found to range from 8 to 15 per cent of the dry leaf material. The uronides are extracted with water, precipitated with ethanol, purified by repeated solution in water, and reprecipitated with alcohol. The product obtained in this manner is principally in the form of calcium salts with about 25 to 30 per cent uronic acid. On further purification, a water-soluble polyuronic acid is obtained, composed principally of galacturonic acid. The method of hydrolysis in which 90 per cent formic acid is employed, and which gave very satisfactory results with compounds composed of mannuronic acid and of hexoses and pentoses, is of no value with compounds containing galacturonic acid. This is apparently due to the fact that mannuronic acid readily forms a lactone which crystallizes well and the acid can be isolated in this form, whereas galacturonic acid does not form

a lactone. Although polygalacturonides can be hydrolyzed with 90 per cent formic acid, on concentration of the acid hydrol-

ysate the galacturonic acid rapidly undergoes condensation or polymerization to form insoluble compounds.

EXPERIMENTAL TAXONOMY

JENS CLAUSEN, DAVID D. KECK, WILLIAM M. HIESEY, AND PAUL GRUN

Broadly interpreted, the experimental taxonomy program has as its threefold objective an understanding of the various kinds and degrees of kinships between plants, of the relations between plants and their environments, and of the evolutionary processes that have produced the diversity between plants and their fitness to the environment. Progress toward this objective becomes more and more a co-operative effort, not only within the staff itself but with outside individuals and institutions.

These investigations advance along an extended front, and during the year the groundwork has been laid for new approaches. Previously, the transplant studies had shown that widely distributed species are able to occupy their diverse environments because they are composed of many physiologically and genetically distinct races, each fitted to live in a different sector of the area occupied by the species as a whole. On the other hand, the experiments on the Madiinae and other plants had revealed the presence of various kinds of barriers to free interbreeding that have arisen during the process of speciation, giving rise to species and other units in various stages of differentiation. The processes through which plants become fitted to their environments remain to be explored. It is known that this fitness depends upon their physiological characteristics, which in turn are governed by genes, but the details of this interaction are unknown. Exploration of the genic-physiological relationships of appropriate plant groups may therefore hold an important key to our understanding of evolution.

Possibly no single organism can be found that fulfills all the requirements of a program in physiological and ecological genetics with these objectives, and probably several groups of plants are needed, each bringing to light features not so readily discovered in others. During the year progress has been made in the search for such groups of plants by testing the suitability of several different genera. These tests meanwhile serve to extend our knowledge of plant relationships, as all have interconnected objectives and aim toward the solution of related problems.

PERSONNEL AND GUEST INVESTIGATORS

During the year a larger group than ever before has participated in this program. Dr. Paul Grun, of Cornell University, joined the staff in March 1949. Dr. Herbert G. Baker, Lecturer in Botany at the University of Leeds, England, spent the year from October 1948 with the group on a Carnegie Institution Fellowship, investigating the evolutionary patterns in some plants having both an Old and a New World distribution.

Dr. Pierre Dansereau, Director of the Service de Biogéographie at the University of Montreal, was with the Division from March through August 1949 on a Guggenheim Fellowship. He was drawing upon the program in experimental taxonomy for material to include in a book on the taxonomy of the vascular plants, in which the aim is to correlate cytotoxicology, ecology, and experimental methods with conventional taxonomy. He also familiarized

himself with the plant communities of the Pacific coast region, especially of the transect marked by the Division's stations, as a background for the correlation.

Dr. Hedda Nordenskiöld, of the Institute of Plant Systematics and Genetics of the Royal Agricultural College, Uppsala, Sweden, was a visitor in the spring of 1949 under a National Swedish Research Fellowship, collecting and studying western American forms of the wood rush, *Luzula*, to correlate with studies on that genus in western Europe. Two Stanford graduate students, Mr. Robert K. Vickery, Jr., and Mr. George H. Ward, have utilized the greenhouses and other facilities in researches intimately connected with the Division's program. These various visitors have brought many new points of view into the group.

POTENTILLA GLANDULOSA

This species of the rose family has many contrasting ecological races and is well adapted for genetic experiments, as was brought out in Year Book No. 47 (1947-1948). It is also a good subject for transplant experiments, since the segregating and cloned second-generation hybrids between races from lowland and high-altitude climates have proved excellent for the study of principles in ecological genetics. During the year the statistical analysis of the long-term selection experiments has been completed with the efficient aid of Dr. Helen K. Sharsraith, and a manuscript on these experiments is now in preparation. The physiological properties of the races and hybrids have not been investigated, however, as this species is difficult to clone and grows slowly, and hence is less well adapted for physiological studies than for the other approaches to ecological genetics.

HYBRIDIZATION IN ACHILLEA

The members of the *Achillea millefolium* complex occur almost throughout the northern hemisphere and have evolved an unparalleled series of contrasting climatic races. They clone easily and are excellent for physiological investigations (Year Book No. 45, 1945-1946, pp. 112-117), but they have only a few distinctive morphological characteristics that can be used as markers in a genetic investigation. The first seven Fi hybrid combinations between contrasting races were made in 1948 and grown in 1949, as follows:

Achillea borealis, 72=27 chromosomes:

San Joaquin Valley race, 36° N. X Kiska Island coastal race, 52° N.

San Joaquin Valley race X Kiska Island alpine race

San Joaquin Valley race X California coastal race

Achillea lanulosa, $n = 18$ chromosomes:

Port Orford, Oregon, coastal race, 43° N. X Vera Cruz, Mexico, montane race, 7000 ft., 20° N.

Port Orford coastal race X California redwood region race

Port Orford coastal race X Great Basin race

Achillea millefolium X *borealis*:

Northern Iceland coastal race, 65° N. X San Joaquin Valley race, 36° N.

The most contrasting cross is between the giant San Joaquin Valley race of *A. borealis*, which is 150 cm. tall, winter-active, and from a continental subtropical climate in California, and the diminutive (8 cm. tall), winter-dormant, alpine, subarctic race of the same species from treeless Kiska Island in the Aleutians. It is expected that recombinations of the genes in the second generation of the hybrid of parents from such contrasting climates will produce offspring of great physiological variation.

EXPLORATORY STUDIES IN MIMULUS

A new plant being tested for its applicability to experimentation is *Mimulus guttatus*, the common monkey flower. It and its close relatives compose a highly variable group of plants that has evolved races all over western North America west of the Rocky Mountains from Alaska to Baja California, and from sea level up to 11,000 feet. Most of the races of *guttatus* are perennials, but some are annuals. Both perennial and annual forms may flower as soon as four months after sowing. They clone easily and cross easily, most forms are self-compatible, and a single pollination may result in hundreds of seeds. Cultivation is difficult, however, for this plant occurs naturally in moist soils or even in running water, although it can be grown to maturity without trouble under greenhouse conditions. Its adaptability to garden culture is now being tested.

Four Fi hybrids between races of the *Mimulus guttatus* complex from very different climates along our station transect were grown this year, namely, coastal X Sierran foothill; coastal X alpine (*Mimulus Tilingii*); coastal X subalpine Great Basin; Sierran foothill X alpine (*M. Tilingif*). The crossings were easily made except those involving the alpine form, which has been known as a distinct species; and the hybrids appear to be fertile.

Since October 1948 Mr. Robert Vickery has carried on these investigations as a project for his doctor's degree through a co-operative arrangement between the Division and the Department of Biology of Stanford University. The experimental work is being done at our stations. Thirty-nine populations of 5 species of the *guttatus* complex have been grown in addition to 57 strains of 23 other miscellaneous species, and in all 7 of the 10 sections of the genus have been represented.

Seventeen races and forms of the *Mimulus guttatus* complex, mostly from our transect, have been crossed during the year in all possible directions. These strains came from the immediate coast, the coastal mountains, and a series of localities over the Sierra Nevada from the foothills on the west to 10,000 feet elevation near the crest and to the plateau of the Great Basin beyond, and included the closely related annuals *M. nasutus* and *M. laciniatus*. A Chilean form of *M. luteus* was also included.

In addition to these crossings, designed to explore thoroughly the interrelationships within a single section of the genus, these 5 species and 9 others, representing 7 sections, were less intensively hybridized to ascertain the genetic relationships among species of various sections and the limits of crossability between species.

Two major objectives motivate the *Mimulus* investigations. One of these is to determine through experimental means the evolutionary relations between species of distinct sections of the genus, between closely related species of the same section, and between ecological races of one species. The other objective is to ascertain the suitability of *Mimulus*, specifically the members of the *guttatus* complex, for experiments on ecological and physiological genetics. To this end some no selected individuals of races and hybrids of this complex were cloned and transplanted to the three altitudinal stations, and it is hoped that a few key individuals can be tested next spring in the controlled greenhouses of the Earhart Plant Research Laboratory of the California Institute of Technology. The two major objectives dovetail and supplement each other.

POA INVESTIGATIONS

The background of the range-grass breeding program, employing members of

the genus *Poa*, and aiming at both practical and basic scientific objectives, has been detailed in previous Year Books (cf. No. 46, 1946-1947, pp. 95-103). The purpose is to explore the possibilities of creating new species of range grass having new sets of qualities and suited to new environments, by combining the inheritances of existing species. It entails the production and study of hybrids between species belonging to different sections of the genus and from very contrasting environments. The parental species and many of the hybrids set most of their seed without fertilization, and thus reproduce as apomictic clones rather than through ordinary genetic segregation.

The *Poa* hybrids often had as one parent a wild strain obtained from the extensive collections in the Pullman Nursery of the U. S. Soil Conservation Service. From the beginning of these experiments we have received the wholehearted co-operation of the Soil Conservation Service through Dr. A. L. Hafenrichter, chief of the nursery division of the Pacific Coast Division, and members of the staff. Accordingly, the *Poa* hybrids are under test not only at the three altitudinal transplant stations of the Institution, but also in a series of nurseries of the Soil Conservation Service extending from southern California to northern Washington. The members of our staff are following the progress of the tests in the nurseries of the Service in addition to those at our own stations. The most extensive of these plantings are at the Pullman Nursery, where about 5000 individuals of some 70 different forms have been grown this year.

Intercontinental transplant experiments. The apomictic clones of *Poa* offer exceptional advantages for transplant experiments on a geographically widely extended scale because they can be propagated by seed. In response to a request for seed of

winter-active grasses, samples of the new *Poa* hybrids and their parental species were sent to Dr. J. W. Gregor, in charge of grass research, of the Scottish Society for Research in Plant Breeding, at Corstorphine, Edinburgh. More than 2000 individuals of 15 strains were grown in an experimental plot with 8 replications, and Dr. Gregor has supplied an analysis of detailed notes and measurements on these strains made throughout the season of 1948.

Dr. Clausen, who was in Europe in 1948 attending the Eighth International Congress of Genetics in Stockholm, had the opportunity of seeing this experiment and conferring with Dr. Gregor on the results. Plants that had come from places having warm summers and severe winters did not grow well in the cool, moist summer climate of Edinburgh, but plants from cool coastal regions made a better showing. The summer climate of Edinburgh is not duplicated at any of the other stations where these grasses are being tested, and it appears to bring out differences between those plants that require heat for their best growth (continental-type plants) and those that thrive under cool conditions (plants fitted for the west coasts of the continents). These characteristics are transmitted from the parents to their hybrids. In view of the favorable early results, it was decided to augment the experiment by testing additional strains and hybrids.

Through personal contacts made in Europe by Clausen it was also possible to arrange for a co-operative intercontinental transplant experiment on an extensive scale. The seeds were shipped to be sown in 1949, and the plants will be ready for measurement in 1950. About 40 largely apomictic species and hybrids of *Poa* are included in this experiment, and they are being tested in 13 environments that range from southern California, at 34° N., to

central Sweden, at 61° N., from sea level to 10,000 feet, and from coastal to continental climates, and that include various seasonal combinations of temperature and moisture.

The places at which these clones of *Poa* are now being tested and the collaborators in charge of them are as follows:

Western United States:

San Fernando, California, Dr. Paul Lemmon, Soil Conservation Service Nursery; coastal, 34° N. latitude.

Central California, Carnegie Institution stations, at sea level, 4600 feet, and 10,000 feet altitude, 38° N. latitude.

Pullman, Washington, Mr. John L. Schwendiman, Soil Conservation Service Nursery; continental, 2400 feet altitude, 46° N. latitude.

Bellingham, Washington, Mr. W. E. Chapin, Soil Conservation Service Nursery; coastal, 48° N. latitude.

Eastern United States:

Raleigh, North Carolina State College, Dr. Ben W. Smith; continental, 36° N. latitude.

Northern Europe:

Aberystwyth, Wales, Dr. T. J. Jenkin, Welsh Plant Breeding Station; very coastal, 52° 30' N. latitude.

Edinburgh, Scotland, Dr. J. W. Gregor, Scottish Association for Research in Plant Breeding; coastal, 56° N. latitude.

Kapelle (Rotterdam), Holland, Dr. A. J. Th. Hendriksen, of D. J. van der Have, seed growers; subcoastal, 52° N. latitude.

Ötoftegaard, Denmark, Dr. H. N. Frandsen, Danish Associated Farmers' and Cooperatives' Experiment Station; intermediate, 55° 40' N. latitude.

Uppsala, Sweden, Dr. E. Akcrberg, Ultima Branch of the Swedish Seed Association (Svalöf); continental, 59° 50' N. latitude.

Volbu, Norway, Dr. Paul Solberg, State Experiment Station for the Mountain Districts, Løken; intermediate, 1500 feet altitude, 61° N. latitude.

Western Asia:

Seeds were earlier sent to Amman, Transjordan, Jubeiha Experiment Station (Mr. M. A. Haddad); continental, 3200 feet altitude, 32° N. latitude.

Each of these experiment stations is interested in discovering new plants for grazing purposes, and we are interested in determining the ranges of tolerance of the hybrids and their parents. These two objectives can be achieved through the experiment as planned, for the responses of each form indicate fairly accurately its range of tolerance, and each station is free to retain, multiply, or use for crossing any form that is well fitted for its own environment.

Two of the hybrids are intended for a selection experiment. They are sexual and therefore will segregate. In the very different climates of this experiment, selection will be made for the best strains. It would be of importance in the study of evolution to determine whether seeds of a single hybrid can give rise to new strains that fit contrasting climates.

Most of the stations have arranged to take brief notes on the performance of the plants through the various seasons, from which the seasonal periodicities of these forms can be determined. It is furthermore hoped that a member of our staff may be in a position to take more detailed and more fully comparable notes at a time when the plants are mature.

Physiology. As another step in this unique experiment, it is hoped that during the coming year key forms may be tested under controlled conditions in the greenhouses of the California Institute of Technology, where Dr. Hiesey will co-operate with Dr. F. W. Went. Through these tests it should be possible to discover some of the environmental factors that determine the physiological responses of these *Poas* in various climates and to correlate the

responses with the heredities of the plants. Such tests will also indicate to what extent the experimental results under the controlled conditions of this unique new laboratory can be translated into predictable field performances in various climates.

Cytology. One other approach to the study of the Poas included in the intercontinental experiment is through cytology. Dr. Grun is now studying the influence of the environment on chromosome distribution in selected clones of *Poa* in the transplant series of gardens, and it is hoped he will be able to study similarly the forms to be grown under the different controlled conditions at Pasadena. It is known that environmental conditions do affect chromosome pairing in some species. By using the cytologically unbalanced *Poa* species and hybrids, it may be possible to analyze further the patterns of environmental influences which cause variation in the behavior of chromosomes.

Embryology. Plants such as *Poa* that produce seed without fertilization can follow various methods in developing their seed, but how and where the asexual embryos arise can be determined only by detailed microscopic studies. Dr. Axel Nygren, of the Institute of Plant Systematics and Genetics of the Agricultural College of Uppsala, Sweden, who is making such studies on the embryology of European species and hybrids of *Poa*, expressed interest in studying our hybrids also. His request was welcomed, because such studies on the development of the embryo would complement Gran's investigation of the male sex cells of the same hybrids. Dr. Nordenskiöld prepared and shipped material for Dr. Nygren's preliminary studies while she was at our laboratory. Additional materials will be available to Dr. Nygren in the plot of the intercontinental transplant experiment at Dr. Akerberg's neighboring institution in Uppsala, so that

these plants will serve a double purpose.

Genetic studies. Our understanding of the inheritance patterns in *Poa* has been advanced through the study of some 8000 F₂ and F₃ individuals at Stanford, in addition to the 5000 plants tabulated at Pullman. All together, 175 progenies have been analyzed. These represent offspring of 97 F_i individuals from 22 different crossings and 9 kinds of interspecific hybrids, as listed in table 1.

All hybrids of which a sufficient number of progenies were grown produced not only apomictic but also sexual offspring in somewhat variable proportions. In all crossings but one, both parents were apomictic. In the exception, the paternal parent was sexual, but this combination produced at least as large a proportion of apomictic offspring as the others. Among 97 F₂ progenies, 43 were apomictic to a greater or less degree, and 54 were purely sexual, as listed in table 1. This proportion between apomicts and sexuals might suggest a very simple genetic formula for the inheritance of apomixis in *Poa*, but it is not so simple as that, for the apomictic F_i individuals are apomictic to very different degrees. This is indicated by their F₂'s, in which the proportion of maternal-type offspring ranges from 10 to 90 per cent of the total

The derived apomicts, in turn, produce asexual offspring of their own type and, in addition, aberrants which probably arise by means of the still active sexual process (table 1). This behavior resembles that of their naturally occurring apomictic parents. Unlike the parents, however, which produce weak aberrants that are quickly eliminated in competition, the derived apomicts may produce aberrants that are either stronger or weaker than themselves. Since some of these aberrants may, in turn, prove to be apomictic again, this source of

variation must be considered in breeding work.

A very vigorous, fertile, and adaptable apomict has arisen in this manner from a weak apomict hybrid of *Poa ampla* X *pratensis alpigena*. The particular Fi parent of this new apomict was itself apomictic, but it was a nonflowering dwarf at Stanford, though fairly vigorous at Mather and Timberline, where it ripens seed. Most of

south of the habitat of either of its grandparents. It is one of the apomicts now being tested in all the environments of the intercontinental transplant experiment.

Another promising apomict, developed from a sister Fi of the *ampla* X *pratensis alpigena* cross just mentioned, had quite a different history. This apomictic line was of an agriculturally highly desirable type, combining some of the best characters of

TABLE 1

NUMBER OF PROGENIES OF INTERSPECIFIC HYBRIDS OF POA

HYBRID COMBINATIONS	F ₂ PROGENIES		F _a PROGENIES				TOTALS
			FROM ABERRANTS IN APOMICTIC F ₂ 'S		FROM SEXUAL F ₂ 'S		
	Apomictic	Sexual	Apomictic	Sexual	Apomictic	Sexual	
<i>ampla</i> X <i>pratensis</i>	20	15	7	2	6	7	57
<i>ampla</i> X <i>compressa</i>	1	5	4	4	14
<i>ampla</i> X <i>arida</i>	1	1	2
<i>Canbyi</i> X <i>pratensis</i>	1	3	4
<i>scabrella</i> X <i>pratensis</i> ...	14	25	..	2	20	23	84
<i>scabrella</i> X <i>compressa</i>	1	1
<i>scabrella</i> X <i>arida</i>	2	1	3
<i>scabrella</i> X <i>ampla</i>	2	2	4
<i>scabrella</i> X <i>gracillima</i> ...	3	3	6
Totals.....	43	54	7	4	30	37	175
	97		11		67		

its offspring are uniformly weak and vegetative at Stanford like itself, but a few of its aberrants are vigorous and flower freely. One of these is the new apomict, which is both very fertile and highly apomictic. It is vigorous like *ampla*, rhizomatous like *alpigena*, rust-resistant, and apparently better adapted for contrasting climates than either parent of the original hybrid. This derivative of a plant from the Palouse Prairie in Washington and another from Lapland thrives at Stanford and also at the two mountain stations, at a latitude far

each parent. It was only 10 per cent fertile, however, and some of its aberrants were as vigorous as itself or even more so. In an attempt to produce a more fertile line. Fa populations were grown from four of its best aberrants, which had presumably risen sexually. One of these was considerably improved over its Fi parent. It is 70 per cent fertile—twice as fertile as its *ampla* grandparent—and it is 85 per cent apomictic, a proportion comparable with that found in wild Poas. Moreover, its aberrants are weak, so they will be eliminated

in competition with the vigorous apomictic type. In the gene exchange it also gained longer rhizomes, an advantage in many climates. These examples illustrate selection methods that can be applied in improving plants that already are apomictic.

The situation is different in the sexual fraction of the F_1 interspecific hybrids in *Poa*. They produce segregating, variable F_2 progenies. Tests on a series of individuals from such F_2 populations showed that some F_2 plants had become apomictic like the grandparents, but others had remained sexual. Among 67 F_3 progenies from sexual F_2 's, it was found that 30 were more or less apomictic and 37 were still sexual (table i). The degree of apomixis in such F_1 's varies as in the apomictic F_2 progenies, a fact which suggests that the segregation for this characteristic is still fairly intricate. From the breeder's point of view it is most fortunate that a sexual period of recombination of genes or blocks of genes may intervene before the inflexible hereditary pattern of apomixis is regained.

An example of the effect of such recombination is afforded by a sexual hybrid out of *Poa scabrella* from coastal southern California X *P. pratensis* from the Athabasca region of Canada. This sexual F_1 inherited winter activity and the bunchgrass habit from its *scabrella* parent, and increased tillering and slight summer activity from *pratensis*. Its periodicity limits it to environments with mild winters, where it outperforms its *scabrella* parent because of its longer period of activity. At Mather, where the winter is long and cold, it is forced into winter dormancy and is scarcely able to survive. A highly variable F_2 was grown from this plant at Stanford, all the individuals of which were winter-active. Four of the best F_2 plants, which had the longest rhizomes and were most summer-active, were selected for progeny tests. The best of these shows fertility in-

creased from 25 to 90 per cent, has long rhizomes, is active most of the summer, is apomictic, and survives the Mather winter successfully. This strain is also included in the intercontinental transplant experiment.

The apomictic, nonsegregating *Poa* progenies are on the whole much more vigorous than the sexual, segregating ones. Many of the variable offspring of the latter are so weak that they are unable to survive even in the experimental field. This difference in performance is understandable when we remember that these are hybrids between very distinct species. Each parental species has a balanced combination of genes selected in the long process of evolution. In different species, however, these balances are produced by different sets of genes. The F_1 hybrids have received an unbroken set of genes from each parental species, and in so far as these sets can produce a harmonious physiological development when together, the F_1 is successful. The apomictic offspring of the F_1 are also successful, for they actually represent an unaltered clone containing an intact chromosome set from each parental species.

By contrast, the offspring of the segregating, sexual sister hybrids arise from an exchange of genes. This causes great variation, because the offspring receive various proportions of the parental genes. Probably a number of the exchanged genes govern physiological processes that run counter to each other, as, for example, photosynthesis and respiration. If such processes become unbalanced, growth may be adversely affected. Many offspring of these sexual hybrids actually die in the seedling stage, and others make almost no growth or are very susceptible to disease. This is the same pattern of weak and diseased offspring already observed in many other interspecific hybrids previously investigated. Occasionally, however, a vigor-

ous, well adapted plant arises through such interspecific gene shuffling, and if that event coincides with the reinstatement of apomixis, a new apomictic species or an agronomically important new grass may appear.

Taxonomy of Poa. The experimental program has dealt with relatively few species in *Poa* and principally with the members of but two of its sections. In part because of the presence of incomplete apomixis, and in part because of a wealth of minor morphological characters that appear in a bewildering array of combinations, *Poa* is a highly variable genus, and the specific and even the sectional lines within it are rather indistinct. This has meant that a sound taxonomic treatment of *Poa* was required for presenting the experimental results.

Poa is of almost world-wide distribution, but for the present purpose Dr. Keck has made a systematic study of the 50 or so species occurring in the western United States, where it is the largest genus of grasses represented. The resulting treatment, which is helping to clarify the relationships in this complex group, has utilized the cytological and experimental results thus far available.

CALIFORNIA PLANT COMMUNITIES

Dr. Keck has been co-operating with Dr. P. A. Munz, of the Rancho Santa Ana Botanic Garden, on a classification of the plant communities in California as a prelude to the writing of a new type of regional flora for California. The plant communities reflect the climatic differences within a region in much the same way as do their component species and climatic races, a fact which suggests that similar environmental factors govern the distribution of all. The results of experiments on California plants are to be utilized in the writ-

ing of the manual, and, conversely, the preparation of the manual is a means of discovering groups of plants that are in need of experimental attention.

ARMERIA STUDIES

H. G. BAKER

The genus *Armeria*, the thrift or sea pink of the Plumbaginaceae, is almost restricted to Europe and the Mediterranean borders of North Africa. The only species which ranges outside this area is *A. maritima* (Mill.) Willd. The wide, discontinuous distribution of this species includes stations in northern Asia and North and South America, as well as in Europe. There is evidence which suggests that the disjunct nature of this distribution is connected with extensions and contractions of the range of the species during the Pleistocene epoch. These isolated populations form promising material for the study of local adaptation and of the relation of this to the particular breeding mechanisms that are found in *Armeria*.

Discontinuity in the distribution of *Armeria maritima* is particularly striking in North America. Here forms are scattered from Newfoundland and the Gaspé Peninsula through the Canadian and Alaskan Arctic, and along the Pacific coast as far south as southern California. A single, very isolated population of *A. maritima* var. *labradorica* Lawr. occurs at an altitude of about 12,000 feet on Hoosier Ridge in the Colorado Rockies, more than 1500 miles from the nearest known population of its closest relatives in the vicinity of Hudson Bay.

Apart from those in the far north, populations of each of the European forms of *Armeria maritima* contain balanced proportions of two kinds of plant, which appear to differ only in the morphology and physiology of their pollen and stigmata.

The flowers of one kind of plant bear stigmata that somewhat resemble miniature maize cobs, and anthers containing pollen grains (type A) with coarsely reticulate ornamentation. The stigmata of the other kind of plant are clearly papillate, and the pollen grains (type B) show an ornamentation of fine spines. Both kinds of plant are self-incompatible but cross-compatible, for type A pollen germinates only upon "papillate" stigmata, and type B pollen only upon "cob" stigmata. Such populations are said to be dimorphic.

The races which inhabit northern Norway and the Kola Peninsula, northern and northeastern Asia, and North and South America contain only one kind of plant and are therefore monomorphic. These plants have type A pollen and papillate stigmata, and are self-compatible.

More than 1500 individuals of *Armeria maritima* are being grown in a uniform garden and in the greenhouses for the purpose of analyzing their heritable differences and of correlating these with the environments from which they came. These represent 41 natural habitats in Europe, the Faeroes Islands, Iceland, Greenland, and North and South America, and include some of those which geographically are most disjunct.

Three major groups are recognizable in the cultures of this species. The first comprises the plants from western Europe. These are dimorphic, obligatorily outbreeding, with large, showy flowers, and their populations are very variable. Several distinctive races have developed in this group, and they occupy sea cliffs, pebble beaches, and salt marshes. Farther inland, they occur in high montane habitats and rarely in the lowlands. Within this group, populations from the Shetland and Faeroes Islands and Iceland in the North Atlantic may constitute a distinct oceanic racial complex.

The second group, inhabiting arctic Eurasia and northern North America, is composed of monomorphic, self-compatible plants that have small, inconspicuous flowers containing relatively few pollen grains. Within this group, var. *lahradorica* Lawr. is in culture from localities ranging from the Gaspé Peninsula, through Labrador and by Hudson Bay, to Great Bear Lake in the Northwest Territories and the remarkably disjunct Colorado station, together with representatives of the more highly arctic var. *sibirica* Lawr. from Greenland.

The forms of the third major group of *A. maritima* inhabit the coasts of Pacific North America and northeastern Asia. They have conspicuous flowers like European races, but are monomorphic and self-compatible. They show less variation within populations than does the European dimorphic complex, although there may be considerable difference between populations. The plants from the north, from Puget Sound northward and westward along the coast to Alaska and Sakhalin, have, among other distinctive characters, ciliate leaves. They have been recognized as var. *purpurea* Lawr. Rarely in the Puget Sound area and more commonly southward to southern California, the populations usually have glabrous leaves and belong to var. *californica* Lawr. Unlike the European dimorphic members of this species, the members of this group have not evolved forms adapted to salt-marsh conditions or to alpine environments.

It is possible to intercross all the forms of *Armeria maritima* which have been cultivated, irrespective of their geographic origin. Such crossings are successful only when the appropriate kinds of pollen and stigmata are brought together, even when self-compatible and self-incompatible forms are being intercrossed. For example, pollen of the self-compatible race from the

California coast (type A/papillate) will germinate upon the stigmata of only one kind of the European self-incompatible var. *typica*, namely type B/papillate. On the other hand, the Californian form can be successfully pollinated only from the other European kind (type A/cob). Crossings already performed between European var. *typica* and Canadian var. *lahradorica* suggest that the genetical determination of this breeding mechanism is relatively simple, and resembles that of dimorphic heterostyled genera such as *Primula*.

These rules apply also to the production of interspecific crosses. By following them, successful crossings have been achieved between *Armeria maritima* and each of the following species: *A. plantaginea* Willd., *A. canescens* Host., *A. pseudarmeria* Lawr., and *A. Welwitschii* Boiss. The last crossing is notable because it is intersectional and indicates that the boundaries of intercrossability may reach far into the genus.

In addition to these experiments, material for pollen analysis of the Plumbaginaceae was obtained in some of the major American herbaria.

INVESTIGATIONS IN THE GERANIACEAE

H. G. BAKER

Geranium. The section *Robertiana* of the genus *Geranium*, as defined by Knuth, is probably unnatural. Two of its species, however, *G. Robertianum* L. and *G. purpureum* Vill., are certainly quite closely related, and they have been the objects of this study. *Geranium purpureum* is diploid ($2n=32$) and strictly annual. Its distribution is essentially Mediterranean, although fingers reach through France to south-western England and Ireland, and also into eastern Africa and Macaronesia. *Geranium Robertianum* is annual or biennial and is distributed widely through Europe (except in the north) and along

the mountain chains of Asia to China. Diploid forms ($2n=32$) have been found in the British Isles, but all forms examined from continental Europe are tetraploid ($2n=64$).

Seventeen representative forms of *G. Robertianum* from coastal and inland British populations, and 3 races of *purpureum*, have been in cultivation in Leeds, England, for periods up to 5 years. A dozen of the cultures of *Robertianum*, 3 of *purpureum*, and 7 of hybrid origin were planted in the garden at Stanford and have provided information concerning their performance in a completely different environment. A race of *Robertianum* from Ithaca, New York, which differs from any known in Europe, has been added to the cultures, and it is hoped to extend these further to include races from northern India. Further crosses have been made this year between races of both species, whereby it will be possible to study the genetic barriers between them.

Er odium. The abundance of plants of *Erodium*, the filaree, is particularly striking to a visitor in California, and some of the problems which they pose are very challenging.

Three species of this genus have long been known to be important members of the introduced flora of California and to have considerable value as forage plants. In all probability, *Erodium cicutarium* L'Her. reached California with early settlers from southern Europe, and was plentiful in the Central Valley by 1844. *Erodium botrys* Bertol. and *E. moschatum* L'Her. followed later. Until 1943, two distinct forms had been confused under the name *E. botrys*, and very recently one of them has been separated by J. T* Howell as *E. obtusifolium*. This form may have been introduced from North Africa.

Forms of these four species have been

grown, studied, and intercrossed, and, in addition, probable natural hybrids have been detected in a mixed natural population of *botrys* and *obtusiplicatum* on the Stanford campus. These putative hybrids appear to be fertile, although *botrys* and *obtusiplicatum* frequently occur together, and there appears to be little difference in their ecological requirements. There is

some evidence that the prevalent self-pollination of these species may account for the restricted amount of hybridization between them in many localities. Seed has been gathered for the cultivation of *E. cicutarium* from a series of habitats, which should give some indication of the part played by direct environmental modification in the polymorphism of this species.

PALEOBOTANY

RALPH W CHANEY

The nearest American relatives of the Chinese redwood, *Metasequoia glyptostrochoides*, are *Taxodium distichum*, the swamp cypress, and *Sequoia sempervirens*, the coast redwood. Superficially similar in foliage and other characters, these two species differ fundamentally in their climatic and topographic requirements. The swamp cypress lives in regions of summer rainfall in the eastern United States, and is limited to lowlands, largely to river swamps. The coast redwood occupies areas with winter rainfall and summer drought, and is confined to the shores of the Pacific where summer fog is prevalent; primarily a tree of well-drained river flats, it is also abundant on valley slopes to an altitude of 3000 feet. *Taxodium distichum* ranges north into regions of continental climate, where there are wide seasonal extremes in temperature, with several cold winter months; *Sequoia sempervirens* does not live beyond the limits of a marine climate characterized by low daily and annual ranges in temperature, where freezing is seldom prolonged. In view of these differing regimes of rainfall and temperature, it is natural to find that the coast redwood has an evergreen habit whereas the swamp cypress is deciduous.

During the middle part of the Tertiary period, from twenty to forty million years

ago, fossil representatives of *Taxodium* and *Sequoia* were widely distributed over the western United States, as was the now Asiatic genus *Metasequoia*. In the John Day Basin, where the most complete sequence of Tertiary floras is to be found, *Metasequoia* was at the outset the most abundant of these conifers; at Twickenham its leafy shoots make up over three-fourths of the specimens recently collected, with katsura (*Cercidiphyllum*) and birch (*Betula*) also abundant; both these trees are modern associates of *Metasequoia* in central China. The gradually rising Cascade Range, which involved the piling up of lava flows and pyroclastics during this later Tertiary volcanic climax, appears to have altered the environment in eastern Oregon both as to climate and as to topography. Judging from the Miocene vegetation, this barrier to the west was at least partially responsible for reduced precipitation and greater extremes of temperature. With the blocking of streams, many lakes and swamps were formed. In the Dayville region, *Taxodium* was a predominant tree, together with oaks, hickory, and others now found living with it in the eastern United States. *Metasequoia* survived there in limited numbers, apparently in better-drained habitats on adjacent slopes. *Sequoia* is never found in direct association, but is well represented in volcanic

ash deposits of the near-by Blue Mountains, in association with other conifers and with angiosperms which suggest a higher elevation. Within a distance of forty miles there are Miocene records of the occurrence of these three genera of the Taxodiaceae, now widely scattered and in diverse habitats. The continued uplift of the Cascades and the consequent changes in environment on their eastward flanks have eliminated a majority of the Tertiary tree genera from the John Day Basin and from other interior localities in western North America. *Sequoia* was first restricted to the coastward slopes, where it ranged as far north as Portland during Pliocene time, and is now limited to the coast of southwestern Oregon and California. Current studies of its living conditions at the south end of its range, in Monterey County, suggest that if a trend toward drier climate were to continue into the future, the coast redwood might make its last stand in the coastal mountains of central California, in a habitat much like that which can be reconstructed for the region of last occurrence of Miocene redwoods in the John Day Basin.

With the change to a drier climate, *Taxodium* and many of its associates have disappeared from western North America. A study of its modern distribution in the southeastern United States, using funds made available by a grant from the American Philosophical Society, has added many significant data regarding the occurrence and associates of *Taxodium distichum*, and is providing a basis for comparisons with the limited forests of *Metasequoia glyptostroboides* in central China. The rainfall regime there is the same, and the Chinese redwoods are likewise deciduous; but the mountainous setting introduces major differences which can be interpreted only after further field work has been completed. In view of the present unsettled

political situation, plans for work in China during 1949 have been postponed. Fortunately there was a continuation of field studies in the *Metasequoia* forest by Chinese botanists during the summer of 1948; there is now a basis for determining that this relict tree occupies a zone of ecological transition between the temperate forest of higher altitudes and the subtropical forest below. Additional collections from the Tertiary of Oregon and British Columbia, and extensive studies of material already available, are indicating that many of the fossil occurrences of *Metasequoia* in western America represent ecological transition zones similar to that in the mountains of Hupeh and Szechuan. This region will continue to provide critical data regarding Tertiary climate and topography, containing as it does one of the most significant forest survivals ever discovered.

Under the auspices of the Save-the-Redwoods League, and with the assistance of the University of California College of Agriculture, a large number of seedlings of *Metasequoia glyptostroboides* have been propagated. These are being distributed over a wide area in western North America, from southeastern Alaska to British Columbia along the coast, and at numerous localities at varying altitudes in western Washington, Oregon, and California. Seedlings have been sent also to Mexico and Guatemala, where they will be planted in upland areas now occupied by survivors of the Arcto-Tertiary Flora. This Flora, which appears to have had its origin in Alaska and other northern lands in Cretaceous and early Tertiary time, formerly included *Metasequoia* as one of its most abundant members. The subsequent migration of this forest southward, and the gradual elimination of *Metasequoia* and other genera, is a matter of substantial record. It is now proposed to plant

Metasequoia wherever there are existing constituents of the Arcto-Tertiary Flora. In addition it will be replanted in as many of its localities of Tertiary occurrence as possible, leaving out—at least for the present—the areas east of the Cascades and Sierra Nevada where existing conditions are now too arid to offer much hope for the growth of a tree with such high moisture requirements. At this time there is no basis for confidence that the redwood of Asia will survive Alaska winters, though its deciduous habit carried it through the exceptionally cold winter of 1948-1949 as far north as Portland, Oregon. In any event we shall gradually learn whether the factors which resulted in the extinction of *Metasequoia* in North America during

later Tertiary time are still operating, or whether this tree may again become a resident of a continent from which it disappeared some fifteen million years ago.

Dr. Daniel I. Axelrod has continued his study of Pliocene floras of Nevada and California. Several of these represent vegetation of a more arid type than is elsewhere found in the fossil record of North America. As a result of these investigations, knowledge of the origin and relations of desert and steppe vegetation is being greatly enlarged. A group of papers by Dr. Axelrod under the general title "Studies in Late Tertiary Paleobotany" has been approved for publication by the Carnegie Institution in its Contributions to Paleontology.

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DEPARTMENT OF EMBRYOLOGY

Baltimore, Maryland

GEORGE W. CORNER, *Director*

The Department of Embryology continued its work during the year without serious interruptions or distractions. Changes in the group were few. Mrs. Faith Wilson LaVelle, Fellow of the American Association of University Women, left the laboratory in July 1948, having practically completed her research project under the direction of Dr. R. K. Burns. She received the degree of Doctor of Philosophy from Johns Hopkins University in June 1949, offering a thesis based on her work in this Department, which will be published in the Contributions to Embryology. Dr. Jerome S. Harris was appointed to a fellowship of the Carnegie Institution, which he relinquished for part of the year to serve as intern in obstetrics in the Johns Hopkins Hospital. Dr. Edward C. Gillespie came following an internship in the Johns Hopkins Hospital, Department of Obstetrics, to spend a year in research with Dr. S. R. M. Reynolds. Dr. Charles L. Schneider, of Wayne University Medical School, Detroit, came for several months for the same purpose. Dr. Jorgen U. Schlegel, a member of the Department of Anatomy of the University of Copenhagen, spent the year here on a fel-

lowship of the Rockefeller Foundation. Dr. R. Mahanti, Professor of Anatomy at Orissa University, Cuttack, Hindustan, was guest of the Department for several months, observing technical procedures and studying research problems. Professor M. H. Toosy, of the Medical School at Lahore, came for four months by request of the Embassy of Pakistan. Dr. E. Carl Sensenig, Associate Professor of Anatomy in the Medical College of Alabama, again worked in the Department in the summer of 1948. Dr. L. J. Wells, Associate Professor of Anatomy in the University of Minnesota, returned for several weeks early in 1948 to complete the preparation of a monograph on the development of the human diaphragm begun during his tenure of a Guggenheim Fellowship in the Department in 1946-1947. Toward the end of the period of this report, Dr. Árpád Csapó, an experienced pupil of Professor Szent-Györgyi in the biochemistry of muscular tissues, arrived from Budapest via Sweden to be a fellow of the Carnegie Institution and to work in consultation with Dr. Reynolds and Dr. Corner on the physiology of uterine muscle.

PROGRAM OF INVESTIGATIONS

Morphology and experimental embryology. Dr. C. H. Heuser, Curator of the Embryological Collection, prepared during the year about a dozen valuable early human embryos in serial sections for addition to the collection. He continued his studies on the morphology of early human and baboon embryos, with special regard to the formation of the yolk sac. Dr.

Heuser also devoted much time to the task of preparing for press an article ("Developmental horizons in human embryos," stages xix to xxiii) which was left unfinished by Dr. G. L. Streeter at the time of his death.

Under Dr. Heuser's direction Dr. M. H. Toosy continued the study of an interesting double monster, one of the earliest

known specimens of this anomaly in man.

Dr. R. K. Burns devoted his time during the year to the completion of two extensive review articles on the embryonic differentiation of the reproductive tract in vertebrates, and to supervising the preparation for publication of papers by Dr. Faith Wilson LaVelle (on the development and response to hormones of the reproductive tract of the hamster) and Robert J. Faulconer (on the embryology of the ostium of the Müllerian duct in man).

Dr. Joseph Gillman, Research Associate, Johannesburg, South Africa, persisted in his efforts to obtain very early baboon embryos of known age. The difficulties and uncertainties familiar to workers in this field are such that the youngest baboon embryo yet obtained for the Collection by Dr. Gillman is 13 days old. Efforts to obtain the critical stage of 10-11 days are being continued.

Dr. Elizabeth M. Ramsey, following the completion of her studies on the uterine vessels of the rhesus monkey in pregnancy (see below under "Published research") began intensive study of the same problem in man. Through the co-operation of the Department of Obstetrics of Johns Hopkins Hospital, several pregnant human uteri have been sent in directly from the operating room and have been injected by Dr. Corner with India ink through the uterine arteries. These valuable specimens, to which additions may be expected from time to time, have been placed at the disposal of Dr. Ramsey.

Dr. J. U. Schlegel was occupied during the year in perfecting a technique for the demonstration of blood and lymph vessels by the use of an injected dyestuff which stains the endothelial lining of the vessels while it is passing through their walls. The substance used is fluorescent and thus becomes visible in ultraviolet light, so that the vessels are seen as shining channels in

otherwise unstained tissues. The method is aimed at demonstrating the arteriovenous anastomoses in the endometrium recently described on the basis of observations by other methods, by Schlegel, Dalggaard, and Okkels at Copenhagen. It offers also a ready means of determining the time necessary for the passage of the solution of dyestuff from the blood into the lymph stream. The method was described in a preliminary report at the 1949 meeting of the American Association of Anatomists.

Mrs. Dorcas H. Padget made further progress during the year on her studies of the development of the cerebral veins, begun several years ago under a grant to Dr. Corner from the Life Insurance Research Foundation.

Dr. E. Carl Sensenig during his annual summer visit made an investigation of the early development of the spinal meninges in the human embryo, and presented a preliminary report on this subject at the 1949 meeting of the American Association of Anatomists.

Very early human embryos and associated corpora lutea. Dr. Arthur T. Hertig, Research Associate, has actively continued the program of collection of human embryos in association with Dr. John Rock, and with the co-operation of Dr. William J. Mulligan and Dr. F. A. Pemberton, at the Free Hospital for Women, Brookline, Massachusetts and the Boston Lying-in Hospital. During the year, uteri and oviducts from 17 fertile patients subjected to hysterectomy for various reasons, and for whom one or more coital dates were recorded at about the presumed time of ovulation before the operation, were searched. Four embryos were recovered, three of which are of extremely early age. All these are now in the collection of the Department of Embryology, as follows:

No. 8698. A dividing ovum in the 2-cell stage. This is the youngest human cm-

bryo, developing under normal circumstances, ever seen. The specimen was recovered from the oviduct at operation 60 hours after coitus. An ovary with the corpus luteum was also removed. Dr. Hertig estimates the endometrium as of the early part of day 17 of the standard cycle, i.e., day 3 after ovulation. (A closely similar 2-cell egg, inseminated and cleaved in vitro in the laboratory of Dr. John Rock in Boston, is also in the Carnegie Collection; see Year Book No. 47, 1947-1948, p. 117.) Dr. Corner estimates the corpus luteum as about 48 hours old. The two symmetrical blastomeres of this wonderful specimen are enclosed in the zona pellucida. One polar body is clearly visible, the other if present is obscured. The specimen has been adequately photographed and will be cut into serial sections by Dr. Heuser.

No. 8630. An abnormal 5-cell segmenting ovum from the cavity of a uterus corresponding to day 19 of the standard cycle, or about day 5 after ovulation. The blastomeres are multinuclear and show other evidences of abnormality and delayed development.

No. 8663. A normal blastocyst from the cavity of a uterus corresponding to day 19 of the standard cycle or about day 5 after ovulation. This is the second youngest known normal human embryo at present writing. The specimen, which is probably a little shrunken, measures 0.134 by 0.115 mm. and has a cavity 0.057 mm. in diameter. It closely resembles the embryos of other mammals at a similar stage of development. There is an inner cell mass composed of 15 or 20 cells. The rest of the blastocyst wall is composed of a single layer of large cells. It has been perfectly sectioned by Dr. Heuser.

No. 8672. A 13-day ovum measuring 2.34 mm. in diameter, implanted on the posterior wall of the left lateral sulcus of

the uterine cavity. The tissues of this specimen, in the embryonic disk as well as in the trophoblast, are in poor cytological condition, and the chromatin of mitotic figures in dividing cells is clumped. The embryo was probably dying. It shows early unbranched chorionic villi, a bilaminar germ disk, and an early definitive yolk sac which has been formed by an hourglass constriction of the large space enclosed within "Heuser's membrane" (coelomic or exocoelomic membrane of various authors). In this respect the specimen sheds light on the problem of the peculiar origin of the yolk sac in man, long debated in this laboratory and by those outside it who have followed the advancing knowledge gained from the Hertig-Rock and other early human embryos.

The corpus luteum. Dr. Hertig reports that the work on the histology and histochemistry of the corpus luteum of pregnancy, referred to in the last Year Book, has been carried on during the year by himself, Mr. Roger F. White, Dr. John Rock, and Miss Eleanor C. Adams and will soon be ready for publication. About 40 selected human corpora lutea of pregnancy and 50 nonpregnant specimens have been used.

Dr. John Rock, of the Free Hospital for Women, Brookline, Massachusetts, has continued to give his advice and help in the program of collection of early embryos. His own studies on living human eggs are made in association with that program, though supported by other organizations, chiefly the American Cancer Society through the Committee on Growth of the National Research Council. He reports having recovered 158 human ova from 49 patients; 123 ova were Inseminated in vitro and cultured for 48 hours in serum. Three of these eggs divided in vitro, two of them reaching the stage of 2 blastomeres and one of them dividing into 3 blastomeres.

Two patients received pituitary extract treatment before the ovaries were removed. Their ovaries yielded 9 ova, recovered from follicles over 1 cm. in diameter, all containing spindles of the second polar division, as do fully mature follicular ova in most mammals that have been studied. It appears that these ova were caused to mature by the pituitary extract.

Embryonic pathology. In recent years it has become known that one of the common contagious fevers, rubella or German measles, a disease which is of trivial concern to the patient, may be the cause of dire consequences when the patient is a woman who happens to be in the first $\frac{1}{2}$ months of pregnancy. In such a case the infant is frequently born blind, with congenital cataracts, often with abnormally small eyes (microphthalmos), and sometimes even with microcephaly and mental retardation. Congenital deafness and anatomical abnormalities of the heart may also occur. Some infants of mothers who acquire German measles at the stated period of gestation may no doubt escape damage, and possibly there is a difference in the virulence of epidemics. At any rate, the discovery of this hitherto unsuspected relationship has caused so much concern among physicians that some of them have advised the termination of pregnancy when the mother has had German measles early in gestation. The Department of Embryology has received three fetuses from such preventive abortions, and has been entrusted with the preliminary study of two more from another laboratory. Dr. Corner and Dr. Heuser have undertaken the investigation of these specimens. The examination will be laborious and may for various technical reasons yield little information, but the gravity of the problem seems to make it a duty to see what can be learned from these fetuses.

Physiology of the uterus. Dr. S. R. M.

Reynolds and a group of fellow investigators have been at work on various problems in the physiology of the uterus, making particular use of the tokodynamometer, to which reference has been made in recent reports. Dr. Reynolds himself put a good deal of effort, with members of the Johns Hopkins Hospital obstetrics staff, into testing improved models of the tokodynamometer looking to a design suitable for wide practical use. He and the clinical observers have begun to use the instrument in a comparative study of the uterine factors involved in the induction of labor by the use of various oxytocic drugs. With Dr. Louis M. Hellman and Dr. Bruce A. Harris of the Hospital staff, he has completed the collection and analysis of data on the first stage of labor, which show the step-by-step development of the expulsive muscular force of the parturient uterus and the developing dominance of the fundus. Dr. Hellman has employed the tokodynamometer in studying the effects of pituitrin given during labor, with a view not only to explanation of its action, but also to improvement in the practical use of pituitrin in labor.

Dr. Reynolds has given much thought to the problem of the mechanical factors involved in maintaining the circulation of blood through the umbilical cord from the placenta to the fetus. Inasmuch as the anatomical structure of the umbilical cord is an important element in the problem, an anatomicophysiological study of the cord has been begun, in which the structure and relative size of the fully distended umbilical vessels will be studied, together with the status and function of "Wharton's jelly," the connective tissue of the umbilical cord. An extensive program of research on this important and hitherto neglected phase of the fetal circulation is planned.

Dr. Jerome S. Harris, working with Dr. Reynolds and Dr. Edward C. Gillespie,

completed a study of the changing form of the human uterus as it enlarges during pregnancy. Using repeated X-ray pictures, he observed two phases of the development of the gravid uterus. Until the 20th week of gestation, growth occurs in all three dimensions, but thereafter little growth occurs in thickness, and after the 30th week the width actually diminishes, and enlargement continues in length only. Dr. J. Tyler Baker, of Easton, Maryland, in the past a frequent contributor of embryonic material to the laboratory, was influenced by Dr. Reynolds' publications to compile a large series of direct measurements of the enlarging uterus obtained with obstetrical calipers. Though his data are not so precise in individual cases as measurements from X-rays, the large number of cases permits statistical treatment. The results of detailed analysis agree with Gillespie's data in showing that the 7th month is a critical time with respect to the provision of space in the uterus and favorable conditions of uterine blood circulation for the infant.

Dr. Gillespie also undertook experimental studies on the effect of alloxan diabetes on sexual receptivity in rabbits, on fertility, and on the growth of the uterus and the fetuses.

Dr. Charles L. Schneider spent 3. few months observing the work of Dr. Reynolds' group and conducting experiments on the experimental production of toxemic conditions in pregnant rabbits.

Mrs. Lyla T. Bradin continued her studies, previously reported, on the relation between the length of the phases of gestation in different species and the relative maturity of the fetus at birth. A preliminary report on the times of appearance of the various ossification centers in a number of small animal species, with respect to the rate of growth and the changing form of the uterus, was presented

at the 1949 meeting of the American Association of Anatomists.

General physiology of the embryo. Dr. Louis B. Flexner and his associates made further progress during the year in their program of correlating the physiological with the morphological development of the embryonic tissues. With Virginia B. Peters, Dr. Flexner studied the growth in volume of the nerve cells of the brain cortex (of the guinea pig) and the relation of the volume of the cell to that of its elements, the nucleus and cytoplasm, taken separately. By such observations a critical period of change in the development of the guinea pig's brain cortex is found to occur at the 41st to the 45th day of gestation. Dr. Leonard Gallant, of the Henry Phipps Psychiatric Clinic, Johns Hopkins Hospital, and Dr. David B. Tyler, working with Dr. Flexner, find that electrical activity of the cortex first occurs at about the same time.

Dr. Josef a B. Flexner and Dr. Louis Flexner are studying the permeability of the cerebral blood vessels to sodium and its changes during development of the fetal brain.

Dr. David B. Tyler has been investigating the enzyme systems that effectuate metabolic processes, particularly those concerned with carbohydrate metabolism, in the fetal brain, and the changes in their activity during development. He is applying to fetal tissue (rat's brain) the method usual in such work on adult tissues, i.e. that of counteracting the various enzyme systems in the tissues by the use of chemical substances known to interfere with the action of specific enzymes. Striking quantitative differences in the sequence of the chemical steps in the metabolism of carbohydrates, occurring at different stages of development, are thus being observed and subjected to interpretation with the aim of locating the sites of action of the metabolic

enzymes and the history of their differentiation in the embryonic tissues.

co-operative activities. Some of the more extensive co-operative activities of the Department have already been referred to, namely, Dr. Heuser's study of early human embryology in association with Dr. Hertig and Dr. Rock, and the joint work of Dr. Reynolds and associates with the Department of Obstetrics of Johns Hopkins Hospital. Dr. Roger B. Scott, of the Department of Gynecology, Johns Hopkins Hospital, continued to use the monkey colony and operating-room facilities in his experimental studies on the production of endometriosis.

One of the most important co-operative services of the Department of Embryology has not been specifically mentioned in recent reports, though it continues unceasingly. This is the examination of specimens sent in by physicians, mostly abortive embryos and fetuses which come with urgent requests for information as to the cause of death or of abnormalities, the age of gestation when aborted, and other questions, some answerable and some unanswerable at the present stage of knowledge. This work is carried on chiefly

by Dr. Heuser, and to a less extent by Dr. Corner. In addition to the specimens sent for such study, there are frequent requests for information on matters concerning human and animal embryology and concerning the physiology of the reproductive system and its disorders.

Dr. Corner is acting as general adviser and consultant to the American Academy of Ophthalmology in the planning and preparation of a motion-picture film explaining the embryology of the human eye. Dr. George K. Smelser, of Columbia University, has been made a welcome guest of the laboratory in order to facilitate his work as technical adviser on the film.

In April 1949 Dr. Lawson Wilkins, of the Department of Pediatrics, Johns Hopkins Medical School and Hospital, gave two talks at the Department of Embryology on developmental and functional disturbances resulting from sex hormone deficiencies in human subjects. In June Dr. Alfred Yost, of the Collège de France, spoke at the laboratory on his experimental researches on the relation of the sex hormones to embryonic differentiation of the reproductive system.

PUBLISHED RESEARCH

HUMAN EMBRYOS OF EIGHT AND NINE DAYS

In Year Book No. 42, for the year 1942-1943, mention was made of the acquisition, through Drs. Arthur T. Hertig and John Rock, of two human embryos, nos. 8155 and 8171, thought to be about 8 and 9 days old respectively. These specimens were in due course successfully sectioned by Dr. Heuser and the sections were photographed by Mr. Heard for subsequent reconstruction on plastic sheets by Miss Eleanor C. Adams under Dr. Hertig's supervision in Boston. A detailed account of the two specimens has now been pub-

lished in the Contributions to Embryology, volume 33. These two specimens represent the stage of human development when the ovum has become well implanted and the uteroplacental circulation is just beginning to be organized. In the 8-day ovum, definitive trophoblast of both types is actively forming, and in the 9-day specimen further differentiation of the syncytiotrophoblast has resulted in the formation of a network of lacunar spaces for the reception of a small amount of stagnant maternal blood from enlarging capillary sinusoids eroded by the invading trophoblast. The embryo

in each of these ova is a bilaminar germ disk without axial differentiation. Amniogenesis has barely begun in the 8-day ovum, but is well advanced in the 9-day specimen. The so-called exocoelomic membrane ("Heuser's membrane"), which is not yet formed in the 8-day specimen, is present in the elder ovum and is described as being delaminated from the adjacent cytotrophoblast.

The two specimens here described lie between the 7-day and 9-day specimens described by the same authors in 1945 (Carnegie nos. 8020, 8004). The two younger specimens have solid trophoblast, but the two 9-day ova show stages of lacuna formation. The four present all the essential stages in amniogenesis, which consists in the progressive *in situ* delamination of amniogenic cells from the cytotrophoblast dorsal to the germ disk.

EMBRYOLOGY OF THE SKELETON; HISTOGENESIS OF CARTILAGE AND BONE

The late George L. Streeter was preparing, at the time of his death in July 1948, another section of his series of "horizons" or classificatory stages of human embryogenesis. This section was to cover horizons xix to xxiii, that is to say, the last phases of the embryonic period, as distinguished by Streeter from the fetal period of development. It was his intention to trace certain features of the development of each major organ system through the five horizons, and he had already prepared for publication a special article (see bibliography) on the histogenesis of cartilage and bone, in which he used the embryonic humerus as an index of differentiation. The following extract from Dr. Streeter's own summary will serve to indicate his line of thought:

"By confining attention to one bone and following it in all its stages one obtains a

more complete history of the consecutive factors that play a part in the development of the skeletal system. . . .

"In studying the humerus during its cartilaginous period, it was found that cartilage cells, as they grow older, pass through an orderly series of transformations. These include proliferation and growth of the cells, a characteristic vacuolization of their cytoplasm, formation and increase in amount and character of the intercellular substance, and terminal liquefaction or disintegration of the cells. By dividing these consecutive transformations into five arbitrary phases, it was possible to plot maps of the humerus showing the areas of distribution of the respective phases of cartilage that characterize the bone as it increases in size.

"It was found that there is always a growth center in the shaft in which the oldest cartilage cells are located. Adjoining it, above and below, are zones of successively younger phases, the youngest always at the ends of the bone.

"When the cells at the growth center reach the terminal phase of disintegration, the cells of the inner coat of the periosteum penetrate the primary osseous shell and make their way into the cartilage, which is thereon abruptly transformed into marrow.

"The time when this invasion occurs is arbitrarily adopted as the conclusion of the embryonic and the beginning of the fetal period of prenatal life. It occurs in specimens about 30 mm. in length."

EARLY DEVELOPMENT OF THE HUMAN VERTEBRAL COLUMN

The early development of the human vertebral column, and indeed of the mammalian spine in general, has received very little attention. Later stages of human development, after the appearance of car-

tilage and during ossification, have been more thoroughly studied, and the osseous stage has been extensively investigated particularly by roentgenologists, who need to understand the adult details and congenital anomalies seen in their diagnostic films. This situation has resulted in much confusion of description and terminology through the attempt to designate embryonic structures which are not fully understood or well defined, with reference to the adult structures of which they are forerunners. Dr. E. Carl Sensenig, now of the Medical College of Alabama, came to the Department of Embryology in 1944 on a special grant from the Carnegie Institution for the purpose of reinvestigating the early development of the vertebral column. His work was aided in several subsequent summers by the Joseph Henry Fund of the National Academy of Sciences. Results of the investigation appear in volume 33 of the Contributions to Embryology.

The embryology of the vertebrae is a very complicated matter, as might be expected in view of the peculiar demands upon the vertebral column for a combination of strength in protection of the spinal cord and in support of the body with flexibility in movement, firm anchorage for the ribs, and safe exit for the spinal nerves. The development of the individual units of this structure is indeed so complex that it baffles any attempt to discuss it in non-technical terms. For this reason it is impossible in a report intended largely for nonbiological readers to summarize the admirable monograph of Dr. Sensenig. It must suffice to say that he gives a carefully detailed description of the earliest mesoblastic (embryonic connective) tissue and its division into the primitive somites or sclerotomes. Each sclerotome is divided into a cranial and a caudal half by the appearance of a sclerotomal fissure through

the somite. Each of the vertebrae is formed (roughly speaking) by the fusion of the caudal half of one somite and the cranial half of the next tailward somite. This much has been known, for the vertebrates in general, since the description of Remak in 1855. Dr. Sensenig now fills in the details for the human species. Technical readers must study his work for themselves. They will note that he discards elaborate classifications of the stages of vertebral development because of the great overlap between successive periods, and finds that three periods, those of formation of membranous tissue, cartilage, and bone, suffice for descriptive purposes. He has not been able to trace the development of the nucleus pulposus with certainty, but leans to Luschka's theory of origin from the notochord.

Dr. Sensenig's observations on the origin of the ribs disagree with the reports of previous authors except von Bochmann, in that he finds that the caudal sclerotome-half as well as the cranial half contributes tissue to the developing rib. Part of the capitulum of the human rib is found to arise from the preceding vertebral segment. The same is true of the anterior zygapophysis of the vertebra. Sensenig states, contrary to the views of previous writers, that the early embryonic plane of separation between vertebral rudiments is not represented in the adult by the mid-point of the vertebral disk, but rather by the caudal surface of the disk where it articulates with the centrum or body of the vertebra. He gives a detailed account of the myocoeles (cavities of the somites), showing that in man they do not regularly connect with the coelomic cavity.

Two types of chondrification in vertebral development are described. In one type, which is slightly earlier in appearance, cartilage formation occurs directly in the loose areas of the primitive centrum. In other

parts of the vertebra, chondrification passes through a typical precartilag stage.

EMBRYOLOGY OF DEFECTS OF KIDNEYS AND URETER

Variations in the number and arrangement of the ureters, with or without associated abnormalities of the form of the kidneys, are not unusual in adult human subjects. Because they offer a variety of difficulties when encountered in the diagnostic clinic and the operating room, a voluminous clinical literature has grown up on the subject. The embryological development of an anatomical anomaly, when it can be ascertained, often makes the adult conditions more comprehensible. Such anomalies are rarely seen in the embryonic stage, however, for the number of embryos subjected to detailed study is infinitely smaller than the number of persons examined by physicians. The Carnegie Collection contains one early embryo (no. 6516, 9.6 mm. CRL) in which a double ureter is developing on one side of the body. After the study of this specimen was begun by Dr. Lawrence H. Wharton, Jr., while he was a student in Johns Hopkins Medical School, Professor Sidney I. Kornhauser, of Louisville, Kentucky, very kindly made available another embryo (H9S of the University of Louisville collection). These specimens are among the youngest thus far reported that exhibit anomalies of the ureter. In the Baltimore embryo the left ureter is duplicated, and there are two renal blastemata (early kidney-forming tissue masses) corresponding to the two left ureters. The Louisville specimen has developed an accessory rudimentary ureteric bud on the right side, anterior to a normally located ureter and blastema. Although the factors which caused these variations from the normal cannot be established without a larger

series of cases, and indeed probably not without experiments on embryos of other more available species, the specimens described by Dr. Wharton bring out the fact that the formation of a normal kidney depends upon the occurrence of certain critical conditions in time and space, beginning with the formation of a normal mesonephric duct which gives rise to a normally situated ureteric bud. During this same period, the metanephrogenic condensation must be organized locally, and the bud must make contact with it to initiate further differentiation of the blastema, which in turn stimulates formation of the primitive collecting system from the pelvic part of the ureteric bud. Thus there are many opportunities for one or another failure of growth in rate or pattern, which may cause fundamental anomalies of the urinary organs.

ATRESIA OF THE OVARIAN FOLLICLE

In the ovaries of mammals many more egg-containing follicles are formed than can be used in the whole reproductive life of the individual female. The ovaries therefore contain at all times a number of follicles that are being obliterated before they reach maturity. The process of regression, which is called atresia, consists (generally speaking) in loss of the egg cell and the lining of granulosa cells, and in proliferation of the connective-tissue elements of the theca interna and theca externa until the cavity is obliterated. In the case of large follicles, absorption of the fluid and consequent shrinkage assists in the reduction of volume. The process of atresia differs considerably in detail from one species to another. For this reason it is useful to have a description of it as it occurs in a primate species. Little is known of the rate at which atresia takes place, or of its timing with respect to the ovulation cycle.

Dr. Somers H. Sturgis, now of Massachusetts General Hospital, undertook a study of atresia in the rhesus monkey while at the Department of Embryology in 1942 on a Rockefeller Fellowship which was interrupted by the war. Dr. Corner's collection of rhesus ovaries of known reproductive history, in complete serial section, was placed at his disposal. Sturgis begins his account by describing and illustrating three successive stages of atresia: The first is characterized by dissolution of the granulosa; the second ends with loss of identity of the egg cell, with full development of a zone of hyaloid tissue from the theca interna; and the third progresses to disappearance of all distinctive elements.

Sturgis was able to form an idea of the rate at which atresia proceeds by the study of certain peculiar examples of atresia occurring in small follicles that were partially luteinized. In other words, at the time when a normal mature follicle ruptured and was converted into a corpus luteum, a small follicle in the same animal responded by some chance to the same hormonal influences that were affecting the large follicle, so that while part of its wall was undergoing atretic changes, another part was converted into corpus luteum tissue. The atretic process could thus be tentatively dated from the cyclic history and from the corpus luteum tissue of determinable age. Sturgis concludes from his 3 specimens of partially luteinized atretic follicles that stage 1 of atresia probably develops in 24 to 48 hours, and is certainly over in 5 days; stage 2 requires about 3 weeks. The follicle then gradually regresses and probably reaches the terminal phase of relative quiescence in about 5 weeks after the initiation of atretic changes. These estimates refer to atresia beginning about, the time of ovulation and may not apply to the speed of the retrogressive

process under other circumstances. Evidence is presented showing that atresia of relatively large follicles starts just before ovulation, probably serving thus to prevent multiple ovulation in animals which, like rhesus, normally shed only one ovum at a time. The theca interna of these degenerating follicles appears to function for only a few days just before and just after ovulation. Sturgis conjectures that such activity may augment the production of estrogen at a time when that hormone is necessary for ovulation and for luteinization of the follicle that is destined to rupture.

THE CERVICAL MUCOSA OF THE RHEBUS MONKEY

The exit canal of the uterus (cervix uteri) is very important in reproductive physiology, for through it the sperm cells must travel inward and subsequently the infant must pass outward at the time of birth. It guards the entrance of a passageway that opens ultimately into the peritoneal cavity, which must be barred against invading microorganisms while permitting the entrance of the sperm and the exit of uterine secretions and (in menstruating animals) the catamenial flow. For these various functions the lining of the cervix must be adaptable to changing physiological needs.

Dr. Clara E. Hamilton's article in volume 33 of the Contributions to Embryology describes the changes in the epithelial lining of the monkey's cervix uteri in the ovulation cycle, in pregnancy, in castrated and amenorrheic animals, and under various experimental conditions of hormone administration. The work was done at the University of Illinois under the direction of Dr. Carl G. Hartman, member of the Department of Embryology from 1923 to 1941. Although Dr. Hamilton's work was extramural as regards the Carnegie labora-

tory, her material came chiefly from Dr. Hartman's and Dr. Corner's collections.

The observations deal with the cellular morphology of the epithelium (height, position of nuclei, content of mucus, regression, and secretion). It was found that the cell height is increased by estrogen, whereas sudden drops in estrogen level cause secretory changes and subsequent regression of the epithelium of the canal and the cervical glands. No inhibition of the effect of estrogen on the cervix by progesterone or testosterone was found with the doses used. Progesterone but not testosterone enhances the mucus content of the cells. In the light of these findings, the characteristic changes of the cervical epithelium in the ovulatory cycle, pregnancy, and castration are explained.

BLOOD VESSELS OF THE PREGNANT UTERUS

This year saw the culmination of a laborious program of research, marked by the publication, in volume 33 of the *Contributions to Embryology*, of Dr. Elizabeth M. Ramsey's monograph on the vascular pattern of the endometrium of the pregnant rhesus monkey. It has long been evident that the blood vessels of the uterus, and particularly those of its endometrial lining, undergo highly important changes during pregnancy. The peculiar coiling of the arteries of the human endometrium, first noticed by William Hunter in 1774, which is found also in the Old World monkeys, has aroused great curiosity as to its possible usefulness in the implantation of the embryo and the subsequent development of the placenta. Peculiar problems are also presented by the endometrial veins, with respect to the source of nourishment of the implanting embryo. The pathways by which the menstrual blood leaves the intervillous space of the placenta have been much discussed, though without full agreement by the various investigators.

Dr. Ramsey therefore began some years ago, at the suggestion of Dr. Carl G. Hartman, an ambitious attempt to study and describe the vessels of the endometrium and the maternal part of the placenta in the rhesus monkey in order to provide a sequential account of their functional changes throughout pregnancy. In all, 13 female rhesus monkeys were bred to secure a series of pregnancies of known dates. At the chosen time in pregnancy each animal was anesthetized and the blood vessels of the uterus were injected with India ink or other injection mass. Serial sections of large blocks from the implantation sites were made, and the blood vessels and other relevant structures were painstakingly modeled by Dr. Ramsey, using the technique of serial reconstruction on transparent plastic sheets. This project illustrates the effective way in which a specialized research laboratory can deal with certain kinds of work requiring organized co-operation, for it has demanded not only Dr. Ramsey's skillful and persistent efforts, but so much expert service of the departmental staff, from the breeding of the animals through the injecting and sectioning of the uteri to the illustration of the final publication, that a list of those who contributed special skills in furtherance of Dr. Ramsey's research would be almost a list of the whole personnel.

The monograph must be read and the illustrations must be studied to gain an idea of the instructive findings of the research. Summarizing briefly, the coiled arteries are found at first to become more coiled at the implantation site, but afterward to become extended and straight. This uncoiling, which takes place at the beginning of the last third of pregnancy, coincides with the end of the period of uterine growth and the commencement of the period of stretching without growth

that has recently been investigated by Reynolds (see this and recent Year Books). At the same time, the number of arteries connecting with the intervillous space decreases.

The spiral arteries of the placenta undergo a very peculiar change early in pregnancy, by which their endothelial lining becomes greatly thickened by increase of its cells. Dr. Ramsey devotes much attention to this phenomenon, arriving at the conclusion that the proliferation of lining cells is truly a proliferation of endothelium, rather than an invasion by cytotrophoblast cells of fetal origin as it has been considered by some workers.

Dr. Ramsey finds, with respect to the veins of the endometrium at the implantation site, that as pregnancy advances the number of venous channels draining the base of the placenta diminishes, until much the greater part of the venous drainage is into the marginal sinus.

This evidence from a whole series of monkey placentas, graded by age from relatively early to late in gestation, helps to explain the failure of certain workers (e.g. Spanner) on human placentas to find basal placental drainage in the relatively late specimens at their disposal; but on the whole the findings confirm the general theory of placental circulation put forward by Spanner (1934 to 1940) and show that his "overflow" type of filling and emptying of the intervillous space operates in the rhesus monkey as well as in humans, despite minor structural differences.

PHYSIOLOGY OF THE UTERUS

Uterine contraction pattern during labor. Last year's report (Year Book No. 47) included a description of the strain-gauge tokodynamometer invented by Dr. Reynolds for the primary purpose of recording the movements of the human uterus dur-

ing the advanced stages of pregnancy and especially during labor. The instrument, applied without harm and without great inconvenience to the abdomen of the patient, simultaneously records the activity of the muscular wall of the uterus at three different positions, the fundus (i.e. the upper part of the uterus, which is the bottom of the uterine bag), the middle, and the lower end near the outlet. Thus it provides information not only about the strength, duration, and rate of the contractions of the uterus, but also about the relative strength and rate at the three levels. Gradients of force from one point of the uterus to another, and the origin, spread, and dissipation of the contraction waves, are recorded on graphs which can then be conveniently subjected to intensive study and analysis, of the progress of labor, its normal or abnormal character, and the effects upon the uterus of drugs administered to the patient. The instrument has been in active use in the Johns Hopkins Hospital maternity clinic since it first became available, and has been widely copied for use elsewhere.

The results of research with the Reynolds tokodynamometer carried out in the Johns Hopkins clinic have now begun to appear in published form. Dr. Reynolds, with the collaboration of Dr. Louis M. Hellman and Dr. Paul Brans, of Johns Hopkins Hospital, laid the foundation for subsequent clinical reports by describing in the *Obstetrical and Gynecological Survey* the patterns of uterine contractility observed in normal labors and in some of the common types of abnormality (premature and false labor, uterine inertia). Normally progressing labor, as indicated by progressive dilatation of the cervix, is characterized by a gradient of diminishing physiological activity from the fundus to the lower uterine segment. Deviation from

this pattern is associated with prolongation of labor or failure of dilatation of the cervix. The gradient of activity is associated with a high tension in the tissues of the uterine wall at the fundal end, exceeding that in the cervix by about 3 to 1. This favors stronger contractions in the fundus than in lower parts of the uterus and hence contributes to the orderly emptying action of the uterus at parturition. The effect of rupture of the membranes is such that the relative tension at the fundus increases with respect to that at the lower uterine segment. The delicate balance of forces which prevents the untimely emptying of the uterus is thus tipped suddenly, by spontaneous or artificial rupture of the membranes, in a direction favoring delivery of the uterine contents.

Work done by the uterine muscle in labor. The same authors have presented a method of estimating the work done by the different uterine segments during labor. Their article was appropriately published in a Festschrift article for Professor Dr. E. Röthlin, of Basel, an eminent investigator of the pharmacology of the uterus. Graphs recorded by the Reynolds tokodynamometer from a normal labor provided the data for analysis.

The work done by the uterine muscle during the contractions could be computed by planigraphic measurements of the area of the curve under the graph of each contraction. To avoid the impracticable task of analyzing all records with a planimeter, a formula based upon the integration of the intensity, duration, and frequency of the uterine contractions was established, whereby the work per hour could be calculated from the graphs. Such calculated values proved to be parallel to those computed from planimeter measurements, and when corrected by a constant factor could be used to study the work done by various segments and the effect

upon the work of drugs administered to the patient. The formula may therefore be applied with confidence in subsequent studies upon records made with the tokodynamometer.

Recording uterine contractions in animals. Dr. Reynolds and Dr. Irwin H. Kaiser (formerly Fellow in the Department of Embryology) have shown how a dynamometer employing the strain-gauge principle can be used in measuring the responses of uterine muscular tissue of laboratory animals. In an anesthetized rabbit, for example, the uterus is exposed by an abdominal incision and a blunt needle is inserted into its cavity. Pressure changes within the uterus are thus communicated through a rigid tube to the strain gauge. The resulting graphs can be analyzed for the force of the contractions and for the work done. With this apparatus Reynolds and Kaiser have been able to study the quantitative effects of oxytocic (uterus-stimulating) and antihistaminic drugs. The latter (Pyribenzamine and Benadryl) were found to be in general oxytocic, affecting both muscular tone and rhythmic contractility. The hormonal status (pregnancy, nonpregnancy) does not affect the responsiveness of the rabbit's uterus to Pyribenzamine as it does to the pituitary oxytocic substance.

Uterine circulation time. Many recent investigators have suspected that there is a relation between deficient blood circulation in the uterus and toxic conditions during pregnancy. Thus there is a need for measurement of the uterine blood flow. Dr. Edward C. Gillespie and Dr. Reynolds have developed a very ingenious method of making such measurements in experimental animals and have applied it to the rhesus monkey. The technique depends upon observing and measuring the rate at which a substance injected into the muscle of the uterus is cleared away from the site

of injection by the circulating blood in comparison with the clearance of a similar injection into the abdominal muscles. By use of a new high-speed jet injection ("Hypospray") it is possible to propel fluids under the skin almost without pain. The depth of penetration is controllable by the operator. Gillespie and Reynolds found that such injections of the radiopaque dyestuff Diodrast could be made twice weekly in pregnant monkeys without harm. In a typical series shown in their paper (106 days gestation) the clearance of 1 cc. of the dye from the monkey's uterus required 34 minutes, from the abdominal muscle 25 minutes.

Adaptation of uterine blood vessels and accommodation of the products of conception. As the fetus grows, the uterus in which it lies must enlarge with it. The process of uterine growth is not, however, one of mere stretching and enlargement until some extraneous agent causes the uterus to empty itself; it involves an elaborate series of adjustments of the uterus in form and function, resulting in exact adaptation to its contents. For some years Dr. Reynolds has been giving his attention to the various elements of this adaptive process. In volume 33 of the Contributions to Embryology he presents a detailed study of the part played by the blood vessels of the uterus in its accommodation to the products of conception. The data presented were obtained from observations and experiments on rabbits by a wide variety of technical methods involving chiefly injections with diffusible dyes and corrosion preparations. A summary of the results shows, first, that there are two distinct phases in the adjustment of the uterine vascular system to the shape and size of the conceptus. The first of these involves progressive stretching of the blood vessels about the spheroidal conceptus. A maximum degree of distention

is attained about the 22d day of pregnancy in the rabbit. At this time, the second phase of the vascular adjustments takes place after quick conversion of the conceptus to a cylindrical form. The period of rapid fetal growth which ensues involves only lengthening of the uterus. The effect of this upon the blood vessels in the uterus is to cause them, by virtue of their special arrangement, to be separated from one another in the latter part of gestation, without further increase in length. As a consequence, the hemodynamic work involved in moving blood through the uterine blood vessels is no greater when the fetus weighs 80 gm., near term, than at the time of conversion, when it weighs a fourth or less of that amount.

A special mechanism exists by which blood is shunted through the placenta at the time of uterine ischemia, prior to conversion of the conceptus from a spheroid to a cylinder. This shunting takes place by virtue of the existence of two more or less discrete vascular areas in the uterus arising from a common arterial supply. One area (placental) is along the mesometrial border of the uterus, the other (intramural) is over the lateral and ventral aspect of the uterus. The uterus, by virtue of the shape imposed upon it by the conceptus, is subjected to greatest tissue tension on its lateral aspect. That this tension offers resistance to the flow of blood is shown by resistance to passage of dyestuffs and other substances injected experimentally through vessels on the ventral surface of the uterus around spheroidal conceptuses.

Hydrostatic factors within the uterine tissues affect the flow of blood through the blood vessels within them. Although there is a pattern of hormonal changes within the maternal organism throughout pregnancy which affects at any given time the general level of tonus of the uterine muscle,

these changes are superimposed upon a morphological relationship by which the tension to which the tissues are subjected is a function of the factors of the internal pressure and the radius of curvature at any point. Hormonal factors exert themselves by changing the pressure within the uterus through their action on the myometrium, whereas the shape and size of the conceptus influence the tension within the uterus by affecting its radii of curvature.

The uterus in premature birth. The observations on the physiology of the uterus made by Dr. Reynolds and his co-workers during the past several years, all of which have been summarized in the Year Books, could not fail, of course, to yield results of interest to obstetricians. Dr. Reynolds has been in great demand as a lecturer before obstetrical societies. In one of his lectures, given at Denver in May 1948, he outlined the bearing of his findings upon the problem of premature birth, pointing out that the phenomenon of "conversion" or elongation of the uterus during the sixth month of pregnancy, and the associated changes in the circulation, constitute a critical period at which premature birth becomes a special danger. In such studies as he has been making, however, lies the best hope of finding ways and means to recognize impending premature birth and ultimately to ward it off.

ANATOMY AND PHYSIOLOGY OF THE OVARIAN ARTERIES

In the last two Year Books (Nos. 46, 47) reference was made to the observation by Dr. Reynolds that in the human ovary, as well as in the ovary of the rabbit (and presumably of other animals), the ovarian artery has a conspicuously spiral course as it runs along the hilum of the ovary. Evidence that this peculiar condition is dependent upon the presence of estrogen

secreted by the ovary, mentioned last year, has now been published by B. Delson and S. Lubin, of the Cumberland Hospital, New York City, in collaboration with Reynolds.

The same three authors, writing on the vascular patterns in the human ovary, note that the vascular system is more complex than in the rabbit. The branches of the main ovarian artery are helical, with gradually diminishing diameter. The functions of spiraling in these vessels are thought to be (1) adaptation of the vasculature to growth of the ovary and (2) provision of a mechanism for the reduction and regulation of blood pressure within the ovary. When ovarian function diminishes with age, the spiraling decreases. The occurrence of spiraling in the prenatal ovary is presumably stimulated by maternal estrogenic hormones, and its regression, which occurs during several months after parturition, is thought to be due to the removal of their influence.

The hypothesis that coiling of the ovarian artery affords a means of rapidly reducing blood pressure in the ovary as compared with that in the systemic arteries is subject to analysis by hydrostatic theory from measurements of the arteries. Dr. Reynolds points out in the Swiss journal *Acta anatomica* that there are several mechanisms which on physical grounds may be involved in such a reduction. One of these is the rapid decrease in diameter of the ovarian artery after it enters the ovary. Another is the effect which the coiling has in increasing the length of vessel to be traversed by the blood per unit distance along the hilum of the ovary. Reynolds then proceeds to show by hydrostatic theory that the coiling acts also to favor streamline or axial flow under conditions which would give rise to turbulent flow if the vessels were straight.

Angles of branching in the ovarian ar-

tery. In the ovary, more than in most other organs, there is much internal change of structure. The rapid growth and regression of the follicles and the corpora lutea, and the frequent occurrence of minor or major pathological structures (cysts), continually produce alterations in the pattern of the organ and of its constituent elements, particularly the blood vessels. In spite of this, the ovary seems to obey the general law that the blood flow is approximately equal in all parts of an organ. Older writers (Thoma, Hess) long ago laid down certain general principles which are involved in the equalization of blood flow in organs, basing them upon the ratio of the diameters of arterial branches to those of their main stems, and upon the angles formed between the stem and its branches. Dr. Reynolds, in the same article to which reference was made in the preceding paragraph, has sought to find a formula for the angulation of branches of the ovarian arteries. Using one of his corrosion preparations of the rabbit's ovary, in which the channels of the blood vessels are preserved as casts in solid plastic, he measured the angles of branching. The result confirmed a general principle set forth by Sir D'Arcy Thompson in his famous book *On growth and form*, which states that the normal pattern of the blood vessels is such that the circulation can be maintained with minimum effort and a minimum of wall surface. The essential factors in branching arteries which govern this relationship are (i) the diameter of the branch relative to that of the stem, and (2) the angle of branching. It follows from these facts that there must be a relation, expressible by a mathematical formula, between the diameters of arteries and the angles of branching. Dr. Reynolds finds that the following formula fits the specimen he has intensively studied: K (coefficient of arterial branching) =

diameter of branch divided by diameter of stem X cosine of angle of branching.

The article is illustrated with a beautiful three-color half-tone stereoscopic picture, removable for use in the stereoscope, from a photograph by Chester F. Reather.

PHYSIOLOGY OF MENSTRUATION

Prostigmine. In 1940 Soskin, Wachtel, and Hechter reported that the cholinergic drug prostigmine, when administered hypodermically to women suffering from delayed menstruation, will bring about menstruation within three days. Since a woman who is not menstruating (because of pregnancy) does not respond in this way, Soskin and his colleagues suggested that the finding could be made the basis of a test for pregnancy. They explained the induction of bleeding by prostigmine by certain elaborate pharmacological assumptions involving the mechanism of menstruation and its failure in amenorrhea. The finding has been widely advertised in pharmaceutical literature, and there have been numerous clinical papers supporting the observation, though few of them are scientifically critical. For this reason Dr. Corner tried prostigmine in monkeys, soon after the Soskin report appeared, administering it during the summer amenorrhea that is characteristic of rhesus monkeys in captivity in our climate. Menstruation was not induced. These unpublished experiments were greatly extended by Dr. Irwin H. Kaiser during his recent incumbency of a fellowship in the Department of Embryology. He found that prostigmine does not induce menstrual bleeding in spontaneously amenorrheic animals, or in castrated animals during estrogen treatment; nor does it alter hormone-withdrawal bleeding following courses of estrogen, estrogen with progesterone, or a sequence of estrogen-

progesterone-estrogen simulating the normal cycle.

These conditions under which prostigmine failed to induce menstruation in monkeys are not exactly similar to the kinds of amenorrhea in human patients in which it is claimed to be effective. They do not therefore of themselves invalidate the results on humans, but they do call for greater caution than has been used by some of the clinical writers.

Estrogen and the endometrial coiled arterioles. Many students of menstruation during the past twenty-five years have been tempted to assume that the coiling of the arteries of the endometrium is in some way causally related to the bleeding of menstruation, because (speaking approximately) the phenomenon of menstruation and the phenomenon of coiling of the endometrial arteries both occur only in the higher primates (man, apes, and Old World monkeys). In Year Book No. 47 reference was made to Dr. Kaiser's observation that a process resembling menstruation occurs in certain New World monkeys which do not have coiled endometrial arteries.

In a subsequent publication he now reports having produced experimentally in rhesus monkeys, which normally have coiled endometrial arteries, a condition in which they lack such arteries, and yet undergo bleeding of a type usually thought identical with menstruation. This was done by giving massive doses of estrogenic hormone to castrated rhesus monkeys. Discontinuance of the injections was followed, as expected, by menstruation-like bleeding. Post-mortem examination of the uterus under similar experimental conditions (i.e. after 4 weeks of massive dosage) revealed excellent proliferative development of the endometrium but virtual absence of coiled arteries.

In the *American Journal of Obstetrics*

and *Gynecology* for December 1948 Dr. Kaiser reviewed the newer concepts of menstruation. It has been known for some years that the menstrual blood flow results from a sudden drop in the concentration of ovarian hormone (estrogen) in the blood at the end of the menstrual cycle, but just why the hormone deprivation causes the blood vessels in one particular organ, the uterus, to break down with ensuing hemorrhage has been a matter of much conjecture and of largely unsuccessful experiment. A number of recent workers have set up new hypotheses involving details of the uterine vessels, i.e. the coiled arteries mentioned above; supposedly numerous arteriovenous anastomoses; an apparently inadequate lymphatic drainage. Dr. Kaiser's article reviews these hypotheses in the light of his own observations of the nonessentiality of the coiled arteries to the menstrual process. The problem remains one of the outstanding mysteries of human biology.

ENZYMES IN EMBRYONIC TISSUES

Dr. Josefa B. Flexner and Dr. Louis B. Flexner, working in part under an American Cancer Society grant recommended by the Committee on Growth of the National Research Council, have added another contribution to their series on biochemical and physiological differentiation during morphogenesis. They are currently interested in studying the turnover of phosphorus in the metabolic processes of growing tissues, in relation to the rate of growth and the degree of differentiation of the tissues in form and function. The present article deals with the presence and activity, in the fetal brain and liver of the guinea pig, of two enzymes that are concerned with phosphorus metabolism, namely adenylypyrophosphatase ("apyrase") and acid phosphatase.

The first of these (apyrase) is a substance or group of substances found in animal cells which has the property of releasing energy for use in bodily work from energy-rich phosphorus compounds such as phosphocreatine and adenosine triphosphate. A similar enzyme in muscle, adenosine triphosphatase (myosin), has been isolated and identified chemically. In other tissues the chemical nature of the enzyme is not so clearly defined, hence the use of a different name, adenylypyrophosphatase, or apyrase for short.

The authors find that a critical time with regard to apyrase in the brain cortex of the fetal guinea pig is reached at the 42d day of gestation. At this time the activity of the enzyme suddenly begins to rise from a relatively low level toward the high concentration which is found at the time of birth and in young animals. Much the same is true of the fetal liver. The activity in both tissues is still higher in older animals.

Acid phosphatase, which splits the phosphates in acid environments, is widely distributed in the body. It presents a challenge to the student of animal metabolism by reason of our ignorance of its function. In the fetal liver this enzyme was found by Flexner and Flexner to follow a curve similar to that of apyrase. In the brain cortex, however, it behaves differently, for here it is at a constant level of activity about twice as high as that found in the adult.

PERMEABILITY OF THE HUMAN PLACENTA

Frequent reference has been made in these reports to the researches led by Dr. Louis B. Flexner on the function of the placenta, and to the joint efforts to apply his methods to the human placenta in which Dr. Louis M. Hellman and Dr. G. J. Vosburgh, of the Department of

Obstetrics of the Johns Hopkins Hospital and Medical School, have been associated. These three, together with Dr. W. S. Wilde and Mr. N. K. Proctor, have now published another contribution in which they report on the permeability of the human placenta and the supply of water to the human fetus, as studied by the use of deuterium oxide (heavy water) as the tracer substance. Observations were made upon 7 patients whose pregnancies were being terminated by abdominal operation at various stages of gestation, because of serious conditions involving danger to the mother. Measured small amounts of deuterium oxide made isotonic with sodium chloride were injected into the mother's veins about 10 minutes before the obstetrician delivered the fetus and clamped the umbilical cord. The amount of the heavy water which passed from the mother's blood through the placenta to the fetus was ascertained in the case of the older viable fetuses from blood samples, and in the case of the younger, nonviable fetuses by direct extraction of the fetal tissues. The result, as expressed in a graph showing the rate of transfer of the water, agrees with the experience of Flexner and his colleagues with regard to the transfer of sodium through the human placenta, and of several other substances in animals. In view of the laborious nature of the experiments the authors feel that they are justified in presenting conclusions from only 7 patients. There was a fivefold rise in permeability of the placenta to water from the 14th week of pregnancy (the earliest case in the series) to a peak at the 35th week, and thereafter a sharp decline to term. At the peak, the very large quantity of 3.6 liters of water per hour crossed the placenta to the fetus. The placental transfer coefficient for water is five times as great

as that for sodium at corresponding periods of gestation. The human fetus receives across the placenta at the 14th week of gestation 700 times and at the 31st week 3800 times as much water as is incorporated in the growing tissues.

CAPILLARY PERMEABILITY

The use of substances such as heavy water and radioactive salts, which do not significantly differ from the ordinary water and salts of the body in their physiological activities, but are easily identified as they travel through the body by their weight or radioactivity respectively, is not of course limited to the study of special problems, such as the rate of placental transfer, in which Dr. Flexner first used such substances. He and his colleagues have in fact for some years been applying the tracer method to the more general problem of the permeability of the blood capillaries. As participants in the Cold Spring Harbor Symposium on Quantitative Biology in the summer of 1948, Dr. Flexner, Dr. Dean B. Cowie of the Department of Terrestrial Magnetism, and Dr. Gilbert J. Vosburgh of the Department of Obstetrics, Johns Hopkins University and Hospital, presented a summary of the joint studies in which they and others (A. Gellhorn, M. Merrell, R. M. Rankin, R. O. Scholz, W. S. Wilde) have taken part.

Among the four major problems to which they have contributed, the first is that of the rate of exchange of water, of sodium, of chloride, and of iron (in the physiologically important form of ferric betai-globulinate). These substances, marked by their weight (heavy water) or by being made radioactive, are injected into a blood vessel and the rate of their escape, as measured by the amount still in the blood stream after various time intervals, is determined. The results show in the

first place that the substances named pass through the walls of the capillary blood vessels into the surrounding tissues at different rates for different substances. The average capillary wall of the guinea pig is 23 times as permeable to water as to sodium and to chloride, and at least 100 times as permeable to water as to ferric betai-globulinate.

In man, 78 per cent of the blood-plasma sodium and 105 per cent of the plasma water is exchanged per minute with extravascular sodium and water. An amount of water equal to a man's entire weight passes out of his blood capillaries, and is replaced by an approximately equal amount, every 20 minutes. The capillary part of human blood circulation, seen in the light of these facts, is a system of fine tubules with permeable walls through which floods of water bearing salts and other metabolic substances are pouring at every moment throughout life.

A second problem which is largely solvable by the tracer method concerns a much debated hypothesis that whereas water and dissolved gases pass through the whole of the thin protoplasmic wall of the capillary, i.e. through the endothelial cells as well as the intercellular cement, the only important avenue for escape of the electrolytes is the cement substance, much as if one should say that water running through hollow tiles could leak through both the tiles and the joints between them, but salt only through the joints. Flexner, Cowie, and Vosburgh make a calculation based on an estimate of the area of the capillary wall to which 1 ml. of plasma is exposed, on the diffusion constant of a salt ($KC1$), and on the concentration gradient of the salt in the blood plasma. From these data the theoretical amount of potassium chloride which can diffuse across the interface per day can be worked out. The amount of radioactive chloride which actu-

ally moves across the capillary wall in a unit of time was previously measured (Cowie, Flexner, and Wilde, 1941). This proves to be about what would be predicted if the electrolyte were diffusing through the whole available wall, rather than through the far smaller area of the intercellular cement. The same deduction may be made for water and for sodium.

A third problem is to determine whether, as has been thought by some workers, the dissolved substances of the plasma pass the capillary wall "in bulk"; that is, do all go across in the same concentration that exists in the plasma, or do they cross the wall in different proportions? Results show that the latter supposition is correct. The fluid passing through the capillary wall is found to contain a far smaller proportion of ferric globulinate than of chloride to the amount of these respective substances in the plasma. The iron-protein compound is thus largely held back within the capillary while the water and salts freely interchange with the same substances in the tissues.

Finally, the method permits deeper insight into the physical processes involved in passage of the capillary wall by these substances, indicating that diffusion rather than filtration is the essential process.

Transcapillary exchange of iron; assay of iron. A separate paper by the same three authors, presenting the observations on which the above statements about the transcapillary exchange of iron were based, will be found listed in the bibliography appended to this report. In order to make accurate determinations of radioactive iron in biological material, an improved method of assay had to be developed. This also is cited in the bibliography.

THE AMNIOTIC FLUID

The fluid that exists about mammalian embryos and fetuses within the amniotic

membrane, serving to float the embryo and protect it from physical shock, presents interesting problems concerning its formation and the rate of exchange of its water with the water of the mother's blood, from which it is ultimately derived. The amniotic fluid has generally been considered to be rather stagnant. Some writers have thought that it consists mainly of urine discharged by the fetus. That dyestuffs may be made to pass into it from the mother's blood has been known. Flexner and Gellhorn applied the isotope technique to the problem in 1942, using guinea pigs, and found that the water of the fluid is replaced at the surprisingly rapid rate of about once an hour, whereas the rate of replacement of the sodium is about 50 times slower. The transfer of water and sodium to the human amniotic fluids has now been measured, using deuterium oxide (heavy water) and radioactive sodium (Na^{24}) as tracer substances. The observations required a team of specialists, namely two obstetricians, G. J. Vosburgh and L. M. Hellman, of the Johns Hopkins Hospital, Department of Obstetrics; a physicist, D. B. Cowie, of the Carnegie Institution's Department of Terrestrial Magnetism; and three physiologists, L. B. Flexner, W. S. Wilde, and N. K. Proctor, of the Department of Embryology. The tracer substances were injected intravenously into pregnant women who were undergoing operative procedures involving surgical exposure of the uterus. At measured intervals after the injection, samples of the amniotic fluid were drawn through the wall of the intact uterus with needle and syringe and were submitted to measurement of the deuterium oxide and the tagged sodium. Details of the method and calculations are given in the paper. It was found that the water of the human amniotic fluid is completely replaced on the average once every 2.9 hours. The sodium

is transferred about 5 times more slowly than the water. At term a fetus weighing 3.4 kg. is surrounded by approximately 1000 cc. of amniotic fluid which is exchanging water at the rate of 350 cc. per hour. This is by no means a condition of stagnation; indeed, it is astonishingly rapid. The fetal urine alone cannot account for so much water, which therefore is probably furnished directly through the amniotic membrane.

SEX RATIO OF ABORTIONS

Wherever human birth records are kept, it appears that among newborn infants boys are slightly more numerous than girls. In the United States the sex ratio for live births is 105.6 boys to 100 girls; for stillbirths, 124.1 to 100, and for the two groups combined, 106.0 to 100. The sex ratio at the time of conception in man ("primary sex ratio") is not accessible to direct observation. Dr. E. Carlton MacDowell, of the Department of Genetics, arrived some years ago at the sex ratio of mice at conception by an ingenious method requiring surgical exploration of the mother's ovaries, and found it approximately 1:1. If the primary sex ratio in man were also 1:1, the figure of 105.6 to 100 at birth would mean that a slightly greater number of female embryos than of male succumb during gestation. On the contrary, however, widely accepted reports made during the past hundred years have asserted that many more male embryos and fetuses than female succumb during gestation. The sex ratio of aborted fetuses has generally been reported still higher in the earlier months of gestation than in the later months and at birth. Four of the largest reported series of observations when combined give a sex ratio of 224 (males to 100 females) in the 4th month, 143 in the 5th, 121 in the 6th, and 114 in the 7th

month of gestation. The primary sex ratio has therefore been estimated by various writers, by extrapolation from such figures, at from 125 up to 170. No sound reasons for any such excess of male conceptions, or for such high mortality of male infants in utero, have been put forward.

Dr. Christopher Tietze, a physician and statistician associated with the School of Hygiene, Johns Hopkins University, has reinvestigated the sex ratio of abortions by analyzing the records of the Department of Embryology, which provide the only large series of sex determinations of human fetuses made entirely by trained observers under uniform conditions. A similar analysis published in 1921 by Dr. A. H. Schultz, then a member of the Department, gave an unweighted mean sex ratio of 109 for the 3d to the 7th month of pregnancy inclusive. The Carnegie Collection, having grown greatly, now affords more material for statistical study. Dr. Tietze abstracted from the main catalogue of the Collection records of 5667 fetuses from the 4th to the 7th month. Their sex had been determined by members of the research staff or by trained technicians. This series was supplemented by 120 specimens of the 3d month, the sex of which was determined by microscopic examination of the gonads, largely by the late Dr. George L. Streeter. The help given Dr. Tietze by Dr. Streeter was the last of the latter's many co-operative services to fellow scientists. These 120 specimens of the 3d month had a sex ratio of 93.5 males to 100 females. The fetuses of the 4th, 5th, 6th, and 7th months had ratios of 107.7, 115.6, 109.7, and 100.6 respectively. The mean ratio of the 5787 cases was 107.9. Dr. Tietze's analysis therefore reveals no evidence for a primary sex ratio materially different from that found at the end of pregnancy among the combined live and stillbirths. He tentatively ascribes the

widely different reports based on other material to errors in determining sex made by medical men without special training in embryology, or by midwives. Such errors are the more likely to occur, the earlier the fetuses and embryos studied.

NUMBER OF YOUNG AT BIRTH; NUMBER OF NIPPLES

Dr. Adolph Schultz, of the Department of Anatomy, Johns Hopkins Medical School, a former member of the Department of Embryology, from time to time makes use of the Carnegie monkey colony records and specimens in his biometric studies of primates, as for example in an interesting recent report on the number of young at a birth and the number of nipples in primates. The Carnegie colony of rhesus monkeys has had one twin birth in 188 pregnancies. Schultz has assembled records on twinning in other monkeys, which suggest that twinning occurs in most catarrhine monkeys and in apes with a frequency not far different from that in the human species. A frequently cited theory that twinning in man is a newly acquired character, from the evolutionary standpoint, is not supported by this study.

In the entire suborder of simian primates, 2 pectoral nipples represent the normal condition. Supernumerary nipples, however, have been found in 40 monkeys and apes from 10 species representing all the major groups except the Semnopithecinae. They are not uncommon in human beings. Dr. Harold Speert's Carnegie cases in the rhesus monkey, referred to in Year Book No. 41, and those now reported by Schultz, give a rate of frequency of 14 and 1.1 per cent respectively, which is close to that in man. The general percentage frequency in monkeys, apes, and man is very similar and lies not far above 1 per cent. The primitive character of 2 or 3 pairs of

nipples is more common in the lower primates (prosimians), but the number of nipples varies from species to species and often even within the species.

PALATINE RIDGES OF PRIMATES

In the majority of mammals the lining of the hard palate bears ridges, more or less transversely directed, which differ widely in number and relative size. In the human species the palatine ridges are reduced in number and are usually limited to the area in front of the first molar teeth, but any reader of this report will find, by passing his thumb over the front part of the roof of his mouth, that he possesses 2 to 8 ridges, the average in man being 4.2. Many conclusions have been drawn from the palatine ridges about the evolutionary relationships of mammals, including the relation of man to the other primates. Two great comparative anatomists, Retzius and Gegenbaur, have stated that the human ridges are more numerous in the fetus than in the adult, thus implying that the reduction in number seen in humans occurs ontogenetically, i.e. in the individual, as well as phylogenetically. Volume 33 of the Contributions to Embryology contains a thorough quantitative study by Dr. Adolph H. Schultz of the comparative anatomy of the palatine ridges in primates generally and of their ontogenesis in man. Thirty-two human fetal palates studied by Dr. Schultz were obtained from the collection of the Department of Embryology.

Dr. Schultz concludes that the primitive common ancestor of the primates had numerous large, regular palatine ridges, reaching back to the last molar teeth. This is the usual condition in a great variety of lower mammals. The ridges have become significantly changed in many

groups of primates, and the alterations reveal phylogenetic trends. Dr. Schultz's article must be read to appreciate the variability. He finds that the ridges can be recognized in a human embryo of 28 mm., making their appearance prior to the normal embryonic fusion of the two lateral palatine processes. Thus failure of the latter to fuse, resulting in cleft palate, does not prevent the formation of ridges. The average number of ridges is the same in

fetuses as in adults; this means that there is no reduction in their number during growth of a human individual. The palatine ridges of single-ovum twins show only a limited degree of resemblance, and it appears therefore that the details of the ridge pattern are not closely determined by heredity, although the general degree of regularity and the tendency toward unification and discontinuity of the ridges must have a genetic basis.

DIFFUSION AND POPULARIZATION OF RESULTS

In September and October 1948, Dr. Corner presented lectures, based in large part on work done by himself and other members of the Department of Embryology, before the Linn County Medical Society, Cedar Rapids, Iowa; the Hollywood (California) Academy of Medicine, the San Diego Academy of Medicine, the Southwestern Pediatric Society (Los Angeles), the Los Angeles Obstetrical and Gynecological Society, and the New Mexico Clinical Society. Dr. Burns addressed the Regional Post-Graduate Seminar of the American Urological Association at Buffalo in January 1949, on "The hormones and the differentiation of sex in the mammalian embryo," and also spoke at the University of Rochester on the same subject. Dr. Reynolds gave lectures and talks on problems of uterine physiology

during the year at the University of Maryland, the New York Obstetrical Society, and the Obstetrical Society of Boston. Drs. Corner, Burns, Flexner, Reynolds, and Tyler each gave one or more lectures by invitation before classes in anatomy, physiology, and obstetrics of the Johns Hopkins University, School of Medicine.

Mr. Chester F. Reather, departmental photographer, was given the first award for gross-specimen photography of the Biological Photographic Association for a picture shown at the Association's 1948 annual convention at the University of Pennsylvania. The photograph, a superb picture of a human embryo in its membranes (Carnegie Collection, no. 8537A), has been reproduced by half-tone in *Medical Radiography and Photography* (see bibliography below).

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DEPARTMENT OF GENETICS

Cold Spring Harbor, Long Island, New York

M. DEMEREC, *Director*

Members of the Department continued their studies of the nature of the hereditary materials, genes and chromosomes, using a variety of approaches and several different organisms in their experiments. McClintock's cytogenetic studies on maize have given further evidence of the biological complexity of a gene locus, and are revealing one of the possible mechanisms through which genetic instability may be attained and gene differentiation increased. Corroborating evidence of the complexity of a gene locus has been obtained in the work of Demerec and his collaborators with a strain of the bacterium *Escherichia coli*. The biochemical work carried on by Kaufmann, McDonald, and their group with chromosomes of *Drosophila* and several plants is beginning to throw light on the chemical organization of these structures. Studies by Caspari and Dalton, dealing with pigment development in the meal moth and the axolotl, have analyzed the mechanism of the gene action controlling this process. The work of MacDowell and his collaborators with mice has gone farther in demonstrating the importance of factors such as virus infection and mother's and nurse's age in the development of leukemia. Efforts to discover chemicals capable of inducing mutations in *E. coli* have been continued by Demerec, Bertani, and Flint, using a newly developed method involving reversions in this material from the streptomycin-dependent state. Witkin, also working with *E. coli*, has found that sodium nucleate has a striking effect on the time of expression of certain mutants. Doermann investigated a strain of bacterial virus that shows

a high rate of mutations; and Delaporte made cytological observations of the strains of bacteria being used in our various studies. Dobzhansky spent fifteen months in Brazil, studying the dynamics of *Drosophila* populations in tropical and subtropical environments.

The study of mutable loci in maize was continued by McClintock in an effort to determine the mode of origin of mutable loci from normal loci and the nature of events occurring at such a locus to bring about detectable changes in its phenotypic expression. Investigations this year were confined to the *Ac* (activator) locus and to the group of Δ -controlled mutable loci, which require the presence of this second locus for expression of their mutability. It was concluded that the origin of all mutable loci in this class is associated with the transposition of a single locus, *Ds*, from one position in the chromosomal complement to another. The insertion of *Ds* at or near a normal locus may inhibit the action of the normal locus, and thus change its phenotypic expression. Continued changes in the phenotypic expression of the affected locus are related to subsequent events that partially or completely remove this inhibitory action by removal of the *Ds* locus, or that change the constitution or position of *Ds* with reference to the affected locus. The events occurring at *Ds* to bring about such changes result from compound chromatid breaks at this locus. The change in expression of the affected locus depends on the type of fusion of broken ends that follows such breakage events. In several cases, the insertion of *Ds* into or adjacent to a normal locus has

made it possible to analyze more fully the composition and action of the normal locus. This analysis has revealed that one of the normal loci studied is compound, in that it is responsible for at least two reactions associated with the appearance of a single end product. *Ac* itself is a mutable locus. It also undergoes transpositions from one position in the chromosomal complement to another. The mechanism responsible for the transposition of *Ac* and the production of various alleles of this locus is probably the same as or similar to that associated with transpositions and changes of the *Ds* locus.

The patterns of mutation in *E. coli* to streptomycin resistance and dependence, and of reversion from dependence to non-dependence, were studied by Demerec through analysis of the various properties of about 280 separate mutants. Most of these mutants were found to differ from one another, indicating that a reversion from dependence to nondependence probably comes about not through a reversal of the chemical reaction that originally produced the dependent mutant, but through some other change in the *gtnt* complex. Tests made with the K-12 strain of *coli* by Lederberg's method indicate that either a single locus or adjacent loci are responsible for these various mutants. As a rule, each mutant shows the effect in more than one characteristic.

Bertani, Demerec, and Flint investigated mutagenic activity in several chemicals by determining their effect on the frequency of reversions in *coli* from streptomycin dependence to nondependence*. Induction of mutations was observed with the following chemicals: formaldehyde, acriflavine, phenol, caffeine, ethyl carbamate, ammonia, and ferrous chloride. In comparison with radiations and mustard compounds, all these except ferrous chloride

have a low order of mutagenicity. Ferrous chloride in experiments with *coli* is as strong a mutagen as X-rays, ultraviolet rays, or nitrogen mustard.

Demerec also studied in *coli* the patterns of resistance to aureomycin, chloromycetin, and neomycin, and found that there is a stepwise increase in resistance to each of these three antibiotics.

The effect of sodium nucleate on the phenotypic expression of delayed mutations to phage resistance in *E. coli* was investigated by Witkin and Flint. Exogenous nucleic acid can apparently take the place of division in causing delayed mutations to become phenotypically effective.

A new technique for the study of the mutagenic action of certain chemicals was developed by Witkin. The method involves the treatment of bacteria with an inhibitory or toxic compound, the action of which can be specifically reversed by the addition of another compound. This inhibition-reversal system provides a sensitive method for the detection of mutations induced by the inhibitory agent.

Doermann continued investigation of the intracellular growth of bacteriophage along several lines, with emphasis on the genetic approach to this problem. He showed that genetic recombinants are present among the earliest-formed phage particles in bacteria infected with two genetically different phages. This result, taken in conjunction with other genetic information, shows conclusively that phage multiplication is not accomplished by simple fission of virus particles, but that some other mechanism is responsible. The previously described cyanide-lysis method for studying the intracellular bacteriophage population has been modified in such a way that the intracellular phage of individual bacterial cells can be analyzed qualitatively and quantitatively. With this modified method a

study was made to see whether reciprocal exchange is the mechanism of producing genetic recombination among bacteriophages. Although reciprocal exchange was not ruled out completely, its occurrence was shown to be highly improbable, at least during the later stages of virus development.

Doermann and Dissosway made preliminary studies on a genetically unstable series of bacteriophages. The hereditary changes observed appear to be related to alterations in the requirements of the various types for adsorption cofactors. The fact that four new types invariably appear in one plaque arising from a single phage particle implies a fairly high rate of mutation. The quantitative expression of this rate is very much exaggerated, however, by selective factors acting during the growth of the plaque.

Cytochemical studies were continued by Kaufmann, using purified enzymes. A technique of dissecting the chromosome by successive treatments with nucleases and proteases has indicated that this structure represents an integrated fabric, in which no single protein or nucleic acid may be regarded as the primary structural component. In the course of this analysis it was found that the cellular dissolution usually attributed to the specific action of trypsin is due to the removal by water of degradation products of the action of trypsin in combination with electrolytes.

Kaufmann, in extending his studies of the action of near infrared radiation on living cells, did not detect any modification by this portion of the spectrum in the frequency of mutations induced by X-rays in the bacterium *E. coli*.

McDonald found that when dilute solutions of crystalline trypsin in 0.005 N hydrochloric acid are exposed to X-radiation, the ability of the enzyme to hydrolyze

denatured hemoglobin is partially destroyed. For any one enzyme concentration, the residual activity is an exponential function of the dose. In the range of concentrations thus far studied (3×10^4 to 9×10^{16} molar) the ionic yields are not constant, but increase with increasing concentrations of trypsin.

Further studies by MacDowell with transplanted leukemia of line I, freed from a long-unrecognized virus infection, prove that the virus and not the increasing potency of the leukemic cells has been responsible for the death of hosts of a certain foreign strain that had previously been naturally resistant. Hosts of this strain are now as resistant as ever to these virus-free leukemic cells. The virus is lethal to the foreign strain but nonlethal to the strain of hosts that carry the leukemic cells. The same interpretation holds for the breakdown of a 1:1 ratio of susceptible backcross hosts. With virus-free leukemic cells of this line, this ratio has been re-established. On the other hand, the removal of a virus from another line of leukemic cells (line L) has demonstrated that intrinsic changes within the leukemic cells, and not the infecting virus, were responsible in this case for the previously observed increased susceptibility of a strain of foreign hosts.

MacDowell observed also that the age of the nurse has as certain an influence after birth, upon the incidence of spontaneous leukemia and upon the length of life, as the age of the mother has before birth, according to preliminary but unquestionable results of an experiment testing these variables. In F₁ hybrids from leukemic-strain fathers, leukemia is less frequent and life longer when the nonleukemic mothers and nurses are old. But with young mothers, old nurses transmit as much resistance to the appearance of spontaneous

leukemia and as great lengthening of life of both leukemics and nonleukemics as do old mothers with young nurses.

The mechanism of gene action in controlling development of pigment-pattern differences between the white and black strains of the Mexican axolotl was investigated by Dalton, using methods of tissue culture and embryonic transplantation. Study of chromatophores of both strains *in vitro* showed that the genes *D* and *d* do not affect the chromatophores themselves in such a way that intrinsic differences in capacity for proliferation, migration, or pigment synthesis are demonstrable in tissue cultures. Embryonic transplantation experiments indicate that the genetic differences in pigmentation of the two strains are mediated through differences in tissue environment and not in the chromatophores themselves, the white pattern resulting from an inhibitory effect concerned with the migration of pro-pigment cells. The results fail to support the view, suggested in the literature, that the mechanism of gene action in this case involves a diffusible substance necessary for melanin synthesis, contributed to pro-pigment cells by epidermis of the black strain but lacking in the white strain. Furthermore, the assumption, on which this was based, that pro-pigment cells in the white axolotl migrate as extensively as in the black, but do not produce pigment, is rendered questionable by results of Dalton's investigation. Inhibition of chromatophore migration by surrounding tissues of a particular genotype has not previously been described as a mechanism of pigment-pattern formation in amphibians. Demonstration of such inhibition in the white axolotl raises the question whether the pigmentation effects observed in other species combinations after reciprocal transplantations of epidermis may *not* in part depend on simi-

lar relations between epidermis and pigment cells.

The action of the genes *a* (red eyes) in *Ephestia* and *T* (Brachyury, short tail) in the mouse was investigated by Caspari, using serological methods. In *Ephestia*, serological differences between *aa* and *a*a*⁺ genotypes could be established by titration and absorption experiments. An antigen differentiating the two strains was demonstrated in the euglobulin fraction. In the mouse, antigenic differences between *T*/+ and +/+ organs were found both by the precipitation method and by the complement-fixation method. With the latter method, the *gent Ki*, which is closely linked and phenotypically similar to *T*, turned out to be antigenically allied to but not identical with *T*.

Caspari, breeding normal animals obtained from crosses involving *Fu* (Fused), confirmed the hypothesis that *Fu*/+ mothers adversely affect the penetrance of the gene *Fu*. In the offspring of normal animals from crosses of female normal by male Fused, there were occasional phenotypically Fused animals which bred as normals.

An enzyme system catalyzing the oxidation of tryptophane to kynurenin was studied by Caspari in homogenates from mouse liver. The enzyme was partly purified by fractionated precipitation with ammonium sulfate. The properties of the enzyme system and of the reactions catalyzed by it were investigated.

Delaporte studied the cytology of bacteria exposed to various influences such as changes in culture medium, irradiation with ultraviolet, infection by bacteriophage, culture on streptomycin, and inactivation by ultraviolet rays and subsequent recovery by light treatment. She found that all these conditions produce striking effects in the nuclear element of bacterial cells.

A project for study of population genetics of tropical *Drosophila* flies was developed by Dobzhansky, of Columbia University, Research Associate of the Institution, in co-operation with a group of investigators working, during the year 1948-1949, at the University of São Paulo and at the Instituto Agronomico do Norte, Belem do Pará, in Brazil. Samples of wild populations of *Drosophila* were collected in twenty localities, chosen to represent the various bioclimatic regions of Brazil. The collecting work required extensive travel, the distance covered within Brazil by airplane alone amounting to about 28,000 kilometers. More than 100,000 flies were collected and classified. Two species, *ZX willistoni* and *D. pro salterns*, were subjected to cytogenetical analysis, the results of which will be reported later. For the time being, it can be stated that a great amount of genetic variability, both in mutant genes and in chromosomal aberrations, has been discovered in the natural populations of the two species, and furthermore that the quantity of genetic variants present in a population shows a significant relation to the environment in which the population lives.

For the past two years Dr. Ernest W. Caspari has been with the Department as a research associate, while on leave of absence from Wesleyan University, Middletown, Connecticut. He is returning to his former position in September. While here, Caspari carried out extensive research on the action of genes, investigating the chemistry of gene-controlled development of pigment in *Ephesia*, and studying the behavior of several genes in the mouse. He took part in many of the general activities of the Department, serving as chairman of the Seminar Committee and of the Library Committee. His profound knowledge of the literature made him a welcome

participant in discussions and a helpful adviser.

The fellowship program of the Institution has brought to the Department several young members who have been a great asset to the research program. Dr. A. H. Doermann was here for two years, working on reproduction and mutation in bacterial viruses. He left in the summer of 1949 to take a research position at the Oak Ridge National Laboratory of the Atomic Energy Commission. Dr. H. C. Dalton is remaining for another year to continue his research on pigment development in the Mexican axolotl. Dr. Berthe Delaporte, of the Centre National de la Recherche Scientifique in Paris, stayed at the Department for fifteen months, working on various cytological problems with bacteria. Dr. G. Bertani is now studying mutations in bacteria occurring spontaneously and induced by various chemicals.

The research program has been expanded through a grant received from the U. S. Public Health Service in support of Kaufmann's work, and through a grant of the American Cancer Society, recommended by the Committee on Growth of the National Research Council, supporting work of Demerec. Collaboration with the Biological Laboratory adds four research members to the group. Dr. V. Bryson, biologist at the Laboratory, is studying the biological aspects of the origin of bacterial resistance to various chemicals. Associated with him is Dr. B. Prytz, chemist, who is investigating the chemical aspects of the same problem. Dr. A. Kelnar, bacteriologist, who left this summer, was successful last year in discovering the photoreactivation process, whereby microorganisms inactivated by ultraviolet irradiation partially recover after subsequent exposure to visible light. Dr. Bruce Wallace, geneticist, is studying the effect of continuous ex-

posure to radium on the genetic constitution of *Drosophila* populations.

The Cold Spring Harbor Symposium on Quantitative Biology, organized by the Biological Laboratory, brought more than 180 scientists to the Laboratory this June for a ten-day conference on the subject "Amino acids and proteins." Among the speakers at the conference were Kaufmann and McDonald, of the Department's staff. Seven of the speakers came from Europe,

and there were several other participants from foreign countries. During the rest of the summer, more than fifty scientists and their assistants from various institutions stayed at the Laboratory.

During the year the *Drosophila* stock center sent out 318 cultures to research workers, 53 of them to countries in Europe, Asia, and South America. The number of cultures sent to teaching laboratories in high schools and colleges was 666.

MUTABLE LOCI IN MAIZE

BARBARA MCCLINTOCK

During the past year the study of mutable loci in maize has been continued, in an effort to determine the mode of origin of mutable loci from normal loci and to ascertain the events occurring at a mutable locus that result in detectable changes in phenotypic expression. Progress has been made with respect to both these objectives.

As stated in previous reports, two main classes of mutable loci have appeared and are continuing to appear in the maize cultures. One class includes a number of mutable loci that undergo changes in action only when a second locus, the activator (*Ac*), is likewise present. Mutable loci of the second class do not require such an activator locus. During the past year, study has been continued only on the Δ -controlled mutable loci. The decision to confine efforts to these mutable loci was made because all of them respond to the same *Ac* locus, regardless of the diversities of phenotypic expression they represent. On the basis of this common response to the presence of *Ac*, it could be suspected that the events leading to a change in phenotypic expression are of the same nature in all the *Ac*-controlled mutable loci. What are these events? Also, why do normal,

"wild-type" loci suddenly become unstable in these cultures?

Previous reports have discussed in detail the Δ -controlled mutable *Ds* (dissociation) locus. It was shown that *Ac* may induce chromatid breaks at the *Ds* locus that are followed by fusions of broken ends, and that these fusions may result in the formation of a dicentric chromatid and a U-shaped acentric fragment. It was also pointed out that each such event is comparable, with respect to time and frequency of occurrence, to mutations of other loci that produce recognizable phenotypic changes in gene action. It was concluded that *Ac* must give rise to a specific condition in certain cells of the plant that brings about an alteration in the mode of reproduction of the *Ds* locus in these cells during the mitotic cycle. This alteration eventuates in the production of breaks in the sister chromatids at the *Ds* position, as previously described. By genetical and cytological test methods, it was possible to place this *Ds* locus at a position demarcating the proximal third of the short arm of chromosome 9. Continued study, however, has revealed a type of event involving the *Ds* locus that appears to be responsible

for the origin and subsequent behavior of all Δ -controlled mutable loci. This event brings about a transposition of the *Ds* locus from one location in the chromosome complement to another. In its new position, *Ds* responds to *Ac* just as it did in its previous position. (The position of *Ds* in the short arm of chromosome 9, where it was first detected, has been designated the "standard position.") These transpositions of *Ds* are not infrequent. In the sporophytic tissues, they usually occur late in development and in individual cells of the plant. For transposition to occur, *Ac* must likewise be present. When *Ds* is transposed from its standard position to another position in the short arm of chromosome 9, the new location may be readily determined.

THE MECHANISM OF TRANSPOSITION OF THE *Ds* Locus

A number of cases of transposition of *Ds* are now under investigation. In some of these, a gross chromosomal alteration has accompanied the transposition of *Ds*. By cytological and genetical analyses of the cases involving gross chromosomal aberrations, it has been possible to reconstruct in considerable detail the events that must have occurred to bring about a transposition of the *Ds* locus. These events are similar in all analyzed cases, and can be summarized as follows: During a mitotic cycle a condition may be produced at the *Ds* locus that results in the removal from one or both chromatids of a submicroscopic fragment of chromatin containing the *Ds* locus. Both ends of this fragment are unsaturated; and the mechanism of removal of the fragment may be a tearing process, since unsaturated ends, capable of fusion, are produced in each of the chromatids of chromosome 9 at the position where the fragment was situated. If, dur-

ing the same mitotic cycle, a spontaneous break occurs elsewhere in the chromosome complement, four additional broken ends may be present in the nucleus. Since any unsaturated broken end is capable of fusion with any other unsaturated broken end, a number of different consequences of fusion among the twelve broken ends can arise. If the spontaneous break occurs in the short arm of chromosome 9 at a position other than the *Ds* locus, several types of altered chromosomes 9 can be formed. These may have a deficiency, a duplication of a segment of the short arm—either in a normal or in an inverted order—or an inversion. On the other hand, fusions of broken ends can bring about a transposition of the *Ds* locus without an accompanying gross chromosomal rearrangement. If the spontaneous break occurs in one of the other chromosomes of the complement, a translocation between the short arm of chromosome 9, at the position of the *Ds* locus, and this other chromosome can be produced. A transposition of *Ds* may likewise accompany such an event. Examples of these various kinds of translocation and transposition have been found. Those involving transpositions of *Ds* within the short arm of chromosome 9, either accompanied or unaccompanied by gross chromosomal rearrangements, have been selected for continued investigation.

In the analyzed cases of transposition of *Ds*, the inserted segment of chromatin containing the *Ds* locus is not visible in its new position with the light microscope. It is also too small to affect detectably the percentage of crossing over in adjacent regions in plants heterozygous for the transposed *Ds* locus. Its detection in the new position is easy, nevertheless, because it behaves as it did in its former position; dicentric chromatids and acentric fragments may be produced by subsequent

breaks and fusions that now occur at this new position. Because it behaves in its new position as it did in its former position, transposition from this new position to still another position may occur subsequently.

The discovery of the transposition of the *Ds* locus, and the knowledge gained in determining the principal events responsible for it, have supplied the information needed for understanding the origin of other Δ -controlled mutable loci. It has also become possible to formulate a more direct approach for investigation of the primary effect of *Ac* on the *Ds* locus, wherever it may be, and to determine more fully the various changes that are known to occur at the *Ds* locus itself.

THE ORIGIN OF Δ -CONTROLLED MUTABLE LOCI

In Year Book No. 47 (1947-1948), the sudden appearance of an *yic*-controlled mutable *c* locus was described. It was found in a single one of the tested male gametes produced by a plant having one *Ac* locus. This plant was also homozygous for a normal *C* locus and for *Ds* in its standard position. In this gamete, the action of the *C* locus had changed. It behaved thereafter like the known recessive (*c*) but, unlike this recessive, was capable of mutating back to a normal *C* action when *Ac* was present.

Study of the $c^{TM'}$ locus has been of particular importance in revealing the factors associated with the origin and subsequent behavior of Δ -controlled mutable loci. It is now apparent that the mutable *c* locus arose when the *Ds* locus was transposed from its standard position to a position within or close to the normal *C* locus. This event occurred late in the development of the parent plant, and probably only in a single cell of this plant. No gross

chromosomal rearrangements accompanied the transposition. The chromosome 9 carrying this transposed *Ds* locus is morphologically normal. The transposition of *Ds* was recognized by the altered position of the chromatid breaks associated with *Ds* behavior and the concomitant disappearance of such events at the standard location. Both cytological and genetical test methods, used to determine the location of these breaks, were in agreement in placing the Δ -type activity at the known position of the normal *C* locus in the short arm of chromosome 9. In its new position, the *Ds* locus presumably inhibits the normal action of the *C* locus. The *C* locus, although present, does not appear to function, and as a consequence no aleurone color is produced. With respect to pigment formation, the tissue response is the same as that given by the known recessive allele, *c*, or by a deficiency of the *C* locus. This inhibited *C* locus, however, can mutate to a state that re-establishes its former action. This occurs only when *Ac* is also present in the nucleus. The restoration may be permanent. The restored *C* locus no longer shows unstable behavior in the presence of *Ac*, and it cannot thereafter be distinguished from a normal *C* locus. What occurs, then, at the inhibited *C* locus to restore its normal action?

As stated previously, the studies of a number of different transpositions of the *Ds* locus have shown that *Ds* may be removed from a chromatid and that the mechanism of removal involves compound chromatid breakage at this locus. The removed fragment containing the *Ds* locus has unsaturated broken ends, and the ends formed in the chromatid by its removal are also unsaturated and capable of fusion. It is known that *Ds* activity usually disappears completely at the $c^{TM'}$ locus when a mutation from *c* to *C* occurs. The known mechanism of removal of *Ds* from a

chromatid, gained from a study of transpositions of *Ds*, suggests an explanation of these mutations. An event leading to removal of the inserted *Ds* segment from the *C* locus would give rise to two broken ends in the chromatid. Fusion of these broken ends would re-establish the former normal gene order, and remove the inhibitory action on the *C* locus induced by the inserted segment; and as a consequence a mutation from *c* to *C* would be evident. No further changes at this locus would occur, for no *Ds* locus would be present to produce them. The *C* locus would be completely normal again. If this primary event is responsible for the *c* to *C* mutations, it also explains why a few of these mutations are accompanied by detectable transpositions of *Ds*. Transpositions could take place if a spontaneous chromosome break, elsewhere in the chromosome complement, occurred in the same mitosis that removed *Ds* from the *C* locus.

The analysis of the events occurring when *Ds* is inserted into or close to the normal *C* locus has made it possible to interpret a previously puzzling aspect of *Ds* behavior at its standard location. At this position, two contrasting "states" of the *Ds* locus have long been recognized. When one of these states (state I) is in effect, the majority of mutational events occurring at the *Ds* locus result in the formation of a dicentric chromatid and a U-shaped acentric fragment. In the contrasting state (state II), there is a markedly lower frequency at this locus of breaks and fusions resulting in the formation of dicentric chromatids or other gross chromosomal rearrangements.

The above two contrasting states of *Ds* may be recognized when it is at the *C* locus (c^{*1}). In the original isolate of r^{*1} a state I *Ds* locus was present. This was the same state of *Ds* that had been present in the chromosome before its trans-

position to the *C* locus. In kernels having this state of *Ds*, only a few mutations giving a *C* phenotype appear. This state of the *Ds* locus at c^{*x} changes rather frequently, and by a single event, to one that is comparable to state II of the *Ds* locus at its standard position. The event is made evident by a greatly lowered frequency of dicentric chromatid formation. The rate of *c* to *C* mutations rises to a frequency that is comparable to the previous rate of dicentric chromatid formation. It has been determined that the *c* to *C* mutations are associated with a simultaneous loss of *Ds* activity. This relationship indicates that the change from a *c* to a *C* phenotype is associated with an event involving the *Ds* locus itself. A normal chromosome 9 having a fully active *C* locus but no *Ds* locus is the usual consequence. An interpretation of the event leading to a *C* phenotype has been given above. On this interpretation, the two contrasting states of the *Ds* locus reflect the relative frequencies of alternate consequences of the breakage events occurring at this locus. Both types of consequence are recognized when *Ds* is at the c^{*1} locus but only those giving dicentric chromatids or other gross chromosomal abnormalities are detectable when *Ds* is at its standard position. At this latter position, *Ds* may inhibit the action of the adjacent loci, but the inhibition may not be recognized because it results in no obvious change in a readily detectable phenotypic character. In this case, neither the inhibition of gene action brought about by the insertion of the *Ds* locus nor the release from inhibition following its removal would be evident. Detection of the frequency of breakage events at the *Ds* locus would be confined to the fraction that results in the formation of a dicentric chromatid and a U-shaped acentric fragment. This fraction may be high or low, depending on the state of the *Ds* locus.

That the time and frequency of aberrant events occurring at the *Ds* locus may be the same for each of these contrasting states will be indicated in a later section. The important difference is in the consequences of the breakage events, not in the frequencies of the events themselves.

The recognition of different states of the *Ds* locus makes it necessary to consider the factors responsible for the origin of these states and the conditions present in each. Two clearly distinguishable states of *Ds* have been described above. Other states of this locus have been recognized. When *Ds* is at the *C* locus (c^{m+1}), these several states are distinguishable, one from another, by the relative frequencies of the two main consequences of events occurring at *Ds*—that is, dicentric chromatid formation or *c* to *C* mutations. At the standard position, the comparable states are distinguished, one from another, by the relative frequency of only one of these consequences—"dicentric chromatid formation. These states appear to be intermediates between the extreme state I and the extreme state II. It has been well demonstrated that a *Ds* locus giving a high frequency of dicentric chromatid formation may change at a single mitosis to one that gives a low frequency. A *Ds* locus giving a low frequency of dicentric chromatids, on the other hand, does not change to one giving a high frequency at a single mitosis. This change from extreme state II to extreme state I requires several stepwise events, reflected in the intermediate states. These observations would suggest that the individual states of the *Ds* locus are indications of the number of active *Ds* units that may be present in a small chromatin segment, and that the change from one state to another involves a change in number and/or distribution of these units within the segment. Such changes might be expected to occur as one of the consequences

of the chromatid-breakage-and-fusion mechanism associated with the aberrant events occurring at the *Ds* locus. On this interpretation, it could be concluded that the extreme state II *Ds* locus has few *Ds* units and that the extreme state I *Ds* locus has many such units; for the mechanism could readily reduce the number of units through losses at a single aberrant mitosis, but would require a series of such mitoses to build up a large number of units.

The analysis of the origin and subsequent behavior of *Ds* at the *C* locus has served to clarify some other aspects of this study of mutable loci. Why did new *Ac*-controlled mutable loci arise in these stocks? Why did a normal "wild-type" locus suddenly behave as a mutable locus? What event occurred at the locus to bring about a mutation, that is, a change in phenotypic expression? The analysis of the origin and behavior of c^{771m} has made it possible to approach these questions and to formulate a concise interpretation of the origin and behavior of the other *Ac*-controlled mutable loci. Inhibition of a locus, either qualitatively or quantitatively, by insertion of a foreign bit of chromatin can be followed by release of this inhibition if the foreign chromatin is removed, transposed, or in some manner altered in position with respect to the inhibited locus. The primary mechanism that allows for such changes at a locus is associated with compound chromatid breaks at the locus and subsequent fusions of the broken ends. In its initial aspects, it is only necessary to consider a single locus having the peculiar faculty of undergoing such breakage events, at whatever position it may be located, to account for the origin and behavior of many different mutable loci.

TRANSPOSITION OF THE *AC* LOCUS

During the past year, an extensive study of the inheritance behavior of the *Ac* locus

was undertaken. This study has established that *Ac* is inherited as a single unit. It shows typical Mendelian inheritance, with one important exception. This exceptional type of inheritance behavior is the same as that shown by *Ds*: transposition of the locus from one position in the chromosomal complement to another. Two or three per cent of the gametes of an *Ac Ac* plant may be derived from cells in which a transposition of *Ac* has taken place. These transpositions usually occur relatively late in the development of the plant. Plants derived from zygotes that have *Ac* loci in allelic positions in each of two homologous chromosomes may give rise to a few gametes with either (1) two *Ac* loci showing no linkage with one another, (2) two *Ac* loci completely linked or very closely linked, or (3) no *Ac* locus at all. When an *Ac* locus is transposed to a new position, it shows typical Mendelian inheritance at this new position. Linkage with known genie markers can be established. Here, again, exceptions may arise as the consequence of a few transpositions from this new position to still another position. The frequency of these transpositions is not high enough, however, to distort seriously the statistical data of linkage studies. It is likely that the mechanism producing transpositions of *Ac* is the same as or quite similar to that producing transpositions of *Ds*.

Ac itself is a mutable locus. It can be identified only by its action on *Ds*. Its mutations are made evident by changes in the time and frequency of *Ds* mutations. (The events at the *Ds* locus that result in either dicentric chromatid formation or a change in phenotypic expression of a *Ds*-inhibited locus will be termed "*Ds* mutations" in this account.) It is known that the number of *Ac* loci in the nucleus controls the time and frequency of *Ds* mutations. Increased doses of *Ac* loci (from 1

to 3 in the triploid endosperm) result in an increasingly delayed time of occurrence of *Ds* mutations. Similar changes in the mutational response of *Ds* will be registered after a somatic mutation in a single *Ac* locus. These responses indicate that some quantitative change may take place at the *Ac* locus when it mutates—probably an increase or decrease in the number of subunits at this locus. Thus, superimposed on those quantitative changes that can be produced by additions of whole *Ac* loci through controlled chromosome combinations in diploid tissues of the plant or in triploid tissues of the endosperm are those that can occur at a single *Ac* locus.

There is a ready method of identifying those kernels on the ears of *Ac Ac* plants that are likely to have a transposed *Ac* locus. This involves crossing plants having no *Ac* locus to plants having a single *Ac* locus in which the *Ac* state is known (determined by its effects on *Ds* in 1, 2, and 3 doses). The *F_i* plants are selfed and the *F₂* progeny grown. The *F₂* plants are then crossed by plants having no *Ac* locus but carrying *Ds* at its standard location in each chromosome 9. The ears produced by the *Ac Ac F₂* plants are selected, and an examination is made of the *Ds* mutation rates in the kernels. If, in the *Ac Ac F₂* plants, no mutations have occurred at the *Ac* locus and no transpositions have taken place, all the kernels should show the same pattern of *Ds* mutations. In other words, the control of these *Ds* mutations should be the same, since all the kernels should have two *Ac* loci in the endosperm cells and all the *Ac* loci should be alike. The majority of the kernels on such ears do show a remarkable similarity in the pattern of expression of *Ds* mutations. A small percentage of the kernels, however, are markedly different. These exceptional kernels fall into three classes: (1) those showing no *Ds* mutations at all,

(2) those showing a few very late-occurring *Ds* mutations that suggest an increase in *Ac* dosage, and (3) those showing a time and frequency of *Ds* mutations that suggest a lowered dosage of *Ac*. A preliminary test was made in an attempt to determine the reason for the changed responses of *Ds* in the kernels of types (1) and (2). Twenty-five such kernels were selected from these ears, and plants were grown from them. Tests were conducted to determine (1) the presence or absence of *Ds*, (2) the presence or absence of *Ac*, and (3) the action of *Ac*, when present, in one and two doses. Eleven of the plants arising from these selected kernels gave no evidence of *Ac* at all; the *Ac* locus was either absent altogether or completely inactive. Ten other plants had two independent, nonlinked *Ac* loci. In four plants, *Ac* was inherited as a single unit; but this unit, in a single dose, produced the same effect on *Ds* mutations that two units of the original *Ac* locus, from which it was derived, had produced.

One type of event, the transposition of *Ac*, will account for these results. If, in these *Ac Ac* F₂ plants, transposition of one of the *Ac* loci occurred in a meiotic or premeiotic mitosis, two *Ac* loci would still be present in the nucleus, but they would no longer be allelic with respect to position in the chromosomal complement. If the transposed *Ac* locus were inserted into a nonhomologous chromosome, meiotic segregations could give rise to gametes with either (1) one *Ac* locus, in its original position or its new position, (2) two *Ac* loci, one in each of two nonhomologous chromosomes, or (3) no *Ac* locus. Transposition within the same chromosome (or homologue), or insertion of the *Ac* locus of one chromatid adjacent to the *Ac* locus of the sister chromatid, would give comparable meiotic segregations with respect

to the production of gametes with two *Ac* loci or with no *Ac* locus.

In the given cross, the kernels arising from the megaspores having no *Ac* locus would show no *Ds* activity; for no *Ds* mutations occur without *Ac*. Tests for *Ac* in the plants arising from these kernels would be negative, because no *Ac* locus would be present. Kernels developing from megaspores receiving a single *Ac* locus, either in its original position or transposed but unmodified in its action, would show the characteristic effect on *Pi* mutations produced by the *Ac* locus when two are present in the endosperm. (It should be recalled that the female parent contributes two nuclei to the triploid endosperm tissue, and the male parent one.) Those developing from megaspores with two *Ac* loci, either linked or situated in different chromosomes, would give rise to endosperms with four instead of two *Ac* loci. It is known that increases in the dose of *Ac* will delay the time of appearance of *Ds* mutations, and that this effect is proportional to dosage—the higher the dose, the more effective the delay. With four doses of *Ac* instead of the usual two, the delay may be so effective that either no *Ds* mutations will occur during the development of the tissue or only a few will occur very late in the development of the endosperm. In either case, the kernels having such increased doses of *Ac* will be strikingly different in appearance from the majority of kernels, that is, those with two *Ac* loci in their endosperm cells. It was this striking difference in appearance of a few kernels on these ears that allowed the selection to be made. The analysis of the *Ac* composition of the kernels has led to the conclusion that they develop from ancestor cells in which a transposition of *Ac* has occurred.

For comparison, plants were grown from some of the kernels on these F₂ ears that

showed the characteristic type of *Ds* mutational response known to be associated with the presence in the endosperm of two *Ac* loci. Tests of the *Ac* constitution of these plants gave the expected results. One *Ac* locus was present in each of the tested plants, and its control of the time and frequency of *Ds* mutations, in one or two doses, was similar to that in the parent plant.

These studies have been expanded during the summer of 1949; but the results of the preliminary tests are sufficient to indicate the factors responsible for apparent exceptions to the expected Mendelian inheritance of *Ac*. They have also made possible an interpretation of one of the several kinds of event that occur during the development of the plant or of the endosperm to bring about pronounced changes in the action of *Ac* on *Ds*. These changes are registered by the appearance of precise sectors showing altered *Ds* responses. Tests are now being conducted to distinguish between changes in state of the *Ac* locus—that is, between changes in quantitative action of an *Ac* locus that is inherited as a single unit, and changes that are caused by an increase in numbers of such loci after transposition of *Ac*, as outlined above. The phenotypic effects of these two types of change overlap, but the causative series of events, although related, are nevertheless separable.

The mechanism responsible for transposition of the *Ac* locus has not been analyzed. It is thought likely to be the same as or similar to that producing transpositions of *Ds*. If so, some of the transpositions of *Ac* should be associated with chromosomal rearrangements. A chromosomal translocation was recognized in one of the cases cited, but it has not yet received adequate analysis.

THE ACTION OF *AC* ON THE MUTABLE LOCI IT CONTROLS

It has been emphasized repeatedly that *Ac* controls the occurrence of *Ds* mutations and that its quantitative levels control the time and frequency of these mutations. In this report, it has been shown that the mutable c^{m-x} locus is merely a transposed *Ds* locus situated at or close to the *C* locus. The analysis of this c^{TM-1} locus and of its origin from a transposition of *Ds* has suggested that all \wedge -controlled mutable loci arise from transpositions involving, originally, only one *Ds* locus. According to this interpretation, *Ac* does not control the mutability of many different loci, but only the mutability of a single locus—the *Ds* locus—wherever it may be situated in the chromosomal complement. Mutations of *Ds* in these various positions may result in changes in phenotypic expression that are strikingly different. The change in phenotype, in any one case, depends on the kind of locus that has been inhibited by the insertion of *Ds*. In their normal action, these various *Di*-inhibited loci must control quite different chemical processes. The events at the *Ds* locus that result in a return to partial or complete action of the inhibited locus must therefore involve a different series of changes in chemical processes in each case. Without an integrative understanding of the events that occur at such mutable loci, it would be difficult to understand why *Ac* should control the mutability of loci concerned with such unrelated processes, and why each such locus should respond to a particular *Ac* locus and dosage in an exactly comparable manner. There is no difficulty, on the basis of the given interpretation, in appreciating the apparent nonspecificity of control of mutable loci by *Ac* and the similarity in response of these mutable loci to changes in *Ac* state and dosage.

In order to obtain more specific information about the nature of the action of *Ac* (other than its known effects in producing chromatid breaks at the *Ds* locus and controlling the time and frequency of these breaks), combinations of *Ds* loci at various positions in the short arm of chromosome 9 have been made. These combinations were made in an attempt to answer the following question: Does *Ac* produce a cellular or nuclear condition in a certain cell, at a certain time in development, to which all *Ds* loci will respond? An instructive example for this purpose is a combination of c^{TM^1} (*Ds* at or close to the *C* locus) with *Ds* at its standard location. If a plant carrying c^{TM^1} and *wx* in its chromosomes 9 is crossed by a plant carrying c^s (stable *c*, nonmutable with *Ac*), *Wx*, and *Ds* (standard location, to the right of *Wx*), kernels will be produced that are $c^{TM^1} wx/c^{m \sim x} wx/c^B Wx Ds$. This combination should show whether or not mutations in the several *Ds* loci will occur at the same time in the same cell, and whether this response will be of the same order with one and with more doses of *Ac*. Simultaneous mutations would be revealed in these kernels provided an extreme state II *Ds* locus were present at $c^{mm\%}$ (mutation from *c* to *C* and few if any dicentric chromatid formations), and an extreme state I *Ds* locus were present in the $c^s Wx Ds$ chromosome (high rate of dicentric chromatid formation). If all *Ds* loci respond to some particular developmental change that is brought into being by the presence of *Ac*, then when this changed condition arises in a cell, a mutation of *Ds* at the $c^{m \sim l}$ locus should give a *C* phenotype in the descendent cells. A mutation at the *Ds* locus in the $c^{\circ} Wx Ds$ chromosome should also occur. A *wx* phenotype would then appear in the descendent cells, because a *Ds* mutation in the $c^s Wx Ds$ would produce a dicentric

chromatid and a U-shaped acentric fragment; this acentric fragment would carry the *Wx* locus, and consequently *Wx* would be lost from the nuclei during a mitosis. The effects produced by such simultaneous mutations of the several *Ds* loci should be visible in the mature kernel. Colored areas (the *c* to *C* mutations) should appear, and the underlying starch should be *wx*. Also, the borders of the sectors having both of these altered phenotypes should correspond exactly. In the examined kernels having these given constitutions, a high percentage of the *C* areas had underlying *wx* starch, and the borders of the sectors did exactly correspond. Exceptions were expected, and a number were observed. Some examples were: *C* areas with underlying *Wx* starch, *wx* areas with overlying colorless aleurone, *C* areas with only half of the underlying sector composed of *wx* starch, or *wx* areas with only half of the overlying aleurone layer showing a *C* phenotype. It is hoped that an extended analysis of the various classes of exceptional areas in these kernels will reveal the more unusual consequences of the events that occur at the *Ds* loci in these mutation-producing mitoses, and the resultant organization in the two affected sister chromatids.

Tests have also been constructed to determine the relation between the mutations of *Ac* and those of *Ds*. Although the analyses of these tests are incomplete, it seems apparent that *Ac* tends to mutate in the same cell in which a *Ds* mutation is occurring, or in an immediate ancestor cell. The combined evidence suggests that some condition, under the control of the *Ac* locus and depending on its state and dosage, must develop in specific cells at specific times, to produce a mutational response (chromatid breaks) at *Ds* loci as well as at the *Ac* locus itself. The consequences of such mutation are the observed changes in

genic action, transpositions or losses of *Ds* or *Ac*, and production of gross chromosomal rearrangements with or without accompanying transpositions of *Ds* or *Ac*.

MUTABLE LOCI $c^{m\sim 2}$ AND $wx^{m\alpha}$

The \wedge -controlled mutable loci $c^{m\sim 2}$ and $wx^{m\sim 1}$ were described in Year Book No. 47. A few salient facts and conclusions based on the continued study of these loci are as follows: Both loci express their mutations quantitatively. A series of alleles derived from such mutations, which show gradations of quantitative expression, have been selected for study. When *Ac* is absent, a particular expression of an allele can be held constant, for no somatic mutations of these alleles occur. When *Ac* is present, the alleles may continue to mutate to either higher or lower levels of quantitative expression. For a study of the action of any one allele, therefore, it is important that no *Ac* locus be present.

It has been determined that chromatid breaks may occur at these two mutable loci; in this respect, they are similar to c^{TM^1} . Both $c^{m\sim 2}$ and $wx^{m\alpha}$ were isolated from stocks known to have a *Ds* and an *Ac* locus. Unlike $c^{m\alpha}$ they were not detected at the time of their origin. It is therefore impossible to reconstruct the particular events associated with their origin from a normal *C* locus and a normal *Wx* locus. The presence of Dy-type behavior at these mutable loci points to a mechanism similar to the one associated with the origin of $c^{\wedge 1}$.

In the case of $c^{m\sim 2}$, the position of insertion of *Ds* into or adjacent to the *C* locus may differ from its position of insertion in $c^{m\alpha}$; for two qualitatively different types of phenotypic expression of the *C* locus result from mutations of $c^{m\sim 2}$, whereas only one type regularly follows mutations of $c^{m\alpha}$. Both types of qualita-

tively distinguishable mutations at $c^{m\sim 2}$ result in pigment formation in the aleurone layer. Within each of the two qualitative types there occurs a series of mutants showing various degrees of quantitative expression. The color intensities produced by the different mutants of both types range from a faint pink to a deep red (in *pr pr* constitutions). The two series of mutants are distinguished from each other mainly by the fact that a different diffusible substance (or substances) is produced by the members of each. Both substances are concerned with pigment formation. The diffusible substance produced by type 1 mutants may be utilized by a cell having a normal *C* locus, or by a cell having a type 2 mutant, to intensify the color of the cell pigment. The normal *C* locus and the type 2 mutants, on the other hand, both "produce a diffusible substance that can be used by type 1 mutants to intensify pigment color. Thus, the type 2 mutants and the normal *C* locus are much alike; they both produce a diffusible substance that type 1 mutants can use, and they both can use a diffusible substance produced by type 1. This relationship suggests that a normal *C* locus is probably responsible for the production of at least two diffusible substances, both of which are required for pigment formation. It also suggests that the dosage responses noted for the normal *C* locus may be the consequence of a limited production of one of these substances by a single *C* locus: the more *C* loci were present, the more of this substance would be produced and the deeper would be the pigment color. The quantitative grades of expression of the alleles within the two types of mutations arising from $c^{m\sim 2}$ may reflect the relative quantities of the two substances produced by individual members of a type—limitations in the production of one of these substances conditioning the amount of pig-

ment that can be formed, and thus the depth of color that can appear.

The conclusions derived from study of $c^{m\sim 2}$ regarding the action of the normal C locus are noteworthy, in that they consider a double function of a single unit in inheritance. This unit, concerned with pigment production in the aleurone layer of the endosperm, appears to be composed of at least two qualitatively different subunits, both of which determine the production of substances required for pigment formation. It is possible that this C locus behaves as a unit in inheritance not only because all the subunits are needed for the production of pigment, but also because a particular spatial relation of the units at the locus is required to assure a definite sequence of reactions.

Mutations of the $wx^{m\sim x}$ locus have been similarly instructive in considering the action of the normal Wx locus, but for reasons other than those just discussed for $c^{m\sim 2}$. Here, alleles showing various quantitative levels of expression are produced by mutations of $wx^{m\sim L}$. The levels are expressed by the percentage of amylose in the starch component of the endosperm cells. When only the recessive, wx , is present, no recognizable amylose starch is produced. The selected alleles derived from mutations of $wx^{m\sim x}$ form a series in which a single dose (Wx allele, wx , wx) produces quantities of amylose ranging from very little (less than 1 per cent) to as much as the normal Wx locus produces in three doses. Chemical analyses of the percentages of amylose starch produced by several of these alleles have been conducted by Miss Ruth Sager and Dr. Charles O. Beckmann, of Columbia University. These analyses have shown that the type of color reaction produced by staining with iodine is a relatively reliable indication of the approximate percentage of amylose present, interest in this case centers not so much

in the appearance of alleles having lower activity than the normal Wx locus as in those having higher activity than the normal locus. Is the normal Wx locus partially inhibited, or do the Wx alleles showing greater than normal activity arise from duplications of the locus? In Ac -carrying plants, the chromatid-breakage-and-fusion mechanism associated with mutations at the $wx^{m\sim x}$ locus or its intermediate alleles should give rise, in some cases, to duplications or multiplications of units of the Wx locus. It is hoped that a study of the different amounts of amylose produced by sister chromatids after mutation of $wx^{TM'}$, or one of the intermediate alleles, will furnish some information with reference to this question.

CONCLUSIONS

The purpose of the foregoing sections has been to indicate the progress made during the past year in attacking fundamental aspects of the origin and behavior of Ac^* controlled mutable loci. It was concluded that only two loci are involved in all these cases: the Ds locus and the Ac locus. The origin and subsequent behavior of newly arising mutable loci depends on the transposition of a Ds locus and its insertion into (or adjacent to) a normal locus, and on the constitution of this inserted Ds locus. The gene action of a normal locus may be inhibited by such an insertion. Subsequent events at this new position may remove the inserted segment and its inhibitory action altogether; or changes in the constitution or position of the Ds locus may result in changes in the degree of inhibition of the affected locus. It was also concluded that the events occurring at Ds during a mutation-producing mitotic cycle result in compound chromatid breaks at this locus, and that the observed consequences depend on subsequent fusions of the broken ends.

The fusion phenomenon, of utmost importance in these cases, calls for no new interpretations, since the fusion of newly broken (unsaturated) chromosome ends has been well investigated and could be anticipated.

Both *Ac* and *Ds* are mutable loci, for their mode of action changes as the consequence of events occurring at these loci in certain cells of the plant. Like *Ds*, *Ac* also undergoes transposition from one location in the chromosomal complement to another. The mechanism of transposition, although not directly analyzed, is possibly similar to that associated with the transpositions of *Ds*. The evidence also indicates that changes in *Ac* as well as *Ds* are associated with chromatid breakage and fusion. It is necessary to determine, then, the nature of the events occurring at these two mutable loci, during a particular mitotic cycle, that will result in the observed breakage-and-fusion phenomena. Unquestionably, these events are primarily responsible for all the observed changes at these mutable loci. It is suspected that they are associated with some aberration in the mode of reproduction of a particular type of molecule in the chromosome during a mutation-producing mitotic cycle. Both *Ac* and *Ds* are assumed to have such molecules. If the aberration involves a chemical bonding of the newly formed molecule with the original molecule, which holds at least until after the forced separation of sister chromatids during the prophase period, a rupture of the chromatid could occur at the affected locus during this separation period. It is known that the bonds holding the molecules together in a linear order in the chromosome may be ruptured by mechanical pull, and that the broken ends so produced are unsaturated and capable of fusion with other unsaturated broken ends. It is therefore necessary to assume, in this interpretation,

that the bond connecting the newly formed molecule with the original molecule is stronger than the bond holding the molecules together in linear order.

The study of transpositions of the *Ds* locus has shown that a rupturing mechanism of this type, or at least one that leads to similar consequences, must be involved. It has been established that the transposition phenomenon is associated with chromatid breakage; the *Ds* locus is inserted into a position where a spontaneous break has occurred. The transposition phenomenon is readily explained if it is assumed that break-producing events at the *Ds* locus may sometimes result in the tearing-out of a minute fragment containing *Ds* and having two unsaturated broken ends. The insertion of *Ds* into a new position would result merely from fusion of unsaturated ends. If the broken ends arising from the spontaneous break are labeled 1 and 2 and those of the fragment 3 and 4, the fusion of 1 with 3 and 2 with 4 would accomplish the transposition. The above-described process of mechanical rupture of the chromatid at the *Ds* position could result in just such a torn-out fragment. The consequence of any one rupture would depend on the type of fusion of broken ends that followed. Not only could transpositions occur, but the *Ds* locus could be lost altogether, or two *Ds* loci could enter one chromatid, leaving none in the sister chromatid. Such duplications (altered states of *Ds*) could, in turn, initiate a series of new consequences when the aberrant type of event, leading to chromatid rupture, again occurred in a descendant of this chromatid.

The analysis discussed in this report of the factors associated with the origin and behavior of Δ -controlled mutable loci in maize has led to a relatively simple interpretation of the nature of the events responsible for changes in action of the genes

involved. The types of phenotypic change that follow mutations of non- λ -controlled mutable loci are similar to those shown by the λ -controlled mutable loci. It is quite possible that the same or similar events are primarily responsible for these changed phenotypes also.

Mutable loci have been described in a number of organisms. Many of them show changes in phenotypic expression similar to those now being observed in maize. The events responsible for changes in expression of gene action may be simi-

lar in these organisms to those occurring in maize. The investigations described in this report cast doubt on interpretations that postulate a "true gene mutation," that is, a chemical change in a gene molecule, resulting in a changed specificity of its active product. Phenotypic change may well be related to inhibition of the action of a normal gene followed by partial or total release of this inhibition, together with such duplications or deficiencies of the locus as could be produced by the mechanism outlined above.

THE GENE

M. DEMEREC, B. WALLACE, E. M. WITKIN, AND G. BERTANI

During the past year our group has been engaged in studies of a number of problems dealing with *gent* mutations. In *Escherichia coli* an extensive study was made of the genetic mechanisms responsible for changes to streptomycin resistance and dependence, and reversions from dependence to nondependence. This study revealed a complex system of different mutations that can be recognized by their effect on several other properties in addition to streptomycin resistance. Results so far suggest that the majority of these mutations are due to changes either in a single *gene* locus or in adjacent loci. The effect of sodium nucleate on mutations in *coli* was studied; and the resistance patterns for aureomycin, chloromycetin, and neomycin were investigated. Survey studies of the mutagenic action of chemicals were continued, using both *E. coli* and *Drosophila*.

We were assisted in this work by Misses J. Flint, E. Lively, H. Spring, and L. Hahn, Mrs. J. Buchanan, Mr. R. Millemann, and Mr. W. Reiser, and during the summer by Miss Rada Demerec, of Swarthmore College, and Mr. Norton Zander, of the University of Wisconsin. The work was

aided by a grant from the American Cancer Society, recommended by the Committee on Growth of the National Research Council.

GENETICS OF STREPTOMYCIN RESISTANCE IN *ESCHERICHIA COLI*

Our earlier work (Year Book No. 45, 1945-1946, pp. 152-153) showed that bacteria resistant to streptomycin originate through mutations, and also that completely resistant mutants may be obtained in one step from sensitive bacteria. Even though the rate of mutation to complete resistance is very low, it is not difficult to measure, since several billion bacteria may be plated on a Petri dish with medium containing streptomycin and only the resistant will grow to form colonies. As in the earlier studies of mutation to bacteriophage resistance, extremely large numbers of individuals can be used in these experiments, and thus very exact determinations of spontaneous and induced mutability can be made. Therefore, investigations of streptomycin-resistance mutability were continued, with the purpose of developing another method in mutation studies to

complement the phage-resistance method, which we have been using with good success since 1942.

Rate of spontaneous mutation to resistance. Determinations were made by M. Demerec and E. Lively of the rate of spontaneous mutation to streptomycin resistance, using the method of independent cultures developed by Luria and Delbrück. In each experiment about one hundred broth cultures of 1 ml. each were grown to saturation, and the proportion of cultures without mutants was determined by plating the whole contents of each tube into agar-broth medium containing 25 μg . per milliliter of streptomycin. In addition, 5 cultures in each experiment were assayed to determine the average number of bacteria present. Using Delbrück's formula, mutation rate was calculated in four experiments as follows: with strain B/r, 0.73 and 1 per 10^9 ; with strain B/i, 1.3 per 10^9 ; and with strain B/6, 2.6 per 10^9 . From these data it may be concluded that for the B strain the rate of mutation to streptomycin resistance, using concentrations of 25 μg . per ml, is about 1×10^{-9} .

It has been found that a portion of streptomycin-resistant mutants are dependent on streptomycin; that is, they require it in order to divide. About 60 per cent of mutants in the B/r strain are of this dependent type.

Spontaneous reversions from dependent strains. Dependent mutants are able to pass through only a few divisions on medium without streptomycin. The number of divisions depends on the mutant itself, on the concentration of streptomycin on which it grew, and on the degree of crowding of the cells on the culture. Therefore when dependent bacteria are plated on medium not containing streptomycin, they will soon stop growing; but any mutant to nondependence (a reversion) that occurs during these divisions

will grow and form a colony. Thus when information is available about the number of dependent bacteria plated, the number of divisions passed through on medium without streptomycin, and the number of nondependent colonies appearing, it is possible to calculate the mutation rate from dependence to nondependence. Since large numbers of bacteria can be used, this method is suitable for studies of low rates of spontaneous and induced mutations.

The experimental studies of spontaneous reversion rates were carried on by M. Demerec, G. Bertani, and E. Lively.

The behavior of strain Sd-4 (B/r/Sd-4) has been analyzed in detail. The number of residual divisions on plain mutant agar for Sd-4 bacteria grown on 25 μg . of streptomycin per ml. was determined by washing the plate with 10 ml. of broth and assaying the suspension. The effect of number of bacteria plated on the number of residual divisions has also been studied. When 10^7 to 5×10^7 bacteria are plated, the number of divisions they pass through is approximately 3.5. These divisions are completed in about 12 hours at 37°C . After this time the bacteria stop dividing, but continue to grow in length, forming filamentous polynucleate cells ("snakes"). A high percentage of these long cells are still "alive" (i.e., able to recover and grow normally if streptomycin is added) after 1 to 2 days. Two facts seem to indicate that mutations can take place in the filamentous stage: (a) new mutant colonies are still appearing after as much as 5 days; (b) when cells are irradiated after completion of residual divisions, the number of mutant colonies increases. This means that an estimate of rate of mutations from dependence to nondependence calculated from the number of living bacteria present on the plate will be too high. Under the conditions stated above for streptomycin concentration and crowding, the average

number of reversions, scored after 6 to 7 days of incubation at 37° C, is 36.8 ± 2.8 (average from 17 experiments; 7 to 20 plates per experiment) per 10^8 plated bacteria. Taking into account the residual divisions the bacteria pass through, an estimate of the rate of mutation from streptomycin dependence to nondependence per bacterium per division can be calculated as 37×10^{-5} . This does not take into account the nuclear divisions occurring during formation of filaments. Among the mutants obtained, some (about 25 to 30 per cent for this particular strain) are resistant, some are sensitive. Sensitive mutants are obviously selected against as long as the dependent strain is cultured in the presence of streptomycin. Resistant mutants might theoretically form a "background" in Sd cultures (as happens with mutants resistant to phages); but timing of the appearance of the mutant colonies does not yield any evidence of such "background." In the presence of dependent bacteria and streptomycin, resistant mutants also are probably selected against.

Patterns of residual growth, rate of mutation, and ratio of resistants to sensitives among reversions are different for different dependent strains.

Mutations induced by irradiation. An extensive series of experiments, using ultraviolet rays of wave length 2537 Å and X-rays, was carried on by M. Demerec and E. Lively to determine the rate of induction of mutations from B to resistance and of reverse mutations from dependence to nondependence. Previous work with radiations (see Year Book No. 44, 1944-1945, pp. 115-116) had shown that induced mutations to phage resistance are of two types: the **so-called** "zero-point" mutations, which are expressed before the irradiated bacteria have passed through a cell division; and "end-point" mutations, which are not expressed until after at least one

cell division has taken place. It had also been found (see Year Book No. 45, pp. 143-144) that with X-rays the mutation rate is directly proportional to the dose, giving a single-hit curve, whereas with ultraviolet rays there is a disproportionately rapid increase with increased dose, indicated by a multiple-hit curve.

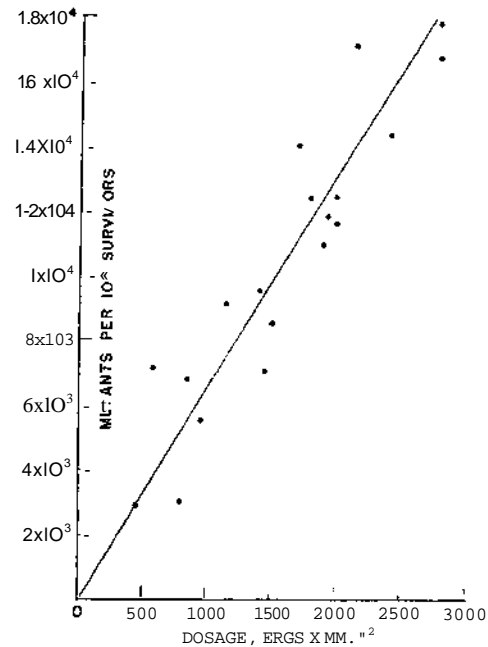


FIG. 1. Reverse mutants induced in a streptomycin-dependent strain (B/r/Sd-4) of *Escherichia coli* by various doses of ultraviolet rays.

This year's experiments showed that in the streptomycin-resistance system zero-point mutants do not appear, either among the resistant mutants or among the reversions. Studies of the relation between dosage and mutation rate, made with reversions in the streptomycin-dependent strain, demonstrated that increase in mutation rate is proportional to increase in dosage in material treated either with ultraviolet rays or with X-rays (figs. 1,2).

Studies of induced mutation from sensitivity to resistance indicated that not all

the induced mutants show up by the time surviving bacteria have doubled in number. This may be due to the same cause as the delay observed in the expression of end-point mutations to phage resistance (see Year Book No. 44, pp. 115-116).

Mutations induced by chemicals. It was found convenient to use induction of reversions from streptomycin dependence to nondependence as an index in studying the mutagenic properties of chemicals.

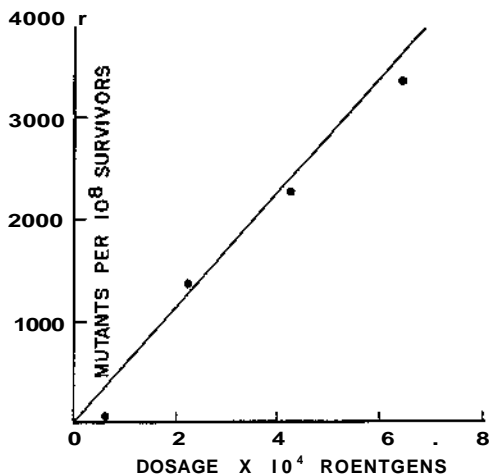


FIG. 2. Reverse mutants induced in a streptomycin-dependent strain (B/r/Sd-4) of *E. coli* by various doses of X-rays.

Resting bacteria of the dependent strain Sd-4 were treated with a certain chemical in aqueous solution, plated on broth agar, and incubated for 7 days; then the number of mutants on each plate was determined. Occasionally the treatment caused the bacteria to clump; and to avoid the variable that might thereby be introduced, the treated bacteria were examined under the microscope and used only if clumping was not evident. Untreated controls were included in each experiment.

This test—because of the difficulty of checking the amount of residual growth—is not the most favorable for quantitative

studies of relations between mutation rate, concentration of chemical, and length of treatment. It is probably the most expedient, however, when the main problem is whether or not a substance is mutagenic.

These experiments were done by G. Bertani, M. Demerec, Jessie Flint, and Eileen Yongen, who is working under a grant from the Jane Coffin Childs Memorial Fund, administered by the Biological Laboratory.

Chemical substances to be tested for mutagenic potency were chosen according to two main criteria: either because they had shown definite or probable mutagenicity in earlier genetical tests (induction of lethals in *Drosophila*, induction of resistance to phage Ti in *E. coli*, etc.), or because they represented particular chemically defined groups of substances. The data collected for various chemicals are summarized below.

Formaldehyde: Previously shown to be mutagenic in *Drosophila* feeding-technique experiments. Streptomycin test showed definite mutagenicity, even at survival levels as high as 60 per cent. Tenfold increases in number of mutations, as compared with controls, were detected in some experiments. Preliminary experiments showed that treatment with formaldehyde has no considerable effect on residual growth.

Acriflavine: Previously shown to be mutagenic in phage-resistance tests. In the present test, mutagenic effect could be detected at a survival level of less than 1 per cent.

Phenol group: Phenol is known to be mutagenic in *Drosophila*, when the treatment is applied by means of special transplantation techniques. With the streptomycin test it was also positively mutagenic, in a survival range of 0.5 per cent to 34 per cent, with increases up to tenfold in number of mutations as compared with controls. Two other substances, chemically closely related to phenol, were tested, namely, picric acid and alpha-dinitrophenol. Both showed mutagenicity of the

same order and in the same survival range as phenol.

Caffeine: Known to be mutagenic for molds. Preliminary experiments showed positive results in the streptomycin test.

Sodium desoxycholate: Previous phage-resistance tests showed a very low degree of mutagenic potency. In the present test, no induction of mutations could be detected. This may indicate that the streptomycin method of testing is less sensitive than the phage-resistance method.

Ethyl carbamate (urethane): Known to be mutagenic for *Drosophila*. In the streptomycin test, definitely mutagenic when survival is less than 10 per cent, weakly mutagenic when survival is as high as 60 per cent.

Ammonia: Detectably mutagenic only when survival is lower than 2 per cent. The same effect seems to be typical of ammonium chloride.

Inorganic acids: Phosphoric, hydrochloric, nitric, and sulfuric acids were tested. None showed mutagenic activity.

Organic acids: Acetic, formic, and lactic acids were tested. Results with this group of substances were very irregular and are still in progress. Formic acid is probably weakly mutagenic.

Alkalis: Sodium hydroxide and potassium hydroxide were tested. Neither is mutagenic.

Heavy metal salts: Copper sulfate, mercuric chloride, and silver nitrate were tested. At high survival levels (more than 10 per cent) none of them is mutagenic. Experiments with low survival ranges (which are difficult to obtain with short periods of treatment, such as have commonly been used in these experiments) are still going on.

Ferrous chloride: This compound gave a very high number of induced mutations at high survival levels. Mutagenic potency compares well with that of nitrogen mustard and radiations,

Mutation pattern of the streptomycin re si stance wstcm.* Mutants of the B strain of *coli* that are resistant to streptomycin (B^rS) or dependent on streptomycin (B/Sr) may be isolated by plating large num-

bers of the bacteria in medium containing streptomycin. Reverse mutants (R) from the dependent strain may be isolated by growing B/Sd on medium lacking streptomycin. By the same process of selection, resistant and dependent mutants can be picked up from the reversions, and from these second-order dependents another set of reversions may be obtained. By continuing the procedure, resistant mutants and reversions of higher orders may be produced. M. Demerec, Ethelyn Lively, and Helen Spring studied such a chain of mutational changes through four orders, and made an extensive analysis of 163 dependent mutants and 120 reversions isolated in this series of experiments. The following properties were studied: (1) mutation rate, (2) mutation pattern (i.e., proportions of B/S and B/Sd mutants and R and S reversions given by the different R's and B/Sd's), (3) growth rate, (4) biochemical deficiencies, which frequently are connected with these mutational changes, (5) sensitivity to ultraviolet radiation, and (6) dependence on certain degradation products of the streptomycin molecule.

From the results of all these tests together, it was evident that hardly any two of the mutants studied were alike; and we used only six out of many possible criteria to detect differences between them. This means that a reversion from a B/Sd to an R does not come about by a reversal of the chemical reaction that originally produced the B/Sd (B \rightleftharpoons B/Sd), but must originate through a change independent of the original one. Therefore there must be a considerable number of independent reactions that give rise to either streptomycin-dependent, streptomycin-resistant, or streptomycin-sensitive mutants.

Genetic mechanisms of the resistance pattern. An effort was made to find out whether one or several gene loci are involved in the control of reactions in the

streptomycin-resistance system. The most direct way of solving such a problem is to determine genetic relationship by intercrossing. Unfortunately, this cannot be done with bacteria, at least not with the B strain of *E. coli*. Lederberg, however, working with the K-12 strain of *coli*, has shown that if two lines, each carrying several heritable characteristics, are plated together, there occur interchanges between the two sets of characters comparable to the crossing over observed in higher organisms. He has worked out a technique whereby the amount of crossing over can be determined and the distances between loci calculated. If no recombination is obtained between two characters, this indicates either that their genes are in the same locus (allelic) or that they are located in closely adjacent loci.

We have partially repeated with the K-12 strain the experiments made with our B strain, and have found that they have a similar pattern of resistance to streptomycin. Intercrosses were made between six mutants of K-12/S and four mutants of K-12/Sd, all of different origin and some having special properties. The results indicate that these mutants are either alleles or located close together. The crosses were carried out by Mr. Norton Zinder, a graduate student of Dr. Lederberg, who spent six weeks with us during the summer. The work is now being extended to additional mutants of higher orders.

Correlation between spontaneous and induced mutation rate. It has already been mentioned that B/Sd mutants differ in their rates of reversion. For example, reverse mutations were observed to occur in Sd-4 with a frequency of about 3.7 per 10^7 plated bacteria, whereas in Sd-12 only one mutant was found among 4.72×10^{10} bacteria. Experiments by Demerec and Lively showed that ultraviolet treatment that in-

duced 6600 mutants in Sd-4 did not induce any in Sd-12.

Streptomycin dependence. In our studies of the streptomycin-resistance system we have accumulated a large collection of streptomycin-dependent strains of separate origin. Of these, 124 were tested by Demerec and William Belser for ability to grow on two streptomycins (dihydrostreptomycin and mannosidostreptomycin) and on five degradation products of the streptomycin molecule: (1) tetrahydroanhydrostreptobiosamine hydrochloride, (2) streptobiosamine hydrochloride, (3) streptidine dihydrochloride, (4) streptidine sulfate, and (5) streptamine dihydrochloride. The two streptomycins, and degradation products (1), (2), (3), and (5), were received from Dr. Josef Fried, of E. R. Squibb & Sons; and degradation product (4) was received from Dr. Amel Menotti, of Bristol Laboratories, and Dr. Karl Folkers, of Merck & Company.

Both the streptomycins were adequate to supply the deficiency in each of the 124 dependent strains. Degradation products (3), (4), and (5) were not adequate to supply the deficiency in any of our strains; whereas 44 strains were able to grow on (1) but not on (2), 9 strains on either (1) or (2), and 1 strain on (2) but not on (1). Considerable differences were observed among the various Sd forms with respect to rate of growth on these compounds, as well as on streptomycin.

General considerations. The analysis of the streptomycin-resistance system indicates that here a series of reactions is controlled by what appears to be a single gene locus. One change in this locus produces a mutant resistant to streptomycin; another change in the same locus produces a mutant dependent on streptomycin; another, a resistant slow grower; still another, a dependent biochemically deficient mutant; and so on. It appears that this par-

ticular locus controls a series of interrelated reactions—a chain of reactions—the end result being determined by the point at which the chain is interrupted or broken by the gene change.

This chain of reactions may be lineal; or it may be branched—that is, some of its links may serve as the starting points of new reaction chains. Our results indicate that, after this chain has been interrupted by a mutation, continuity is re-established (i.e., reversion occurs) not through a repair in the interrupted link, but rather through a change at some other point, which either re-establishes the original reaction or induces the development of a product to replace the one suppressed by the mutation.

According to information now available, this interrelated system of reactions controls the following properties: resistance to streptomycin; dependence on either streptomycin or some part of the streptomycin molecule; resistance to radiations, mustard compounds, and penicillin; some not yet identified biochemical deficiency; mutation rate; and mutation pattern. It is not associated with resistance to the seven phages of the T series. We have tested about 500 streptomycin-resistant and -dependent mutants with phages of the T series and have detected no instance of a change involving both resistance to streptomycin and resistance to phages. This suggests either that bacterial cells possess several completely independent systems of interrelated reactions, or that there is only rarely a connection between the reactions involved in different systems.

BACTERIAL RESISTANCE TO AUREOMYCIN,
CHLOROMYCETIN, AND NEOMYCIN

Our earlier work (see Year Books No. 43> *mrm4*> PP- 109-110; No. 45, 1945-1946, pp. 152-153) has shown that bacteria

resistant to penicillin and to streptomycin originate as genetic mutants. We have also worked out the patterns followed in the development of high-degree resistance to these two antibiotics. This year we studied the resistance patterns of aureomycin, chloromycetin, and neomycin in *E. coli*. The work on aureomycin was done by M. Demerec and Ethelyn Lively, and the rest by M. Demerec and Rada Demerec, who spent the summer of 1949 at this laboratory under a grant from the National Tuberculosis Association, administered by the Biological Laboratory.

Aureomycin. Results of the experiments indicate that high resistance cannot be obtained in one step, but may be built up in several steps. Because of its action on bacteria, this antibiotic was found unsuitable for detailed analysis of the resistance pattern. In addition to killing some of the bacteria, it suppresses division in the survivors. Since it loses some of its potency during an experiment, these suppressed bacteria begin to divide again when the concentration of aureomycin has decreased to a certain point. This behavior hinders the quantitative analysis of resistance.

Chloromycetin. The material used in experiments was obtained from Dr. John Ehrlich, of Parke, Davis & Company. This antibiotic becomes effective—that is, a detectable reduction in number of survivors is observed—at a concentration on agar plates of between 4 and 5 pg. per rnl. With further increase in concentration, the number of survivors drops rapidly, and at 13 pg. per ml. no survivors are found. Strains isolated from survivors of the highest concentrations are more resistant than the original strain (first-step resistance). They begin to be affected at concentrations of between 10 and 13 pg., and require up to 35 pg. for complete elimination. Second-step resistant strains are very resistant. For them the effective concentration is between

30 and 50 jg., and the lethal concentration about 100 ng.

Neomycin. The material for the experiments was obtained from Dr. Gladys L. Hobby, of Chas. Pfizer & Company. The bacteria are affected by concentrations of between 1 and 15 units per milliliter, and about 25 units are required to eliminate all of them. First-step resistant strains show a considerable degree of variability with respect to the concentrations needed to affect them. The observed range among 7 strains tested was from 4 to 15 units. The lethal concentration is between 25 and 35 units; that is, only slightly higher than the lethal concentration for the original strain. Second-step resistant strains, likewise, are only slightly more resistant than the first-step resistant strains.

Cross-resistance tests. Tests were made to determine whether strains resistant to streptomycin are also resistant to chloromycetin and neomycin. All together, 42 strains were tested with each antibiotic. All tests were negative; that is, resistance to streptomycin did not affect sensitivity to the other two antibiotics.

General considerations. Since the development of resistant strains is an important factor affecting the clinical usefulness of antibiotics, information about resistance patterns is essential for effective use of these substances in medical treatment. Our earlier work showed that first-step strains resistant to penicillin are all very uniform and are only slightly more resistant than the original strain. Highly resistant strains can be obtained only in several steps, by always selecting the most resistant survivors for further propagation. Therefore it is relatively simple to prevent the development of highly resistant strains if the concentrations of penicillin used in treatment are sufficiently high to eliminate all the bacteria present.

The situation is quite different with

streptomycin. Here the variability among first-step resistant strains is very great, and some of them are completely resistant. Since high resistance may be attained in one step, the development of highly resistant strains cannot well be avoided.

The patterns of resistance to aureomycin and chloromycetin resemble that for penicillin. Therefore it may be expected that the clinical use of these two antibiotics will not be complicated by the development of resistant strains.

Neomycin, whose antibiotic activity closely resembles that of streptomycin, evokes a resistance pattern that is intermediate between those for penicillin and streptomycin. First-step resistant strains show a considerable degree of variability, and some of them have fairly high resistance. The development of highly resistant strains can be avoided only if a sufficiently high concentration can be used in treatment to eliminate all first-step mutants. For our strain of *coli*, this concentration is not lower than 25 units per milliliter. Since strains resistant to streptomycin are sensitive to neomycin, the use of a mixture of these two antibiotics would greatly lower the required concentration of each, provided chemical action of the compounds is not neutralized in mixtures.

PHENOTYPIC EXPRESSION OF DELAYED MUTANTS

During the past year an interesting genetic effect of yeast ribose nucleic acid was observed and investigated in *Escherichia coli* by Witkin and Jessie Flint. Cultures of strain B/r grown in broth containing 0.5 per cent sodium nucleate (Schwartz) were found to yield, on the average, significantly higher numbers of mutants resistant to bacteriophage T₁ than a series of control broth cultures. Tests made during the logarithmic growth phase showed

that the increase was not due to a heightened rate of mutation during the growth of the nucleate cultures. No difference in number of mutants between cultures grown with and without nucleate was observed until the "stationary" phase of the culture cycle was well under way. The average number of mutants rose continuously, in both broth controls and cultures containing nucleate, for about 72 hours, with the nucleate cultures maintaining a significantly higher average than the controls after the end of the logarithmic growth phase. A careful investigation of the effect of sodium nucleate on the growth rates of sensitive and resistant bacteria ruled out the possibility of differential stimulation of division in resistant mutants, or more active total division in the nucleate cultures.

Observation of individual nucleate cultures led to the formulation of the following hypothesis: that sodium nucleate increases the frequency of Tr-resistant mutants by hastening the phenotypic expression of spontaneous "delayed" mutants, which would ordinarily remain phenotypically sensitive until the occurrence of one or more divisions. This hypothesis was suggested primarily by the observation that large numbers of small-colony Ti-resistant variants occasionally appeared after 48 hours in a nucleate culture known to contain only the more common large-colony type of mutant at 24 hours. Since the small-colony Ti-resistant variant is relatively rare, it is very unlikely that any one culture will contain more than one clone of this type. The amount of division occurring between 24 and 48 hours of incubation is not sufficient to explain the new appearance of a large clone. Therefore, it seems likely that the clone of small-colony Ti-resistant variants must have been present at 24 hours in a phenotypically sensitive state.

A critical test of the hypothesis that sodium nucleate develops the phenotypic expression of delayed mutants is now under way. Ultraviolet-irradiated cultures, which are known to contain extremely large numbers of induced delayed Ti-resistant mutants, are being used in this investigation. Preliminary results indicate that sodium nucleate converts delayed mutants into "zero-point" mutants; in other words, that the ultraviolet-induced delayed mutants can be detected after treatment with sodium nucleate under conditions precluding division. In the absence of nucleate, division is required before the delayed mutants become phenotypically resistant.

Similar results have been obtained with high concentrations of casein hydrolysate. High concentrations of glucose and certain amino acid mixtures fail to duplicate the nucleate effect.

INHIBITION-REVERSAL TECHNIQUE

Sodium nucleate was used also by Witkin in the development of a new technique for investigating chemically induced bacterial mutations. The inhibitory action of a number of antibacterial compounds can be reversed by the addition of sodium nucleate. Among these are acriflavine and caffeine, which have been shown to be mutagenic (see Year Books Nos. 46 and 47), and various analogues of the naturally occurring purines and pyrimidines, which have not yet been tested for mutagenic activity. The method can be illustrated by describing its application to the study of mutations induced by acriflavine. Twenty-five tubes containing 5 ml. of 0.01 per cent acriflavine dissolved in nutrient broth are inoculated with io^T bacteria from a fresh broth culture of strain B/r. The tubes are incubated for 4 hours at 37° C, at which time the survival in each tube is about

10^{-3} . Sodium nucleate is added to each tube to give a final concentration of 0.5 per cent, and the tubes are incubated 18 to 24 hours. The bacteria not yet killed by the acriflavine at the time of addition of nucleate are able to divide at the normal rate to give cultures of full titer after incubation. Each culture is assayed to determine the number of bacteria per milliliter and the number of Ti-resistant mutants per 10^8 bacteria. The frequency distribution of mutants in the nucleate-reversed series of cultures is plotted and compared with the frequency distribution of mutants in a similar series of control cultures. The control cultures are inoculated with the same number of bacteria found to be viable in the acriflavine series at the time of addition of nucleate, and are grown in the presence of 0.5 per cent sodium nucleate without exposure to acriflavine. The distribution of mutants in the control series is extremely constant, with most of the cultures in the range from 10 to 50 mutants per 10^8 bacteria. In the acriflavine-nucleate series, most of the cultures have over 200 mutants per 10^8 bacteria at the end of growth. Experiments with artificial mixtures of sensitive and resistant bacteria have indicated that selection is not responsible for the effect.

Both zero-point and delayed mutations are detected by this technique, as both types of induced mutations contribute to the final crop of mutants in the experimental cultures. The method has other advantages over those used previously: (1) The nucleate reversal effectively removes the acriflavine from further activity, more completely than several centrifugations and washings. (2) Selection due to differential survival of resistant mutants initially present in the treated population can be ruled out directly, by using inocula small enough to contain no "background" mutants. (3) Statistical error in the estimation of muta-

genic potency is considerably less than with methods in which the observation of small numbers of mutant colonies is the basis of calculation.

The inhibition-reversal method, as illustrated by the acriflavine-nucleate system, is applicable to a great many other similarly reversible systems, some of which are already under investigation.

EXPERIMENTS WITH DROSOPHILA

During the past year a great deal of work has been done in continuation of the study of mutagenic action of chemicals on the sperm of *Drosophila*. This research has been carried on by Wallace, assisted by Leona Harf, Helen Spring, Mrs. Jennie Buchanan, and Raymond Millemann. If one examines the data gathered throughout the years this program has been pursued, one finds that they indicate several fairly distinct periods: (1) During the initial period the aerosol technique was being tested with miscellaneous dyes and organic salts. The results of these experiments were uniformly negative' (Year Book No. 45, pp. 156-157). (2) A second period started with the first test of the carcinogenic substance dibenzanthracene. During this period the results indicated that certain chemicals were mutagenic; chromosomal aberrations were induced by these chemicals, and the results of different experiments using the same chemical were fairly consistent (Year Book No. 46, pp. 127-131)* (3) The third period was a short, not too distinct, time during which the variability from experiment to experiment became alarming and only occasionally was it possible to obtain confirmation of previous experiments (Year Book No. 47, p. 170). (4) The fourth period, encompassing all of the past year, has been characterized by uniformly negative results except in those experiments us-

ing nitrogen mustard, methyl- ϵ -V(beta-chloroethyl)amine hydrochloride. A summary of the experiments made during this period is presented here (table i), but a fuller analysis will be published elsewhere.

Testing the data given in table 1 for homogeneity by the Brandt and Snedecor method of computing chi square gives the

ments, we obtain a chi square of 1307 with 19 degrees of freedom; the probability of getting such a chi square from a homogeneous population is negligible, and it can be concluded that nitrogen mustard does induce mutations.

The study of lethals included analysis not only of their frequency, but also of

TABLE 1

SUMMARY OF THE FREQUENCY OF LETHALS OBTAINED DURING 1948-1949 BY TREATMENT OF DROSOPHILA MALES WITH VARIOUS CHEMICALS

Treatment	No. sperm tested	No. lethals	% lethals
Control	39,928	122	0.306
1,2,5,6-dibenzanthracene	60,758	186	0.306
\wedge -hydroxyazobenzene	7,178	19	0.265
beta-naphthylamine	1,980	5	0.253
azoxybenzene	773	1	0.129
azobenzene	1,907	2	0.105
\wedge -dimethylaminoazobenzene	2,674	7	0.262
3,4-benzpyrene	1,499	4	0.267
1,2-benzanthracene	1,185	1	0.084
methylcholanthrene	3,003	9	0.300
acetylaminofluorene	1,172	2	0.171
pyrene	1,146	2	0.175
anthracene	920	4	0.435
alpha-naphthylamine	1,296	6	0.463
pEenanthrene	1,044	7	0.670
\wedge -aminoazobenzene	741	2	0.270
\wedge -diethylaminoazobenzene	859	2	0.233
acriavine	873	2	0.229
sodium desoxycholate	1,713	5	0.292
Subtotal	130,649	388	0.297
nitrogen mustard	3,757	153	4.07
Total	134,406	541	0.403

following results: If nitrogen mustard results are omitted, the chi square for 18 degrees of freedom is 14.06; the probability of obtaining a chi square as large as this from a homogeneous population is 0.74. There is no reason, then, to conclude from these experiments that any one of these chemicals differs from any other, or from the control, in its mutagenic properties. If we include the nitrogen mustard experi-

their location. Of the 388 lethal gene mutations obtained in experiments other than the nitrogen mustard experiments, 372 were located in one or another of the five regions of the X chromosome formed by the mutant genes *ec*, *ct*, *v*, and *g*. No evidence was found that any of these 372 lethals was involved in a chromosomal rearrangement. It should be emphasized that 114 lethals arising spontaneously in

the control experiments were among those tested for position and for the presence of concurrent chromosomal rearrangements. The confidence interval, then, for the frequency of chromosomal rearrangements occurring with spontaneous lethal gene mutations is 0 to 0.03. (This interval is calculated by use of the equation $i-p = \text{antilog}\left(\frac{\log a}{n}\right)$, where p is the upper limit of the confidence interval, a is the level of significance, 0.05, and n is the number of observations, 114.) If we turn now, for example, to experiment 185 (one of the experiments using p-hydroxyazobenzene made during 1947), in which 3 aberrations were found among 35 lethal gene mutations, we can calculate the probability that these were spontaneous gene mutations occurring concomitantly with aberrations. Using the 0.03 upper limit and fitting the normal curve to the binomial with Yates' correction for small figures, it is found that the probability of getting 3 aberrations with 35 lethal mutations is 0.0375. Since this figure is obtained by use of the upper limit, we conclude that some factor other than chance was operating in experiment 185 and that the aberrations observed were not of spontaneous origin.

In conclusion, it may be mentioned that the following factors have been considered in an effort to locate the source of variability in our experiments: genetic constitution of the treated flies, solvents, temperature, nebulizers, length of treatment, killing of treated flies, size of treated flies, condition of chitin, rate of respiration (as controlled by CO₂), and condition of the M-5 tester chromosome as determined by cytological and genetical analysis.

An analysis of the relation between dominant and recessive lethals induced by nitrogen mustard has been undertaken during the past year. The purpose of this

analysis was to obtain data bearing on the theory that dominant lethals are the result of inviable chromosomal rearrangements. The arguments presented by Muller and by Lea (D. E. Lea, *Actions of radiations on living cells*, 1947) are convincing but are based on differential genetic effects of varying X-ray treatment. Since nitrogen mustard induces both gene mutations and chromosomal rearrangements, but in a ratio different from that induced by X-rays (Auerbach and Robson, *Proc. Roy. Soc. Edinburgh* (B), vol. 62, pp. 271-283, 1947), it seemed that dominant-lethal data from nitrogen mustard experiments would be enlightening.

In contrast with X-rays, whose dosage can be measured and controlled accurately, nitrogen mustard, as applied to flies by the aerosol technique, gives variable results. Because of this, it was decided that the ratio of dominant to recessive lethals in a number of experiments should be obtained and compared with similar data obtained for various doses of X-rays.

The dominant-recessive relation for nitrogen mustard (25 separate experiments) was found to be nearly identical with that expected for X-rays. Since viable rearrangements are less frequent with nitrogen mustard than with X-rays, but dominant lethals for the two are equal, it seems profitable to investigate this problem further. The observed difference may be caused either by a greater induction of primary breaks by X-rays or by the greater opportunity for restitution in the nitrogen mustard experiments.

A third series of experiments, involving X-radiation, was initiated by our group and is now being pursued at the Biological Laboratory of the Long Island Biological Association. It was necessary to know whether eggs and larvae can survive a chronic treatment of 133 r units per day.

and whether, if they do survive, an appreciable number of lethal gene mutations can be found in the adult flies.

The eggs of a large number of Oregon-R parents were collected daily for 5 days. Those collected on the first day were irradiated daily for 5 days from the day of collection, those collected on the second day were irradiated for 10 days from the day of collection, those of the third day for 15 days, the fourth for 20 days, and the fifth for 25 days. These treatments covered, respectively, the periods egg to larva, egg to pupa, egg to 5-day-old adult, egg to 10-day-old adult, and egg to 15-day-

old adult. The data obtained are given in table 2, combined with tentative data of a confirmatory experiment carried out at the Biological Laboratory.

The proportion of lethals recovered in the sperm of adult males increases linearly with time until the fifteenth or twentieth day, when it becomes stabilized. This agrees with the work of others (see Demerec and Kaufmann, *Amer. Naturalist*, vol. 75, pp. 366-379, 1941), who have found that sex-linked lethal gene mutations and dominant lethals are eliminated from sperm about 14 to 17 days after treatment of male *Drosophila* with X-rays.

TABLE 2
FREQUENCY OF SEX-LINKED LETHALS OBTAINED BY IRRADIATION OF GROUPS OF FLIES FOR VARYING NUMBERS OF DAYS

Treatment Days	r units	No. sperm tested	% lethals ± S.E.	Expected*
0	0	2107	0.190 ± 0.095	...
5	539	2564	1.21 0.216	1.44
10	1093	1102	3.99 0.590	3.26
15	1617	2454	4.60 0.423	4.32
20	2156	1336	6.14 0.657	5.76
25	2695	327	5.20 1.23	...

* Expected on the basis of a linear increase for 20 days.

CYTOLOGY OF BACTERIA

BERTHE DELAPORTE

To ascertain, if possible, whether the nuclear element seen in all bacterial cells is haploid, diploid, or polyploid—that is, whether it is a single nuclear unit or is composed, at least at certain times during the growth of a bacterial culture, of several fused nuclear units—studies have been made of the bacterial nuclear element under many different conditions of observation, and also in cells treated with radiations or chemical mutagens, during lysis by bacteriophage, and in different strains grown under various conditions.

The technique used most often was staining with Giemsa after fixation with osmium tetroxide vapor and hydrolysis with *N* HCl at 60° C. for 10 minutes. Post-fixation with alcoholic sublimate after the osmium tetroxide treatment gave identical results. Fixation of cells on the agar block before making an impression of the culture gave the same results as fixation after impression, provided the bacteria were not allowed to dry before fixation.

Permanent mounting medium. Since no good technique of mounting was known

that would allow preservation of Giemsa-stained preparations for a few months, several procedures were tried, and Abopon, a water-soluble resin used by E. Lieb to mount amyloid-stained preparations, was found to be convenient and satisfactory. Eight months after mounting, the preparations are the same as on the first day. Staining with Giemsa and with Feulgen gives the same results, but with Feulgen the nuclear element is smaller than with Giemsa.

INFLUENCE OF CULTURE CONDITIONS ON STRUCTURE

Bacteria of the same strain of *Escherichia coli* (B/r/i, for example), grown in different aerated liquid media (broth or the GR medium of Doermann) and subcultured in one or the other medium, show, at the same age, differences in length of cells and in shape of the nuclear element. Culture for 24 hours in broth followed by 105-minute subculture in broth results in very short cells, sometimes almost spherical, with large round or ovoid nuclear elements. Culture for 24 hours in GR medium followed by 105-minute subculture in broth produces medium-length cells with globular nuclear elements. Culture for 24 hours in GR and 105-minute subculture in GR produces long cells in which the nuclear element often appears as an axial rod.

When *E. coli* (B/r) is grown in non-aerated broth, it produces, in a 4-hour culture, medium or long rods with 4 to 8 nuclear elements, isolated but close together, so that they appear as transverse rods (perpendicular to the long axis of the cell), which divide lengthwise and therefore often are seen as V or U shapes.

INFLUENCE OF ULTRAVIOLET RADIATION

When cells of *E. coli* are irradiated, they form "snakes." B-strain cells, irradiated

during the exponential phase of growth, produce snakes in which the nuclear element is seen, most frequently, as a line of adjacent granules along the axis of the filament, or else as an axial line showing no distinct granules. Sometimes the granules are separated, isolated or two by two. The ends of snakes contain no nuclear substance.

ACTION OF BACTERIOPHAGE

When a suspension of bacteriophage is added to a sensitive strain in exponential growth in GR medium (T4 to strain B/r/i, for example), no change is evident for the first 10 minutes, except that in some cells a few granules are visible at the periphery, but after 20 minutes most of the cells are completely and uniformly filled with chromatic substance. (A few cells, resistant to infection, will maintain a normal aspect throughout the process.) One to four small chromatic granules are seen at the surface of some cells. After 25 minutes the membrane has lost its rigidity and sharpness, and small knobs are visible on the cells. After 30 minutes this effect is accentuated. After 38 minutes lysis begins; the cell expands considerably, taking the shape of a racket with a short handle or of a complete sphere in which the whole interior is uniformly chromatic, and then bursts, losing all its chromatic substance. Around newly lysed cells it is possible to see a large number of small points, stained with Giemsa. It is difficult to determine whether or not these are phages, or aggregates of phages, just leaving the cell. These phenomena have also been observed with the phase-contrast microscope. The infected cells remain dark during the entire time of infection and internal multiplication of the phage, and then are seen to swell rapidly into round bodies, which burst a few seconds later and immediately become transparent,

with a remnant of membrane visible as a ghost.

When phage is added to a culture of *E. coli* containing snakes induced by ultraviolet radiation, large, round swellings are observed in the snakes—one or two in short snakes, several in long ones. With the phase-contrast microscope, a few minutes after the beginning of the experiment, one *sets* transparent spaces, approximately square and occupying the whole width of the snake, like complete interruptions of its living substance. A few minutes later, these spaces swell rapidly into round, transparent bodies, sometimes 15 to 20 microns in diameter, sometimes larger. Occasionally smaller round bodies appear on the sides of snakes, but these are filled with protoplasm like the interior. Sometimes the large bodies are in the form of spindles. Staining shows granules of nuclear substance at the periphery of the large bodies. In the remainder of the snakes, the chromatin is in shapeless masses. Some snakes look like a string of beads, which are sometimes closely joined together in an irregular line, but more often separated by short threads of chromatic substance. About 35 minutes after infection, many snakes consist of lengthened masses of chromatic substance, two or three times the normal diameter of cells, separated by thinner, threadlike sections, which are sometimes very long. In living preparations, sometimes one of the large bodies bursts, leaving only debris in its place. In both stained and living preparations, some of the snakes are ghostlike, of normal or smaller diameter, with a few chromatic granules at the periphery.

Lysis by bacteriophage was also observed on a strain of *Bacillus cereus*. Ten minutes after addition of the phage, the nuclear elements of the cell fuse and 10 minutes later the whole cell interior is filled with a chromatic, homogeneous substance. At 60

minutes the cell is considerably larger in volume, the contents always homogeneous and the membrane neatly outlined. At lysis, the chromatic substance recedes from the periphery, in deeper and deeper scallops, until first only an axial rod is left, then only a few granules, which finally disappear, leaving the cell with no chromatic substance. The bacterial membrane keeps its cylindrical shape throughout the process; but the cells, which at first are in long chains, later become isolated. The above-described process occurs simultaneously in all cells of a culture.

ACTION OF STREPTOMYCIN

Antibiotics, when added to a culture, bring about changes in cell form. Penicillin, for example, induces the formation of round bodies on the cell. In certain mutant strains, the cell shape is modified only when cultures are grown on normal medium containing no antibiotic. For example, the streptomycin-dependent strain B/r/Sd, which has normal cell structure when cultured on streptomycin-agar, grows very slowly and forms "snakes" on medium lacking streptomycin. The cells first become elongated, with the nuclear substance in the shape of an axial rod, then gradually develop into long snakes. These are filled with nuclear substance, continuously or in fragments, with occasional swellings or enlargements in which the nuclear substance appears as either contiguous granules, an axial thread, or a loose net of granular filaments.

INACTIVATION BY ULTRAVIOLET AND RECOVERY BY LIGHT

Exposure of a culture of *E. coli* to an appropriate dose of ultraviolet rays causes death of 99 per cent of the cells. These cells do not at once show changes in their structure, except for the appearance of a

few small granules of chromatic substance—perhaps detached from the central mass of nuclear substance—at the periphery of some cells, most often near a pole. Observations were made on strains B and B/r. After about an hour of culture in the dark in aerated broth or agar, the dead cells appear to be smaller, and their chromatin is diffused uniformly throughout (or almost throughout) the cell so that it is more or less completely mixed with the cytoplasm. This effect persists for a number of hours before destruction of the cell is complete. The few cells that do not die maintain their normal structure and begin to multiply normally after a longer than usual lag phase. Later, in B/r (in aerated broth or M-9), some snakes are formed; these have isolated nuclear granules either in an axial line, scattered, or grouped near the middle of the snake. The cells that remain short have only one central granule, or two when they are dividing. In strain B, on agar, almost all the surviving cells form snakes, which lengthen gradually, the chromatin filling the whole interior or being more or less diffuse but localized in the middle part of the snakes. Then the chromatin separates from the cytoplasm, and takes the shape first of reticular structures, which appear as if made up of tiny mixed chromatinic threads, then of separate rectangular fragments having this reticular structure, and finally of granules, more or less closely related, arranged in squares or rectangles. At this stage new cells appear in the culture. They are large and isolated, having a sky-blue cytoplasm and two nuclear elements in the form just described, like the cells of certain young cultures. These isolated cells are found in increasing numbers in aging cultures, and there is a parallel decrease in the number of snakes.

If such ultraviolet-treated cultures are strongly illuminated for about an hour,

soon after irradiation, and then subcultured, a high percentage of the cells recovers (A. Kelner, *Proc. Nat. Acad. Sci.*, vol. 35, pp. 73-79, 1949). In strain B/r, after this light treatment followed by 2-hour culture in the dark in aerated broth, a great many cells begin to form snakes, in which the nuclear substance very often appears as contiguous granules, placed in an axial line. As the snakes lengthen, the nuclear granules are either in an axial line or scattered; exceptionally, they are in two parallel rows. Very few of the cells have the appearance of the dead cells, filled with diffuse chromatin, commonly seen in the same culture* without light treatment. Strain B on agar, given similar treatment, shows a few dead cells with diffuse chromatin, a very few small cells with central, more or less globular nuclear elements, and a large number of snakes, which lengthen progressively, and which have the same nuclear structure as the snakes that form in the absence of light treatment. Later, isolated cells are seen.

OBSERVATIONS ON BACILLI

Formation and germination of spores, and first cellular divisions in a new culture, were observed in *Bacillus mycoides*, *B. cereus*, *B. megatherium*, and *B. macerans*. In *B. cereus*, at the beginning of sporulation (16- to 24-hour culture), the nuclear substance, in almost all cells, appears in the form of several scattered granules. The number and placement of these granules are not constant; perhaps they are linked together, but if so their ties are not visible with the methods used. The one situated nearest an end of the cell becomes the nucleus of the spore. It gradually enlarges, stretching into a short rod; at the same time a homogeneous and dense cytoplasm, stained pink with Giemsa, forms around it and finally takes the ovoid shape and the

dimensions of a mature spore. The cytoplasm then changes its staining characteristics and stains sky blue; the spore is enclosed in a refractive membrane; and the nuclear element moves to the periphery, taking the shape of a short curved rod (in side view) or ovoid ring (front view), or of two or three closely related granules. During this process, the cytoplasm, nuclear substance, and lipid globules that were in the sporangium outside the spore gradually disappear—perhaps assimilated by the spore in formation. The spore is then liberated. In certain species (*B. mycoides*, for example) spores remain permanently inside a remnant of sporangium membrane, which shrinks so that only two short finger-like appendages are seen at the poles of the spore.

During germination, the spore becomes larger, and the nuclear element moves in from the periphery to the center, assuming the shape of a wavy filament or of several closely related granules, which often divide into two symmetrical parts before the

young cell emerges from the spore coat. A second, and sometimes a third, nuclear division often occurs before the formation of a visible transverse membrane inside the rod. A small granule of nuclear substance, which stains Feulgen-positive, is sometimes seen discarded on the empty spore coat; probably it is a remnant of the nuclear substance of the sporangium.

During the first divisions in a new culture of *Bacillus*, the rate of nuclear division is more rapid than the formation of transverse membranes and lengthening of the cell, so that very short cells are formed. Often their width is greater than their length, and consequently the nuclear element is in the form of transverse rods, unless deformed by near-by elements. These transverse rods divide lengthwise, often assuming V or U shapes, and the halves are pulled apart in the direction of the long axis of the cell, as happens in all bacterial divisions. The nuclear element is rarely seen in the shape of an axial thread during the early divisions.

INTRACELLULAR GROWTH AND GENETICS OF BACTERIOPHAGE

A. H. DOERMANN AND CAROLYN F.-R. DISSOSWAY

The biosynthesis of virus particles presents to the investigator a challenging, but extremely complicated, problem. Nevertheless, significant advances have been made in the past few years, especially where bacteriophage has been used as the experimental organism. The finding that genetic recombination is a predictable phenomenon, which occurs when related phage particles are growing in the same bacterial cell (Hershey and Rotman, *Genetics*, vol. 34, p. 44), is perhaps the most notable achievement so far. Not only is the result of fundamental interest in itself, but it also affords to phage-synthesis studies a specificity that is possible only by ap-

plication of the sensitivity of genetic identification.

The approach to the problem of bacteriophage growth that we are trying to develop is one which will make possible the combined application of a genetic and biochemical attack. With this plan in mind, two methods were developed whereby bacteriophage-infected cells of *Escherichia coli* can be disrupted and made to liberate their phage contents at any time during the phage life cycle (see Year Book No. 47, 1947-1948). These methods have been extended and utilized in several ways during the past year. The cyanide-lysis technique has been modified in such a way

that it can now be used to study the phage contents of single bacterial cells at any stage of the reproductive cycle. This modification and the original method have been applied to the study of genetic recombination, with the hope of finding some clue to the mechanism involved.

In addition, another problem has been encountered, which at first sight does not seem related to the plan of investigation mentioned above, but which is nevertheless expected to show a connection with it after fuller study. A genetically unstable phage type has been isolated and preliminary experiments have been made in a study of the mutability pattern of the phages derived from this stock.

TIME OF OCCURRENCE OF GENETIC RECOMBINATION

Hershey and Rotman have shown that when bacteria are mixedly infected with several T2 particles of the types rh^+ and r^+h , the progeny from single bacteria contain all four combinations of these factors, viz., rh^+ , r^+h , rh , and r^+A^+ . Their studies of about twenty independently arising r types have shown that they fall into three categories with respect to amount of recombination with the h locus. With one type there is about 2 per cent recombination. Two others yield approximately 35 per cent recombination. With a third, larger, group, about 15 per cent recombination is found. Our first question concerned the stage of phage growth at which genetic recombination takes place. It seemed likely that recombination might occur only late in the latent period, when the infected bacterium contains numerous new phage particles. Such a result would suggest that recombination takes place between the newly formed phage particles. It appeared possible, however, that the first newly formed phage particles might

already be recombinants, which would suggest that mixing of genetic material must take place even before the presence of phage particles can be demonstrated.

The experiments to test these alternatives were made with mass cultures of stocks received from Hershey. They were carried out in the manner described by Hershey and Rotman, except for incidental technical changes. The phages selected for crossing were T2Hri₃A⁺ and *TiHr⁺k*, for which Hershey and Rotman found about 2 per cent recombination. Bacteria that had been mixedly infected with these phages were subjected to the cyanide-lysis procedure at intervals after infection. Platings were made from the lysed aliquots against mixed indicator strains that permit identification of all four types of phage expected to result from this cross. The results of experiments indicate that, at a time when there are only 2.3 completed phage particles per bacterium, there is already as high a proportion of recombinants as later. It is therefore clear that recombinants occur with about the same probability among the first-formed phage particles as among those formed later in the latent period, indicating that genetic mixing takes place before the completion of any of the phage particles.

DISRUPTION OF SINGLE BACTERIA BY CYANIDE

To make a detailed genetic analysis, it is often important to observe the progeny of single crosses separately. Similarly, in studying the growth and genetics of bacteriophage it is essential in many cases to have data on the progeny from single bacteria. The next problem attacked, therefore, was that of devising a technique whereby single cells might be disrupted and thereby induced to liberate their phage contents. The method finally used is pat-

terned after the original technique of Burnet (*Brit. Jour. Exper. Pathol.*, vol. 10, p. 109, 1929), as modified by Delbrück. It is similar to the cyanide-lysis procedure except for the following details: The medium used is broth made according to Hershey and Rotman. Infected bacteria are distributed by drops to individual tubes, each tube having a probability of about 0.25 of receiving one infected cell. At a designated time the tubes are chilled and cyanide is added. After lysis has been induced, the entire individual samples are plated against mixed indicator strains.

In these experiments, T2Hris and T2HA were again used in mixed multiple infection. The results will first be analyzed on the basis of total yield of phage per cell, and then on a genetic basis. Only those bursts that contained more than one infective center were included in the analysis. Those containing a single particle were omitted for several reasons. Some of them were undoubtedly due to unadsorbed particles of the infecting phage population which escaped antiserum inactivation. Those stemming from infected bacteria fell into two groups, namely, those actually containing a mature phage particle at the time of addition of cyanide, and those not containing one. T6 was omitted from these experiments for technical reasons, and the bacteria lacking a mature phage particle would not be lysed by the cyanide alone. They would thus make a plaque on the plate, because the cell would recover from the cyanide there, resume synthesis of phage, and lyse. To omit the single plaques from the Poisson analysis seemed justified inasmuch as they would not materially affect the estimation or the distribution of the other bursts.

Five experiments were made, and 159 of 558 tubes were found to contain more than one phage particle. By totaling the Poisson analyses of the individual experi-

ments it was found that theoretically 119 of these were single bursts, 18 were double bursts, and 2 were triples. The data indicate a bimodal distribution, corresponding roughly to the bursts from single bacteria and from the accidental doubles. In a general way, it may be said that there is great variability in the amount of intracellular phage growth accomplished in the individual bacteria at a given time. Since the adsorption period was only 2 minutes, and since approximately 10 phage particles were adsorbed in that time, the difference among individual bacteria cannot be ascribed to a difference in the time of infection alone.

These results agree fairly well with those obtained by Delbrück with the virus Ti (*Jour. Bacteriol.*, vol. 50, p. 131, 1945) when he studied the phage yields from individual bacteria allowed to lyse normally. A more appropriate comparison can be made with Hershey and Rotman's results from crosses with T2H. The histogram of 100 of their single bursts lysing normally shows a distribution more concentrated around the mean. Our results show a considerably wider distribution in the relative sizes of yields from individual bacteria, with a concentration of bursts less than the mean. This difference can be explained on the hypothesis that phage growth proceeds linearly in individual bacteria, but that there is a spread in the time at which the first mature phage particles appear in the individual bacteria. If the growth of phage in our bacteria were allowed to proceed up to the time of normal lysis, both the mean and the individual yields would be moved along the abscissa, resulting in a more concentrated distribution of the bursts. This hypothesis would also predict that a curve describing intracellular phage growth in mass cultures should bend upward during the first portion of the rise, since one would add linear rates from in-

dividual bacteria starting at different times. This is precisely what was observed in the earlier experiments (see Year Book No. 47)-

The results of these single-burst experiments add detail to the picture of intracellular phage growth suggested by the mass-culture experiments. It is now clear that the phage obtained in the mass-culture experiments is not derived from a small number of easily disrupted bacteria, but from approximately 100 per cent of the infected cells.

CORRELATION IN THE OCCURRENCE OF ALTERNATE RECOMBINANT TYPES

The single-burst data already described were also studied in an attempt to determine whether the occurrence of one recombinant is correlated with the occurrence of the alternate type. Hershey and Rotman studied crosses between representatives of the three groups of *r* mutants and the *h* mutant. Only in the crosses involving *h* and *r*₇ where about 15 per cent of the progeny were recombinants, was a significant positive correlation found. It appeared surprising that no correlation could be detected between *h* and the more closely linked *ris*, where only 2 per cent recombination was found. It seemed possible that an early correlation might have been obliterated by growth of either recombinant during the latent period. Our experiment could shed some light on this explanation, and the progeny of the single bursts were therefore grouped in four genetic classes, *rh*^{*}, *r*^{*}*h*, *rh*, and *rVi*⁺. Of the 139 samples plotted, the classification of plaques was not dependable in 19 cases, and 12 other bursts contained only one of the parental types. These cases were therefore excluded from the analysis. The remainder were classified, and fell into the grouping shown in table 3. The amount of correlation may

be estimated in two ways. First, the correlated absence of recombinants may be studied. From the table it is seen that 77 of the total of 108 bursts lacked the *r*^{*}*h*^{*} recombinant, whereas 65 lacked *rh*. The number expected to lack both simultaneously, on a random basis, is the product of the above ratios, which is 0.43. This predicts that 46 bursts should show neither

TABLE 3

SINGLE BURSTS FROM CROSS T2Hr₁₃ x T2H/J,
DESIGNED TO TEST CORRELATION IN THE
OCCURRENCE OF ALTERNATE
RECOMBINANT TYPES

Type	Frequency	Type	Frequency
<i>Orh</i> : 0 -f + ...	53	3 <i>rh</i> : 1 j- + ...	1
1 <i>rh</i> : 0 -f + ...	12	2 <i>rh</i> : 2 -f + ...	1
<i>Orh</i> : 1 -f + ...	9	1 <i>rh</i> : 3 -F + ...	2
2 <i>rh</i> : 0 -f + ...	10	3 <i>rh</i> : 2 -f + ...	1
1 <i>rh</i> : 1 -f + ...	7	2 <i>rh</i> : 3 -f + ...	1
<i>Orh</i> : 2 -f + ...	2	4 <i>rh</i> : 2 -f + ...	1
3 <i>rh</i> : 0 -f + ...	2	3 <i>rh</i> : 3 -f + ...	1
2 <i>rh</i> : 1 -f + ...	1	2 <i>rh</i> : 4 -f + ...	1
1 <i>rh</i> : 2 -f + ...	1	6 <i>rh</i> : 1 -f + ...	1
<i>Orh</i> : 3 -f + ...	1		
Total recombinants are 75 <i>rh</i> : 50 ++			

recombinant. The number found, which was 53, is higher, but not significantly so. The result indicates that the presence or absence of one recombinant type is independent of the presence or absence of the alternate type-

On the other hand, the correlation coefficient, *r*_{xy}, between *rh* and *r*⁺*A*⁺ may be calculated. Hershey and Rotman have already shown that a correlation exists between the size of individuals and the number of recombinants in them. For this reason the coefficient of correlation must be calculated using the numbers of recombi-

nants as fractions of the individual burst sizes. When r_{xy} is calculated in this way, no correlation can be detected. The conclusion is that a reciprocal exchange in the later stages in the development of the virus particle is highly improbable. It is nevertheless possible that a reciprocal exchange occurs earlier, and that the correlation is effaced by subsequent reactions which complete the phage particles at random.

From Hershey and Rotman's genetic experiment taken together with the present results, we can draw one conclusion: that phage particles do not reproduce by division as unicellular organisms do. This conclusion is reached as follows. We now know that the first completed phage particles in mass cultures have about the same probability of being recombinants as the particles found later. If division is the mechanism of phage reproduction, recombinants should develop into large clones in those single bursts where they occur early, since an early recombinant should grow into more through subsequent divisions. Clones, however, were not observed by Hershey and Rotman, nor have they been seen in these experiments. The recombinants in Hershey and Rotman's experiments were distributed at random among the bursts.

In agreement with this conclusion is the fact that it is not possible to recover active phage from the infected bacteria during the early part of the latent period. Even the infecting phage particles can no longer form plaques if the cell is disrupted during the first third of the latent period. If reproduction of phage is by simple division, at least one phage particle should be available at all times during the latent period.

A GENETICALLY UNSTABLE SERIES OF PHAGE TYPES

The standard procedure for making bacteriophage stocks with a relatively low

frequency of mutant types is to infect a bacterial culture from a plaque that has arisen from a single phage particle. The lysate obtained in this manner is generally pure, except for a small number of mutant types that invariably arise in handling numbers of this order of magnitude. This procedure was applied to a peculiar scalloped plaque that appeared in an experiment where *Tqr* was used. The resulting lysate was not pure, however, but contained less than 90 per cent of the plaque type that was sought, the remainder being a mixture of other types. The procedure was repeated several times with the same result. Serial plaque isolations were also made, with no better success. It appeared that a mutable phage type was under observation, and on this hypothesis the situation was investigated further.

The first problem was one of identification and differentiation of the several plaque types arising in the plating from a scalloped plaque. The technique used was to suspend an entire plaque in broth, dilute appropriately, and then plate by the agar-layer method. Platings were made of seven apparently different plaques coming from the phage population of a single scalloped plaque. Of these, five proved to be differentiable on the medium used. The other two were probably examples of one of these types. Those differentiable were numbers 1, 2, 5, 6, and 7. They are described in table 4. Stocks were made by infecting bacterial cultures from plaques of these five types.

To verify the fact that these strains were T4 mutants, host-range experiments were made. Bacteria resistant to standard T4 were also resistant to these types, and T4-sensitive bacteria were sensitive to them. To obtain more critical evidence, stocks of types nos. 6 and 7 were compared serologically with the original T4r. They were found to be qualitatively and quantitatively identical with T4.

The composition of the phage populations in plaques of the various types was investigated to learn something about the pattern of mutability among the types of this group. This was done by assay of plaques of the various types described in table 4, followed by classification of at least 100 plaques from each population. The results are summarized in the last column of table 4. From these it can be seen that all five types are different with respect to the populations of their plaques. Type

That this is, in fact, an important consideration will be clear from subsequent data.

During one-step growth studies, differences in rate of adsorption were noted among these T4 types. To study this in a more precise way, a further experiment was made. It is known from the experiments of T. F. Anderson (*Jour. Bacteriol.*, vol. 55, pp. 637, 651, 659) and of M. Delbriick (*Jour. Bacteriol.*, vol. 56, p. 1) that the adsorption of certain strains of T4 is

TABLE 4
CHARACTERISTICS OF THE *Hqr* UNSTABLE SERIES OF PHAGES

Type no.	Approximate plaque diameter (mm.)*	Appearance of plaque	Cofactor requirement†	24-hour plaque contents‡
1	2-3	Clear halo	None; indole-sensitive	Nos. 1, 2, (5)
2	3.5-5	Turbid halo	Broth constituent; indole-sensitive	Nos. 2, (5, 7)
5	3.5-5	Turbid, speckled halo	Not tested	Nos. 1, 2, 5, 7, (6)
6	3-4	Turbid, scalloped halo	Broth or hydr. casein constituent; indole-sensitive	Nos. 1, 2, 5, 6, 7
7	1-1.5	Very narrow halo	Calcium; indole-resistant	Nos. 1, 2, 5, 7

* Absolute values depend on plating conditions and therefore vary considerably from plate to plate. The relative plaque sizes are nevertheless quite dependable.

† Based on preliminary data only.

‡ Parentheses indicate that these types occurred rarely in the plaques assayed.

no. 2 is relatively stable, in the sense that its plaques contain few phage particles of the other types. No. 1, on the other hand, is unstable, since its plaques invariably contained type no. 2 in addition to type no. 1. The other three types are even less stable than no. 1, since their plaques contain phage particles of several other types. If the comparison were based on the percentage of mutants present, the order of stability would be slightly altered. It should be noted, however, that these data may not reflect relative mutational instability, but rather may indicate differences in relative selective advantage of the various types during growth of the plaque.

markedly influenced by the presence of tryptophane, indole, and calcium. To discover whether the adsorption cofactor problem had any bearing on the present case, a preliminary test was made to see whether cofactors influenced adsorption here. A mixture of two of our T4 types was added to a large excess of bacteria in the presence or absence of the substances to be tested. Measurements were made of the percentages of each of the phage types that were adsorbed. Although the results are insufficient at this time to draw any specific conclusions about the cofactor sensitivity of the various types, it is clear that some of the substances tested do influence

the rates of adsorption of the different phages to different degrees.

All plaque analyses described so far were carried out on plaques incubated 20 to 24 hours at 37° C. In the light of information about the effect of adsorption cofactors, it seemed desirable also to study the younger plaques, where the cofactor condition of the plates had not been subjected to so much metabolic alteration. Plaques of T4 are first visible after 3% to 4 hours' incubation at 37° C. The youngest plaques that can be analyzed, therefore, are 4-hour plaques. The most significant factor in the results is that in all cases the 4-hour plaques contained only an insignificant proportion of mutants as compared with corresponding older plaques. It is furthermore significant that in the assays made on type no. 6 plaques of various ages, the percentage of mutant types is correlated with the age of the plaque. The results seem clearly to indicate that the great variety of phage types in the 20- to 24-hour plaques is in large part due to a selective mechanism operating during the growth of the plaque. Since the titer of the plaque from 8 hours to 20-24 hours increases only three- to fourfold, whereas the percentage of mutants increases more than thirty fold in comparable cases, the actual survival or the selective growth of the mutants must play an important role in their relative accumulation.

One technically useful result of these experiments is that it is now possible to prepare stocks of the various types with a

relatively high degree of purity, by infecting a bacterial culture with a 4-hour plaque rather than with an older one. In this way stocks of no. 6 with less than 0.2 per cent mutants have been prepared. It is hoped that these types will now become useful genetic markers for more advanced experiments.

Before the effect of selection among these phage types was discovered, the great variety of types arising from one phage particle in a single plaque seemed best explained by extreme mutability at certain genetic sites in the phage particle. To discover whether the observed mutability is due to instability in specific loci, or whether the hereditary material of these phages is generally unstable, tests were conducted to see whether hereditary antigenic alterations might also occur. The technique was to inactivate aliquots of a stock of type no. 6 with anti-T4 serum to a survival of 10^{-2} to 10^{-6} . Stocks were made from plaques resulting from the surviving phage particles. The rates of serological inactivation of these stocks were compared with the rate of inactivation of the original type no. 6. No significant differences were found in testing fifteen stocks derived in this way. These results suggest that the antigenic properties of these phages must be quite stable, or else that changes become manifest only after the accumulation of many changes, each of which is too small to be detected by these tests.

ORGANIZATION OF THE CHROMOSOME

B. P. KAUFMANN, M. R. MCDONALD, H. GAY, N. C. OKUDA, J. M. PENNOYER, AND S. BLOWNEY

In our approach to problems of the nature and specificity of action of genes in higher organisms, we have continued to focus attention on details of chromosome organization. These studies, originally

formulated at the level of descriptive cytology, have in recent years been supplemented by experimental procedures involving ionizing radiations and chemical mutagens—used independently and in

combination with nonmutagenic agents, such as near infrared radiation—and purified enzymes. All these approaches have been employed in our studies during the past year, primary emphasis being placed on the cytochemical methods. Progress of the work has been facilitated by a research grant from the Division of Research Grants and Fellowships of the National Institutes of Health, U. S. Public Health Service.

CYTOCHEMICAL STUDIES

The cytochemical studies initiated three years ago (Year Book No. 45, 1945-1946) were projected on the assumption that precise information concerning fundamental patterns of cellular organization could be obtained by using purified enzymes in combination with various staining procedures. Since our interest in the gene focused attention on the nucleic acids and proteins, it was necessary to accumulate a stock pile of purified nucleases and proteases; and this requirement in turn led to the development of methods for the elimination of proteolytic contaminants from crystalline ribonuclease (Year Book No. 46). About a year ago Dr. M. Kunitz, of the Rockefeller Institute for Medical Research, published a method for preparing crystalline desoxyribonuclease; and application of his method has enabled us to obtain preparations of this enzyme that are free of measurable traces of proteolytic contaminants. These two nucleases, together with the proteases, trypsin, chymotrypsin, and pepsin, constitute the group of purified enzymes used in our cytochemical studies.

In employing the enzymes cytochemically it is also essential to maintain an extensive series of controls to determine the influence of all variables capable of influencing their hydrolytic activity or interfering with the staining reactions (Year

Books Nos. 46, 47). Only by maintenance of such controls has it been possible to attribute the results obtained to the specific action of the enzyme molecules on substrate molecules. The extreme care required is illustrated by an analysis of the factors involved in the process of dissolution of cells that follows their digestion in trypsin.

The course of tryptic digestion. It has been reported by a series of observers that either crude or purified preparations of trypsin degrade proteins and lead to disintegration of the cell. This type of result is readily demonstrable, as is shown in plate IA. This section of a root tip of onion was treated with trypsin in 0.05 M phosphate buffer, pH 6, for one hour. No such dissolution occurred in the cells of an adjoining section of this root tip that served as a control and was treated only in the buffer. Because of previous experience with the complicating action of buffers in cytochemical reactions (see Year Book No. 46), it seemed essential in an analysis of the course of digestion by trypsin to determine to what extent cellular dissolution was referable to the presence of electrolytes rather than to the primary action of the enzyme in disrupting peptide linkages.

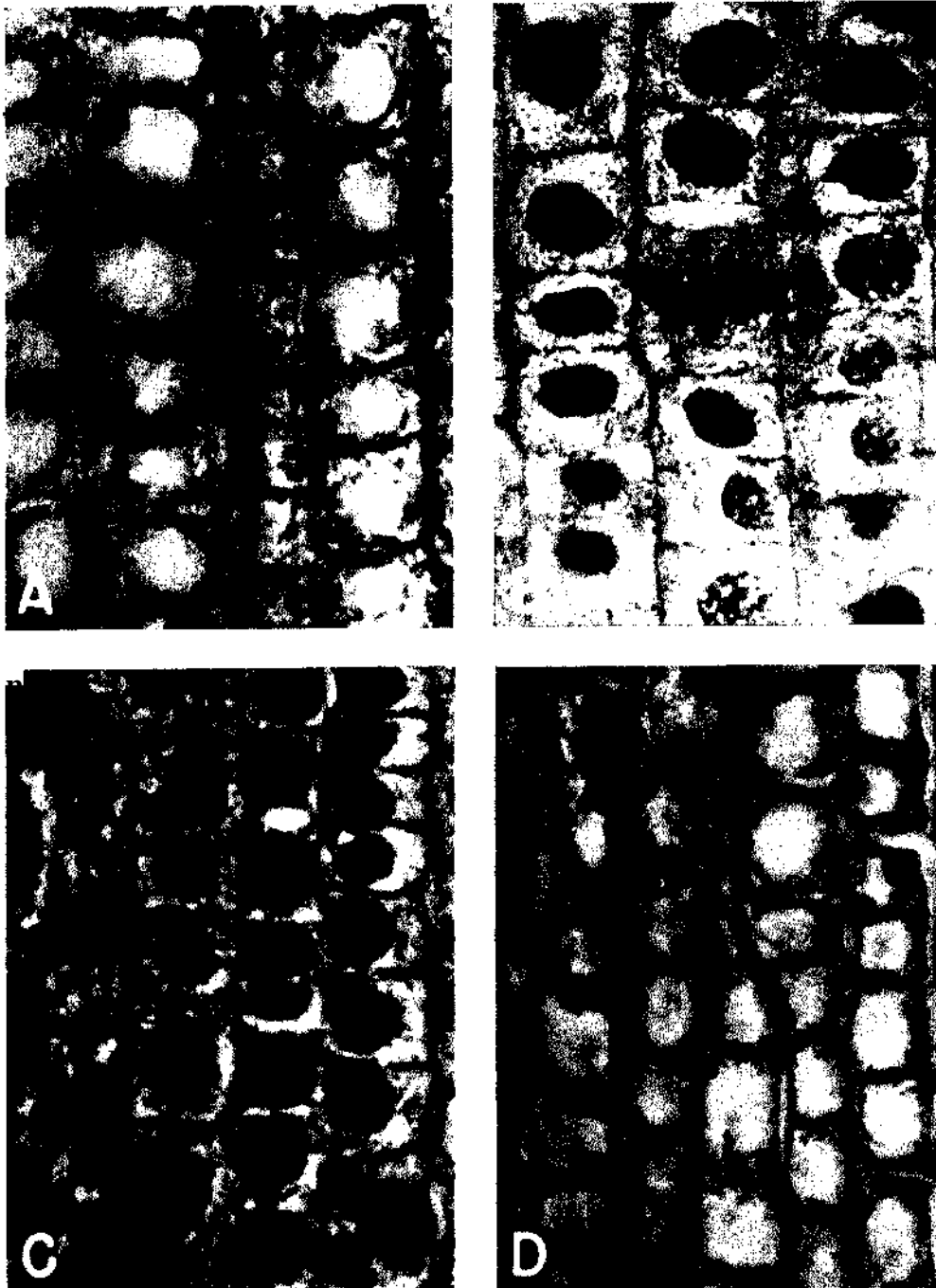
As a first step, it was necessary to determine whether trypsin causes cellular dissolution if used in the absence of electrolytes except for the traces necessary to adjust the pH. It was found that treatment of acetic-alcohol-fixed smears or sections for long periods of time with aqueous solutions of trypsin at pH 6 does not lead to cellular disintegration (pi. iB). That trypsin is active in such solutions is suggested by increase in the stainability of the treated cells with pyronin and other basic dyes. One possible explanation of this increase is that pyronin-stainable nucleic acid is released by tryptic degradation of ribonucleo-

protein (Year Book No. 47). Aqueous solutions of trypsin also reduce the stainability with acidic dyes of the protein released by the action of ribonuclease on ribonucleoprotein (cf. Year Book No. 47). Additional evidence that aqueous solutions of trypsin are not impotent was provided by experiments in which treatment with such solutions was followed, after thorough rinsing, by treatment with 0.05 M phosphate buffer or by 0.04-0.10 M sodium chloride. Dissolution of cellular contents occurred rapidly under these conditions. Obviously the electrolytes play a decisive role in the process; but since treatment with buffers or salt was followed in all cases by prolonged washing in water, further experiments were required to determine the dependence of dissolution on removal of water-soluble materials. Accordingly, sections of root tips were treated with aqueous trypsin, then with phosphate buffer. If they were then transferred to 0.1 M hydrochloric acid before rinsing in water, the contents of the cells were preserved without apparent distortion (pi. iC); but if they were rinsed in water before the treatment with hydrochloric acid, the cellular contents were not detectable (pi. iD).

This phenomenon is strikingly demonstrated by using the phase-contrast microscope, which permits direct observation of gross alterations as they are induced by the various reagents. Immersion in aqueous trypsin (0.1 mg. per milliliter at pH 6 for 1 hour) does not effect any discernible structural alteration in salivary-gland chromosomes of *Chironomus* larvae (blood-worms). Even after prolonged washing in wafer, the precise pattern of banding is clearly defined*. Upon subsequent addition of buffer or sodium chloride solution (in the concentrations indicated above), there occurs an immediate swelling of the chromosomes and separation of the bands

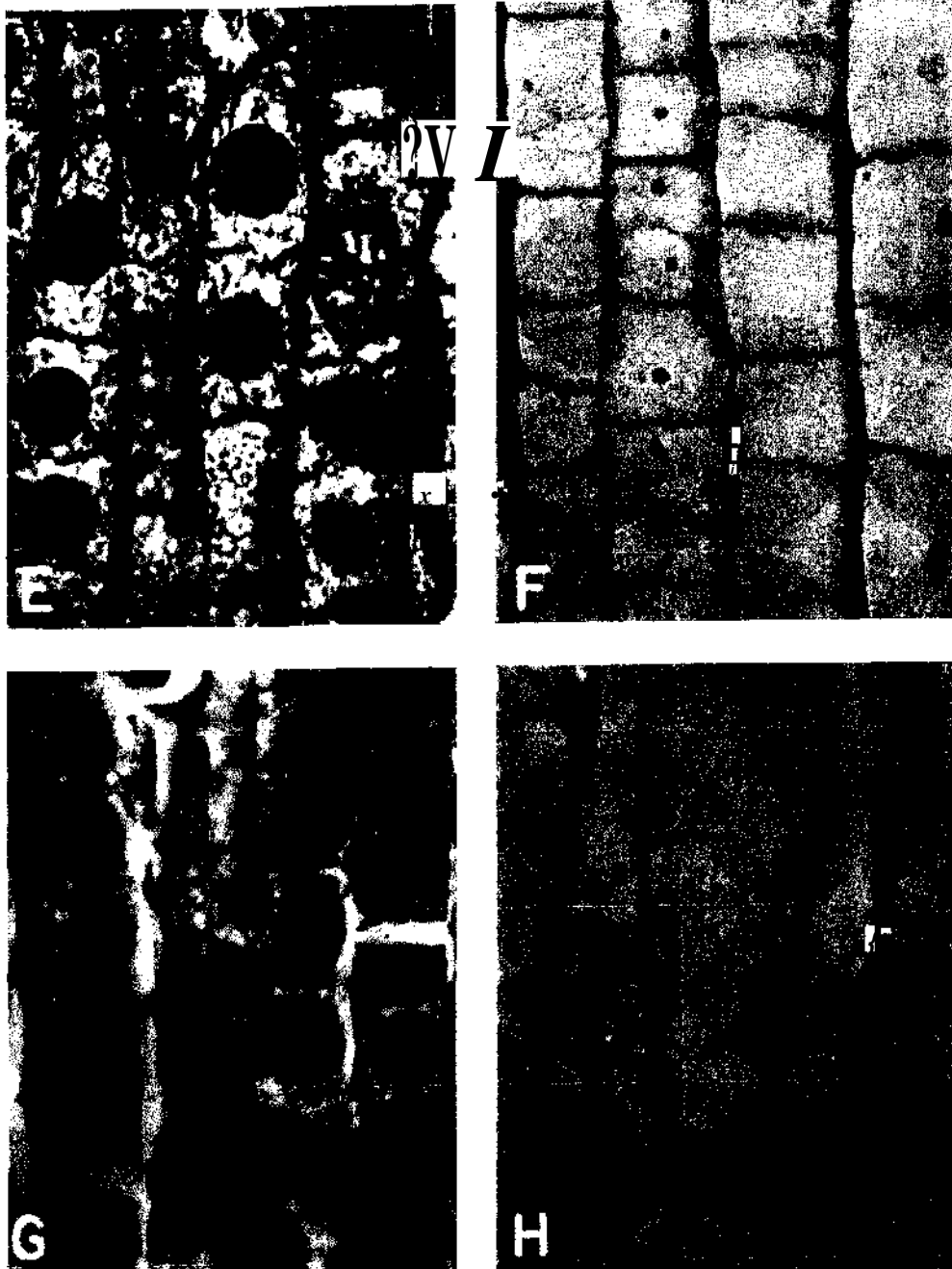
into their component chromomeres. If, at this stage, aceto-orcein is added, the chromosomes contract, and their bands once more become clearly defined and assume the purple color of the orcein stain. If, on the other hand, rinsing in water follows treatment with the buffer, the swelling continues until adjacent chromosomes adhere and their contents become disorganized. Continued washing leads to complete dissolution of cellular materials. It is apparent from this series of observations that the action of trypsin *per se* does not lead to the type of disintegration of substrate materials that has been attributed by other workers to specific disruption of peptide linkages. This in turn suggests a re-examination of the widely accepted concept that protein alone serves to maintain the form and structure of the chromosome. Aside from this application to problems of immediate importance in our cytochemical studies, these observations suggest a method of attack on more general problems of enzymatic digestion by resolving the process into its component phases.

Enzymatic dissection of the chromosome. Recent investigations, including those conducted in this laboratory (Year Books Nos. 46, 47), have indicated that the chromosomes of higher organisms contain histone and nonhistone proteins, and ribose and desoxyribose nucleic acids. Our cytochemical studies have indicated that ribonucleic acid is separable by enzymatic hydrolysis from a tryptophane-containing protein (Year Book No. 47); and chemical studies by various workers have shown that desoxyribonucleic acid is extractable from the chromosome in association with histone. Use of purified nucleases and proteases, independently and in combination with each other, or with various chemical procedures, should provide further information about patterns of association of nucleic acids and proteins as they exist separately and in



Tryptic digestion of onion root-tip sections. 7-micron paraffin sections of root tip of onion, fixed in acetic-alcohol, stained in methyl green-pyronin. X 860.

- A. Nuclear disintegration as produced by a solution of trypsin in buffer.
- B. Cells visibly intact after treatment with aqueous solution of trypsin.
- C. Retention of gross nuclear structure after successive treatments in aqueous solution of **trypsin**, in phosphate buffer, and in 0.1 M HCL
- D. Nuclear disintegration produced by successive treatments in aqueous solution of trypsin, phosphate **buffer**, water, and 0.1 M HCL



Pepsin digestion following removal of nucleic acids. 7-micron paraffin sections of root tip of onion, fixed in acetic-alcohol, stained in fast green. x860.

F. Extraction of all nucleic acids by treatment with 0.3 N trichloroacetic acid for 15 minutes at 40° C.

F. This section treated as F, followed by pepsin digestion.

G. Degradation of nucleic acids by successive treatments with ribonuclease and deoxyribonuclease.

H. This section treated as F, followed by pepsin digestion.

combination within the fixed cell, and should also indicate whether or not their presence is essential for maintenance of structural integrity. It must be kept in mind that cellular materials are preserved by cytological fixatives without gross distortion of the spatial relations that exist in the living cell, and thereby provide a useful basis for initial studies of the chemical organization of the living system.

Trypsin presumably attacks peptide linkages involving basic amino acids that are present in both histones and nonhistone proteins. Chymotrypsin also seems to attack linkages common to both types of proteins, since the action of this enzyme in disrupting cellular organization has been found to parallel that reported above for trypsin. Differences that we have detected in potency and rate of action of the two enzymes promise to furnish information about the specific action of each on histones and other proteins. The evidence now available indicates that disruption of linkages in either type of protein by either of these enzymes (in concentrations as high as 1 mg. per ml.) does not destroy structural continuity of the chromosome unless treatment with the enzyme is supplemented by treatment with a solution containing electrolytes. The importance of different electrolytes in this process is now under investigation.

Pepsin is another proteolytic enzyme that has been used extensively in cytochemical studies. It effects a marked shrinkage of chromosomes without effacing their pattern of structural organization. Using the phase-contrast microscope, we have observed that pepsin in hydrochloric acid (3 mg. per ml. in 0.02 M HCl at 37° C.) will reduce salivary-gland chromosomes of *Drosophila* in 2 hours to about one-tenth of their original volume. On the basis of our studies and those of Mazia and his associates, it appears that the action of pep-

sin on fixed preparations consists primarily in degradation of the nonhistone, or tryptophane-containing, protein. If so, this substance represents a much higher proportion of the chromosome than would be expected from analysis of chemically isolated materials. From the standpoint of structural continuity, the histones and the nucleic acids surviving peptic digestion constitute a clearly defined although markedly shrunken chromosome.

Nucleases—that is, enzymes that degrade nucleic acids—also serve to reveal patterns of structural organization. Ribonuclease reduces stainability of ribonucleic acids with basic dyes, and also degrades ribonucleoprotein to release a protein that stains with acidic dyes (Year Book No. 47). Such treatment does not destroy chromosome structure; nor is it destroyed if treatment with ribonuclease is followed by treatment with pepsin.

Desoxyribonuclease eliminates stainability of chromosomes by the Feulgen method, indicating that this enzyme degrades desoxyribonucleic acid. The structure of the chromosome is not impaired thereby, since it is clearly defined when stained with acidic dyes. Structural continuity is also maintained if all nucleic acid is removed by consecutive treatments with ribonuclease and desoxyribonuclease, or by extraction with trichloroacetic acid at 90° C. (pi. 2G, E). If, however, treatment with pepsin follows either trichloroacetic acid extraction (pi. 2F) or treatment with ribonuclease and desoxyribonuclease, or even treatment with desoxyribonuclease alone (pi. 2H), dissolution of nuclear contents ensues. These results might seem to suggest the existence in the chromosome of two separable complexes, one ribonucleoprotein, the other desoxynucleohistone, either of which is capable in itself of maintaining the integrity of the chromosome. As was indicated in the experi-

ments using hot trichloroacetic acid or the nucleases in succession, however, both complexes may be degraded without effacing the chromosome.

The obvious conclusion from this series of experiments is that the proteins form an interrelated system in the chromosome, and that the nucleic acids are intimately linked with the proteins and perhaps with each other. On the basis of evidence now available, no single protein or nucleic acid may be regarded as the fundamental structural component of the chromosome.

RADIATION STUDIES

Our pioneering studies with near infrared radiation led to the discovery that wave lengths centering around $i p$ can modify the frequency of X-ray-induced chromosomal rearrangements, but not of gene mutations (Year Books Nos. 44-47). With the prospect that near infrared radiation might serve, when applied to organisms in which cytological analysis is not feasible, to distinguish between induced genetic changes that are attributable to chromosomal alterations and those that are not, a series of experiments was undertaken to determine the effects of near infrared rays and X-rays, used independently and in combination, on bacterial cells. In efforts to determine the nature of the "sensitizing" action of near infrared radiation, experiments were also initiated using systems less complex chemically than those of the living cell, namely, solutions of purified crystalline enzymes.

Response of the bacterium Escherichia coli to X-rays and near infrared rays. A series of studies was carried out, with the assistance of Miss Helen Cuneo, to compare the rates of mutation of the B/r strain of *E. coli* from the normal bacteriophage-sensitive condition to a phage-resistant state after treatment with X-rays

alone and after treatment with X-rays preceded by near infrared rays. As a prerequisite to this comparison it was necessary to determine the rate of survival of bacterial cells treated either with near infrared rays or with X-rays. Exposure to near infrared radiation (for periods of either 3 hours or 24 hours at 25° C.) of a culture of *E. coli* in a medium inadequate to permit growth (the solution of salts used in the M-9 medium) did not significantly alter the number of living cells or affect the proportion of mutants as compared with those of a control culture maintained in ordinary light at the same temperature. It thus appears that near infrared radiation alone has no lethal action and no mutagenic potency.

Treatment with X-rays kills bacteria in such proportions that exponential survival curves are obtained. It was found in a series of experiments that essentially a straight-line relationship was obtained when the logarithm of the surviving fraction of bacteria was plotted against the dose of X-rays, but that the slope of these survival curves varied greatly from experiment to experiment. When inocula from the same slant were grown under identical conditions for 48 hours, centrifuged to provide cultures containing approximately 10^{10} bacteria per milliliter, and irradiated under identical conditions at the same intensity, the inactivation doses of the separate cultures (amounts of radiation required to reduce the survivors to 36.8 per cent of the initial number) ranged between 8000 and 21,000 roentgen units. The basis of this variability remains to be determined, but apparently involves biological factors as well as those attributable to dosimetry and technical procedures.

Failure to establish a uniform survival curve has complicated the process of determination of mutation rates among the bacteria surviving X-ray treatment. Only

by assay over a wide range of dosage levels in any one experiment has it been possible to secure the required information. Two methods of measuring mutation rate were employed, namely, the zero-point technique developed by Demerec and Latarjet, and the liquid-culture, end-point technique developed by Newcombe (Year Books Nos. 45, 47). The results obtained by Demerec and Latarjet suggested that mutation rate is proportional to X-ray dosage. The results obtained in our studies were too variable to provide significant statistical verification of this interpretation; in some experiments the mutation frequency was significantly higher than in untreated controls, whereas in others it was not.

The mutation rates in the bacterial populations treated with near infrared rays plus X-rays and in those treated with X-rays alone were not appreciably different in paired experiments. Again, there was considerable variability from experiment to experiment, and more data must be amassed to permit reliable statistical analysis. Inferences concerning the relation of X-ray-induced mutants to chromosomal aberrations in *E. coli* cannot be made until such information is obtained.

The effect of X-radiation on dilute solutions of crystalline trypsin. It has been known for many years that the activity of enzymes can be destroyed by X-radiation. Large doses were necessary, however, for such destruction, and it was therefore generally believed that enzymes were too insensitive to radiation to account for the radiation effects noted in the living cell. In 1940, however, W. M. Dale found that "the effect of X-rays on aqueous solutions of enzymes and of other biologically active compounds depends on the concentration and purity of such solutions, and that doses of as little as 50 roentgens can produce marked effects when concentrations of the order of those occurring in living

cells are irradiated." Work in several other laboratories has since confirmed these general conclusions. Dale postulated that enzyme molecules are affected by the ionizing radiation, not directly, but indirectly through collision with a labile product resulting from ionization of the water. Very little is known about the action of infrared radiation on enzymes. It has been reported as destroying urease, stimulating starch amylase, and having no apparent effect on pepsin. Unfortunately, the experiments on which these reports were based were done with impure enzyme preparations. In our work we have used purified enzymes prepared in our laboratory. The work with near infrared radiation is still too preliminary to warrant even tentative conclusions. We shall therefore report at this time only the results obtained with X-rays on solutions of crystalline trypsin.

Crystalline trypsin was chosen for the original studies for several reasons. In the first place, it can be accurately assayed, permitting precise reproducibility of results. It is stable at pH 2.4, thus eliminating any complicating secondary effects due to spontaneous inactivation. Finally, it appeared to be a suitable enzyme for comparison with carboxypeptidase, the only purified enzyme on which extensive quantitative data were already available, because both enzymes are primarily peptidases, their source is beef pancreas, and their molecular weights are similar (trypsin = 36,500; carboxypeptidase = 35,000).

The effect of X-radiation on solutions of crystalline trypsin in 0.005 N hydrochloric acid has been determined under a variety of conditions, such as varying the radiation dosage while the concentration of trypsin remains constant, varying the concentration of trypsin while the dosage remains constant, and varying both the concentration of trypsin and the dosage, the ratio

of the two being constant. Inactivation has been observed with doses as low as 100 roentgens. As has generally been found for proteins, however, the greater the initial concentration of trypsin, the larger is the dose of radiation necessary for comparable percentages of inactivation. For any given initial concentration of trypsin, the decrease in tryptic activity with increasing amounts of radiation is exponential. It is therefore essential, when comparing various experiments, to do so at a constant level of inactivation. For convenience both of calculation and of comparison with other published data, this level has been taken as 63.2 per cent, the amount of radiation necessary to produce this percentage of inactivation being known as the "inactivation" dose. Calculations of "inactivation" doses show that they increase with increasing initial concentrations of trypsin, but that the increase is not directly proportional in the range of trypsin concentrations so far studied (1.2-344 $\mu\text{g. per ml.}$). This means that in this range of concentrations the ionic yield—i.e., the number of molecules destroyed per ion pair—is not constant. For example, under otherwise identical conditions, the ionic yield for a 9×10^{-8} molar solution of trypsin (3.29 $\mu\text{g. per ml.}$) was found to be 0.0074, whereas for a 9.4×10^{-6} molar solution (344 $\mu\text{g. per ml.}$) it was 0.0376. Drops in ionic yields at low solute concentrations have been noted in other laboratories in studies of the effects of X-rays on oxalic acid, methyl alcohol, glutathione, tobacco mosaic virus, and rabbit papilloma virus. Such results would be expected, according to the "indirect action theory"¹ of the effects of radiation, if the solute concentration is so low that an appreciable proportion of the total number of "active radicals"¹ combine with one another rather than react with the solute molecules. Absolute ionic yields therefore

cannot be stated for radiation effects in very dilute solutions, unless one has ascertained by experiments over a wide range of solute concentrations that they are constant or can be extrapolated from the data. Unfortunately, this has not generally been done, and many of the values for ionic yields given in the literature may therefore be misleading. Barron *et al.*, for example, state that the ionic yield for the inactivation of trypsin by X-rays is 0.025. This value was determined, however, from one experiment at one concentration (50 $\mu\text{g. per ml.}$) and can apply only to that specific case.

Dale noted in his original studies (1940-1943) on the inactivation of carboxypeptidase by X-radiation that, over a wide range of dosage (50 to 400,000 r) and enzyme concentrations (calculated by us from his data to be approximately 1.6 to 540 $\mu\text{g. per ml.}$), the number of enzyme units inactivated by a given number of roentgens and for a given percentage of inactivation was constant. As noted above, in experiments using a similar range of enzyme concentrations (1.2 to 344 $\mu\text{g. per ml.}$) and of X-ray dosage (100 to 100,000 r) no constant value was found for trypsin. This indicated a marked difference between the two enzymes. Since our experiments were completed, however, Dale and his co-workers (1949) have published extended data covering an extremely wide range of carboxypeptidase concentrations, namely from 5 to 150,000 $\mu\text{g. per ml.}$ Here they report that for concentrations below approximately 200 $\mu\text{g. per ml.}$ the ionic yield decreases markedly with decreasing concentrations, although at higher concentrations it is approximately constant and equal to 0.18 enzyme molecule inactivated per ion pair. They obtained similar results with alloxazine adenine **dinucleotide**.

X-rayed 0.005 N hydrochloric acid was found to have no effect on the activity of

solutions of crystalline trypsin. A solution of trypsin, dissolved in hydrochloric acid that had received 35,000 roentgens immediately before use, when assayed after 4 hours at 25° C. had 623 X io⁶ trypsin units (denatured hemoglobin); whereas control solutions in hydrochloric acid that had not been X-rayed had 616 X io⁶ units. Such a solution of trypsin would have lost 84 per cent of its activity had it received this amount of X-radiation directly.

There appears to be no delayed effect, such as R. S. Anderson found with pepsin, nor is there any spontaneous recovery either in the light or in the dark. For example, a solution of trypsin (33 ug. per ml.) that received 18,000 r had, when assayed immediately after radiation, 285 X io⁶ activity units. After 1, 5, and 24 hours at 0° C. in light and in dark, the assays were 285, 285, and 283 X io⁶ units, respectively, for light, and 285, 285, and 286 X io⁶ units for dark.

The rate of inactivation of crystalline trypsin by X-radiation was found to be independent of the intensity at which the radiation was delivered. Inactivation doses for a solution of crystalline trypsin containing 30.4 µg. per ml. were 21,500, 21,200, 20,750, and 21,500 r with rates of delivery of 581, 264, 180, and 129 r per minute, respectively.

All the experiments reported thus far have been carried out at pH 2.4, the point of maximum stability for trypsin. Preliminary experiments indicate that the hydrogen ion concentration of the solution being irradiated affects the amount of inactivation. As yet, however, the optimum pH for X-ray inactivation of trypsin has not been established.

The nature of the change in the trypsin molecule responsible for the loss of activity brought about by irradiation is unknown. It is hoped that an extension of these data

to include trypsinogen, the precursor of trypsin, may shed some light on this problem, as well as on the even more basic problem of what group or groups in the trypsin molecule are responsible for its specific action.

Studies combining treatments with near infrared radiation and nitrogen mustard. As reported in preliminary form in Year Book No. 47, near infrared radiation, when used before nitrogen mustard in treatment of males of *Drosophila melanogaster*, effects an increase over the nitrogen-mustard-treated controls in frequency of chromosomal rearrangements, as determined by genetic tests for translocations between the second and third chromosomes. Posttreatment, on the contrary, does not produce any increase in frequency.

The data obtained in these studies have been subjected during the past year to critical statistical analysis. Since in the pretreatment series five separate groups of flies were exposed to the aerosol of nitrogen mustard, and since each was mated with three groups of females at 6-day intervals, fifteen samples were available for comparison between the infrared-treated flies and their controls. Disproportionate frequencies, and the interaction of the five separate treatments and the three transfers, posed a statistical problem that was solved, at the suggestion of Dr. A. E. Brandt of the Atomic Energy Commission, by use of the method of partitioning of chi square. The analysis indicates that the differences between the separate treatments and the different transfers are significant. The least-square lines derived from the* total data are shown in figure 3. These lines have essentially the same slope. Analysis reveals that the percentages of translocations represented in the experimental and control regression lines differ significantly from each other. The data thus indicate

that pretreatment with near infrared radiation effects an increase of about 50 per cent in the frequency of mustard-induced chromosomal translocations; and this increase corresponds closely to the value obtained in earlier experiments in which near infrared pretreatment was used with X-rays.

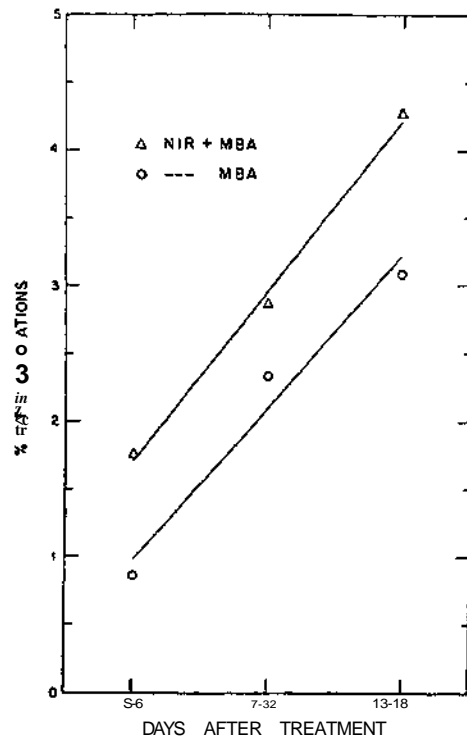


FIG. 3. Least-square lines, for pretreatment series and its controls, derived from frequencies of nitrogen mustard-induced translocations between the second and third chromosomes of *Drosophila melanogaster*. Upper line, near infrared radiation followed by nitrogen mustard; lower line, nitrogen mustard control.

Comparable statistical analysis applied to the posttreatment data indicates that the differences between successive transfers are significant, but that near infrared radiation does not significantly modify the frequency of translocations when it is used after the

nitrogen mustard. This result also agrees with that obtained in the earlier experiments in which near infrared radiation was used after X-rays.

An effort was next made to determine whether the increased frequency of translocations detected in successive transfers was due to a cumulative action of the chemical on spermatozoa that were mature at the time of treatment, or to an effect on cells that were in earlier stages of develop-

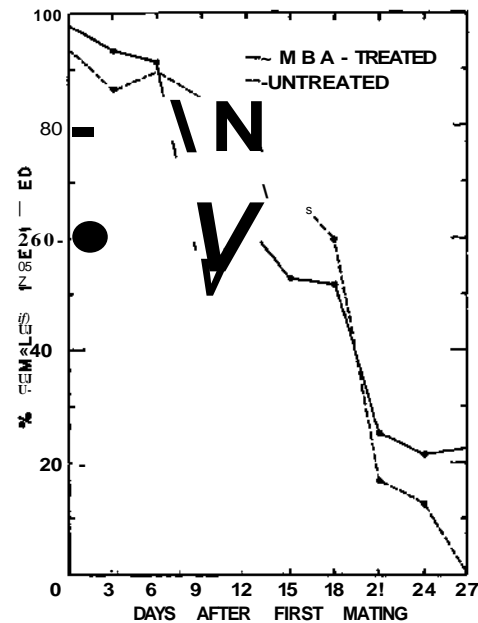


FIG. 4. Proportion of available females inseminated by nitrogen mustard-treated and untreated males at successive three-day intervals after the first mating.

ment at that time. Preliminary experiments were undertaken to determine the length of time after treatment that males remain fertile, as indicated by their capacity to inseminate females and by the progress of spermatogenesis. These studies showed that spermatogenesis is not necessarily terminated by exposure of males to mutagenically potent doses of nitrogen mustard. Cytological examination of sec-

tions of testes dissected from sexually active and inactive males throughout a 30-day period revealed dividing spermatogonia and spermatocytes and an abundance of mature spermatozoa in both. The proportion of available females inseminated by treated males is shown in figure 4. In obtaining these data, each male was mated with 3 virgin females immediately after treatment, and at the end of each 3 days thereafter. Males of the same cultures that had not been exposed to the aerosol of nitrogen mustard served as controls. A comparison of the two curves shown in figure 4 suggests that exposure to nitrogen mustard may temporarily impair sexual

activity, but that recovery occurs in a high proportion of treated males.

Additional work will be required, however, to answer the question whether the higher frequency of translocations detected in later transfers is due primarily to an effect of the chemical on mature germ cells or on immature germ cells. In the latter event, the possibility must be examined that the mustard-induced rearrangements arise in large part as chromosomal mosaics, and that the higher frequency observed in later transfers is due to the facilitation of their detection by the experimental procedure used. Experiments designed to answer this question have been initiated.

MOUSE LEUKEMIA

E. C. MACDOWELL, M. J. TAYLOR, AND L. E. LEWIS

Increasing potency of an invading pathogenic agent or transplanted tumor has frequently been observed to be closely connected with a change in the range of susceptible hosts. Thus the increase in virulence of line-I leukemia seemed to provide a valid reason for the apparent failure of the natural resistance of mice from foreign strain StoLL. During its first hundred passages, line I failed to kill any of its hosts from this strain, but by transfer 147 it was killing a large proportion of these mice.

That the line-I leukemic cells proliferated in these StoLi hosts, and were successfully carried in mice of this strain for a series of 14 successive transfers, seemed ample evidence that leukemia was the cause of death. The difference in genetic constitution between the hosts of this strain and the strain of origin provided a reasonable interpretation of the observed modifications in metabolism of the invaded lymph nodes and in cytological details of the invading cells; for upon subsequent transfer to hosts of the original strain, all the specific characteristics of line-I leu-

kemia, including metabolic rates and cytological details, reappeared. When these interpretations were proposed, the possible association of a virus with line-I leukemia was not even imagined. The two previous reports have recorded the isolation of a virus from line I and the freeing of line I from the virus, which now appears almost certainly to have been transplanted along with the leukemic cells for ten or more years.

Work of the current year with line I, freed from the virus, shows that the virus and not the increased virulence of the leukemic cells was responsible for the death of the StoLi hosts. With the virus eliminated, every StoLi (91) inoculated with line-I leukemic cells has survived, as during the earliest period of this project. But these mice are highly susceptible to the virus, which induces a more violent sickness than in C58 mice and kills every mouse. Thus, strain StoLi is killed by the virus and not by line I, and strain C58 is killed by line I but not by the virus, although an initial proliferation of the re-

spective pathogens occurs in the survivors in each case.

At the time StoLi mice were dying after inoculation with line I, the ratio of deaths in mice of the backcross generation, (C58 X StoLi) X StoLi, rose from the previous fully confirmed Mendelian 1:1 to 4:1. This year, mice (120) of this same backcross generation, when inoculated with line I free from virus, but with virulence as great as ever, once more gave the classic 1:1 ratio (50.8 per cent survived). This ratio is consistently indicated when the data are subdivided according to (1) the four types of matings (reciprocal Fi's, reciprocally backcrossed to StoLi), (2) three pairs of color genes, and (3) sex. Thus it appears that the natural resistance of StoLi to line-I leukemia, and the primary dependence of this resistance upon a recessive gene, are once again demonstrable; and it is highly probable that at no time was there a failure of this resistance.

The discovery that an unsuspected virus was responsible for changing the proportion of Hne-I survivors in a foreign strain and in a backcross does not forthwith demonstrate that all the changes in such proportions that we have observed within other lines in the course of many transfers, and all the differences between lines according to these criteria, can be explained by the intermediation of a virus. For instance, line L, which arose in strain 89 and was long carried by hosts of this strain, killed only 1 per cent of the 92 mice of strain C58 tested in the first 15 transfers, whereas the hosts of strain 89 were all susceptible throughout. With the line-L cells carried exclusively by strain-89 hosts, the proportion of susceptible C58 mice rose by degrees to 95 per cent in the course of 60 successive transfers. Considerably later, line L was found to kill 100 per cent of C58 mice, and for the last 480 transfers

this line of leukemic cells has been routinely carried by C58 mice which have maintained 100 per cent susceptibility.

We have previously reported that line L was found to be carrying a virus, and that the leukemic cells of this line had been freed of this virus. Contrary to the situation found with line I in strain StoLi, however, the elimination of the virus from line L has left the 100 per cent susceptibility of strain C58 unchanged. In this case, changes in the leukemic cells, and not the presence of a virus, were responsible for the changes in the susceptibility of strain C58.

Furthermore, lines E and H after 90-odd transfers in the hosts of the strain of origin (C58) gave susceptibility ratios, in mice of the above-mentioned backcross, that approached 1:1, thus resembling the virus-free line I. Earlier in their histories these two lines showed only 10 to 15 per cent susceptibility in the backcross.

However indicative these previous observations might be, the point seemed of sufficient importance to make a backcross test of susceptibility to leukemic cells that had passed through only a single transfer after removal from a spontaneous case of leukemia. Accordingly, a group of 102 mice of this same backcross were thus inoculated. In the case of line I, the susceptible backcross mice died at the same time and showed the same autopsy picture as the C58 controls; in this case, the intervals before death were so continuously variable and so often greatly prolonged beyond the C58 controls that no sharp line could be drawn between susceptible mice and survivors. Of the C58 controls, 14 died after 13 to 17 days and one after 45 days; of the backcross hosts, 2 died before the 20th day, and the following numbers in successive 10-day periods: 1, 3, 2, 1, 6. At 72 days, observations were discontinued and the remaining mice killed; at this

time the autopsies gave definite evidence of leukemic infiltration in 4 and probable initial stages in 2 others. Although longer observation might have added cases still more delayed, there was no approach to a 1:1 ratio of resistant to susceptible animals, and the contrast with line-I leukemia seemed sufficiently well established. Various degrees of resistance were indicated not only by the intervals before death, but also by the distribution of lesions in the backcross animals. Only 4 of these showed the general enlargements of spleen and nodes found consistently in the C58 controls. In the great majority of cases the backcross autopsies revealed a strong but highly variable concentration of leukemic infiltration in deep lymph nodes and organs (notably the ovary), whereas the superficial nodes and spleen remained relatively small. Obviously, these leukemic cells are resisted in varying degrees by most of the 50 per cent of the backcross mice that are susceptible to line-I leukemia by virtue of their genetic constitution; and it may be concluded that in regard to host susceptibility and resistance these leukemic cells, in their second passage from a spontaneous case, are intrinsically different from cells of line I.

A MILK FACTOR RESISTING SPONTANEOUS LEUKEMIA AND LENGTHENING LIFE

It has been confirmed this year that a resistance factor from mothers of the non-leukemic strain StoLi, rather than a leukemia-inducing factor from mothers of the leukemic strain C58, is responsible for the differing incidence of spontaneous leukemia among animals from reciprocal matings between these two strains. This resistance is transmitted to their hybrid young by mothers over 34 weeks old, but not by young mothers 10 to 18 weeks old. Year Book No. 46 (1946-1947) reported

preliminary results of the experiment designed to verify this mother's-age effect, which had first been recognized in an earlier experiment. The final results show that, with all fathers from the leukemic strain and each one mated to both young and old StoLi mothers, the incidence of leukemia was unquestionably lower in the hybrids from old mothers than in the hybrids from young mothers, and the lives of both leukemics and nonleukemics were lengthened. With young mothers, the final incidence of leukemia, after questionable cases were checked microscopically, was 82.6 per cent of 75 mice; with old mothers, it was 56.8 per cent of 88 mice. The young-mothers group lived from 309 to 891 days (average 595.7 days), with 32 per cent dying between 550 and 650 days; the old-mothers group lived from 546 to 1107 days (average 817.5 days), with 34 per cent dying between 850 and 950 days. The reduction in the incidence of leukemia was direct, and not secondary to the effect on longevity; for longer lives were associated with less rather than more leukemia, and the mother's-age effect on longevity was as certainly manifested by the non-leukemics as by the leukemics.

In the above experiment each mother nursed her own young, but in the next experiment, set up in 1947 (Year Book No. 46), with the same two groups of hybrids from young and old StoLi mothers, some of each group were nursed by their own mothers and some by mothers of the other age group. Although final results are not expected for another year or more, the observations up to date are highly important and significant, leaving little doubt of the validity of the general conclusion that the resistance factor from old mothers can be transmitted by nursing alone quite as effectively as before birth. The control groups from young mothers with young nurses, and old mothers with old nurses,

have closely repeated, up through the 650-day class, the curves of cumulative leukemic incidence by 50-day classes given by the earlier experiments, the gross diagnoses up to date indicating leukemia in 60.8 per cent of the entire group of 120 hybrids from young mothers and young nurses, and in 13.1 per cent of the group of 84 hybrids from old mothers and old nurses. The curve for the critical group, mice from young mothers and old nurses, almost exactly equals that for the old mothers-old nurses group, at 650 days showing leukemia in 11.0 per cent of the group of 109. In terms of those that have already died, the young-young group has yielded 81.6 per cent leukemia, and the young-old group 50 per cent leukemia; these figures are very close to the expected final inci-

dences. The difference in longevity is indicated by death, up to date, of 83.3 per cent of the young-young group against 41.3 per cent of the young-old group.

However real, the resistance to leukemia that depends on the advanced age of the StoLi mother or nurse is clearly not highly potent, in that the incidence is not reduced by even one-half. Yet the discovery of the mechanism by which this moderate reduction in incidence is brought about may well lead to further understanding, if not control, of the manifestation of an inherited tendency to leukemia. On the other hand, the discovery of the mechanism responsible for lengthening life to the extent of $\frac{1}{2}$ per cent by a factor in old nurses' milk would have considerably broader implications.

GENIC ACTION

E. CASPARI AND H. C. DALTON

Analysis of the action mechanism of individual genes has been continued. Besides the genes studied previously—that is, *a* (red eyes) and *wa* (white eyes) in the moth *Ephestia*, and *T*, *Ki*, and *Fu* (tail malformations) in the mouse—the gene $\&$ (white color) in the Mexican axolotl was included in our studies. In addition to the genetic and biochemical methods used previously, the embryological methods of explantation and transplantation were employed; and immunological methods were successfully used for the identification of genetic differences.

We wish to thank Miss Louise Pool for her valuable help in carrying out these investigations. During the summer we were assisted by Misses Louise H. Earle and Barbara C. Wolff and Mr. Noel H. Miller.

MATERNAL EFFECT OF *FU*

Fu in the mouse is a dominant gene with variable expression. As described

previously (Year Book No. 47, 1947-1948), the number of phenotypically Fused animals is lower in crosses of *Fu*/ $+$ females by $+/+$ males than in the reciprocal cross. Evidence seemed to indicate that this phenomenon might be due to the existence in different proportions of *Fu*/ $+$ animals that were phenotypically normal. This hypothesis was investigated by individual test matings of normal animals from crosses involving *Fu*.

The *Fu* strain had previously been outcrossed for 20 to 22 generations to a normal, highly inbred Bagg albino strain. Normal-tailed progeny from crosses of *Fu*/ $+$ females by Bagg albino males and Bagg albino females by $F\llcorner$ - f males were outcrossed to Bagg albino animals. The offspring were observed at birth and discarded, except in unusual or doubtful cases. Animals giving one or more offspring with definitely abnormal tails were counted as genetically *Fu*/ $+$, whereas animals that had at least 22 normal and no Fused off-

spring were counted as $+/+$. This limit of significance was chosen because the cross $Fu/+$ female by $-h/4-$ male gives about 30 per cent phenotypically Fused offspring. With this ratio, the probability of obtaining no $Fu/+$ offspring from a $Fu/+$ mother by chance only would be about 0.0001. Actually, in most cases more than 30 offspring were obtained.

The results of these crosses are given in table 5. The expectations given in column 4 were calculated from the number of phenotypically Fused animals obtained

studying the offspring from individual animals. All "normal overlaps" from the cross $Fu/+$ female by $+/+$ male and from the F2 gave a reasonably high number of Fused progeny, similar to that produced by phenotypically Fused heterozygotes. Six of the offspring from the cross $+•/+$ female by $Fu/+$ male, on the other hand, gave highly abnormal ratios (male no. 683: 51 normal, 1 Fused; male no. 678: 42 normal, 3 Fused; male no. 1565: 116 normal, 1 Fused; female no. 642: 43 normal, 1 Fused; female no. 685: 55

TABLE 5

PROGENY TESTS OF NORMAL ANIMALS FROM CROSSES INVOLVING *FU*

Cross	Genetically		Per cent Fused		X^2 (d.f.=1)	P
	Normal	Fused	Observed	Expected		
1. 9 $Fu/+$ X $cf +/+$	60	21	25.9	28.0	0.177	-0.7
2. 9 $+/+$ X $cf Fu/+$	45	11	19.7	10.0	7.48	<0.01
2a. 9 $+/+$ X $cf Fu/+$ (corrected)	51	5	8.9	10.0	0.175	-0.7
3. 9 $Fu/+$ X $cf F11/+$	9	6	40.0	33.8	1.54	-0.2

previously from the respective crosses, on the assumption that the actual numbers of $Fu/+$ and $+•/-$ offspring were equal, and that the deviations from a 1:1 ratio were due only to the occurrence of phenotypically normal $Fu/+$ animals. Table 5 indicates that the offspring from $Fu/+$ female by $+/+$ male crosses and from $Fu/+$ female by $Fu/+$ male crosses fitted this expectation. Progeny from the cross $+/+$ female by $Fu/+$ male included a significantly higher proportion of animals giving Fused offspring than was expected. The difference between the proportions of $Fu/+$ offspring from crosses 1 and 2 is not significant ($\chi^2=0.745$. d.f.=1, $P \wedge 04$).[^]

This discrepancy between result and expectation in progeny from the cross $-f/+$ female by $Fu/-\text{Y}$ male can be resolved by

normal, 4 Fused, all in the same litter; female no. 421: 55 normal, 2 Fused). Ratios of this type have not been obtained from other crosses involving $Fu/+$ animals, either phenotypically normal or Fused. The question arises, therefore, whether these animals actually are genetically $F\ll/+$, or whether they are $+/-f$ animals that occasionally give offspring with kinky tails.

If these animals are geotypically $Fu/+$, they may be assumed to carry modifiers that suppress the penetrance of the gene Fu . In this case, about half of their normal offspring should carry the gene Fu . Since there is no evidence for the existence of such modifiers in the Bagg albino strain, outcrosses to Bagg albinos should result in the appearance of Fused offspring in about half of the crosses. Actually, from 24

normal progeny of animals giving abnormal ratios, 450 normal and no Fused offspring were obtained. Similarly, if animals carrying modifiers leading to the suppression of the Fused character were crossed to *Fu*^{-V} animals, a reduction of the number of Fused offspring would be expected. Males 653 and 1565 were outcrossed to *Fu*⁺ females, and produced 40 normal and 16 Fused progeny. This ratio is not significantly different from that usually obtained from a cross of *Fu*⁺ female by *+/+* male.

The "Fused" offspring from animals giving abnormal ratios cannot be differentiated phenotypically from *Fu*⁺. The expression of the character is good; in one case it was even stronger than is usual in *Fu*⁺ heterozygotes. Two of these animals were reared and bred. Crossed out to normal Bagg albino females, they produced 60 normal and no Fused offspring. Thus they behaved in crosses as genetically *+/+*, and may be regarded as "Fused overlaps," that is, normal animals exhibiting the Fused phenotype.

The parents of these "Fused overlaps," which otherwise had only normal offspring, must therefore be considered as genetically *+/-K*. It may be assumed that the other 4 animals that gave abnormal ratios, whose Fused offspring were not progeny tested, were also genetically normal. If this assumption is accepted, the values in line *1a* of table 5 are obtained. With this correction, the agreement between expectation and results is good. Furthermore, the difference between the percentages of genotypically *Fu*⁺ animals from crosses of *Fu*^{-h} females by *-f/+* males and from crosses of *+/+* females by *Fu*⁺ males (lines 1 and *1a*, table 5) is significant ($\chi^2=4.917$, *d.f.*=1, *P*=0.025).

Kinky-tailed animals resembling the Fused phenotype occur occasionally in

normal strains. The question arises, therefore, whether the proportion of "Fused overlaps" is greater in the progeny of normal animals from the cross *+/+* female by *Fu*⁺ male than in the normal strain. Assuming that the animals giving abnormal ratios are normal, and their phenotypically Fused offspring are "overlaps," 10 "Fused overlaps" have been obtained among 1457 offspring (0.686 per cent). The lower fiducial limit at the 1 per cent level for this ratio would be 0.331 per cent. A comparison may best be made with the offspring of normal animals from the cross *Fu*⁺ females by *-f/-b* males. Among 958 progeny of this type no "Fused overlaps" were found. It therefore appears with a high degree of significance that particular conditions exist in normal animals from the cross *-f/+* females by *Fu*⁻ males that occasionally result in the appearance of "Fused overlaps."

The results of these experiments demonstrate that the maternal effect of *Fu* is actually due to differential overlapping. *Fu*⁺ mothers influence Fused ova or embryos in such a way as to decrease the penetrance of the gene *Fu*.

TRANSPLANTATION OF EMBRYONIC TAILS IN THE MOUSE

In order to be able to analyze the action of the genes *T*, *Fu*, and *Ki*, we developed a method that allowed us to grow tails of young mouse embryos in the anterior chamber of the eye of adult mice.

The mouse embryos were timed by the vaginal-plug method. Nine to 12 days after the vaginal plug had been observed, the mothers were killed with chloroform and the uteri dissected out and kept in mammalian Ringer's solution at 37° C. The embryos were taken out of the uterus and pieces of the tail or tail bud were cut

off with glass needles. Usually two pieces were prepared from each embryo, one containing the extreme tip of the tail and the other a more proximal section. In some cases pieces were further dissected by a cut dividing the dorsal from the ventral part of the section, so as to obtain pieces that presumably lacked the neural tube and pieces that carried large amounts of neural tube.

The mice used as hosts were mostly Bagg albinos. In some cases strain-C58 and strain-89 mice were used. The hosts were anesthetized by subcutaneous injections with nembutal. Definite strain differences in sensitivity to nembutal were found; C58 mice frequently died of doses that produced anesthesia but not death in Bagg albinos. An incision was made, with an iridectomy scalpel, through the cornea near the dorsal border of the sclera. The transplant was introduced into the anterior chamber in a drop of Ringer's solution by means of a Spemann micropipette. Preliminary attempts to use a metal trocar for transferring the piece of tissue were unsuccessful, because the tissue tended to cling to the metal surface. Usually the transplant was moved, by gentle pressure on the cornea, to the ventral border between iris and cornea.

All operations were carried out under sterile conditions. The Petri dishes used for dissection of the embryos were sterilized by dry heat, the Ringer's solution in the autoclave. The metal instruments for the dissection of embryos were soaked for 14 hour in Zephiran chloride and washed in several changes of sterile Ringer's solution. The glass instruments were dipped in alcohol and washed in sterile Ringer's solution between operations. In many cases the cornea of the host was washed with dilute Zephiran chloride (1:10,000).

The explants were kept in the host for 4 to 5 days; then the host was sacrificed

and the eyes removed. The transplant was dissected out and fixed in Carnoy, or else the eye was fixed in toto. They were then sectioned and stained with hematoxylin-eosin or with azan.

In spite of the sterile precautions, the eye was frequently infected, as judged by the opacity of the cornea or lens. On the whole, 41 out of no eyes appeared to be infected. The infection did not interfere significantly with the growth of the transplant ($\chi^2=0.275$, $P<0.50$). There is no evidence that proximal pieces of the tail grew better than the extreme tail tips ($X^2=0.259$, $P<0.50$). There was no evidence of differences in survival according to the genotype of the graft (heterogeneity $X^2=2.957$, $d.f.=4$, $P<0.50$).

After 4 to 5 days in the host eye, at least some of the grafts showed definite increase in size. All of them showed a certain amount of organization, although the degree was different in different cases. In the best-developed pieces, as shown in plate 3, the neural tube, notochord, and tail gut, in the correct arrangements, could be distinguished. The mesenchyme was sometimes arranged in a structure similar to somites, lateral to the neural tube (pi. 3). The mesenchyme surrounding the notochord frequently assumed a histological structure suggestive of precartilag (pi. 4B).

Grafts from embryos with Bagg albino normal, *T*, and *Ki* parents showed no particular characteristics of differentiation that could be associated with these genes. In grafts from embryos with *Fu* parents, however, although differentiating somite material looked healthy, the notochord and neural tube were either missing altogether or small and poorly differentiated.

Plate 4A illustrates the amount of growth attained by some transplanted tissues. The donor, from a cross of normal

by Kinky, was 9 days old at the time of operation. A small dorsal piece of the tail bud, containing practically nothing but neural plate, was allowed to remain 4 days in the host. Comparison of this neural tube with that of plate 3, which is represented at the same magnification, will give an indication of the growth in this transplant.

An apparent case of induction is illustrated in plate 4B. The graft was a proximal ventral piece of tail from a normal embryo from a mating of *Ki* by *Ki*, 11 days old. It contained somite material from the right side, probably including pieces of notochord but certainly no neural tube. After 5 days in the host the transplant, *on* sectioning, presented the appearance shown in the plate. There is visible a centrally placed notochord surrounded by concentrically arranged mesenchyme cells, which look like precartilage. Adjacent to this, at the edge of the transplant, is a tuberous structure resembling a neural tube. Although it cannot be stated with certainty that no trace of neural-tube tissue remained in the explanted piece, it appears very likely that this structure was induced by the transplanted tissue.

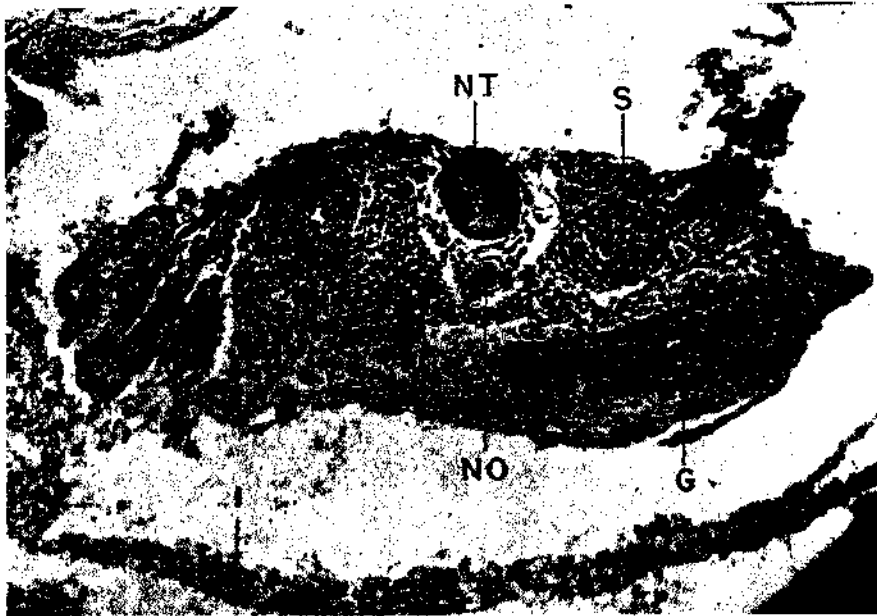
GENE ACTION IN THE AXOLOTL

Amphibian pigment cells offer advantages for the analysis of gene action, because their developmental origin in the neural crest and the subsequent establishment of the larval pigment pattern by migration through the embryonic tissues make possible two types of experimental attack on the problem. First, by explaining in hanging-drop tissue cultures dorsal pieces of neural fold from early neurulae one can obtain isolates of neural-crest material, which in the posterior trunk region consist largely of potential chromatophores. These pro-pigment cells move out from the explants onto the cover glasses, permit-

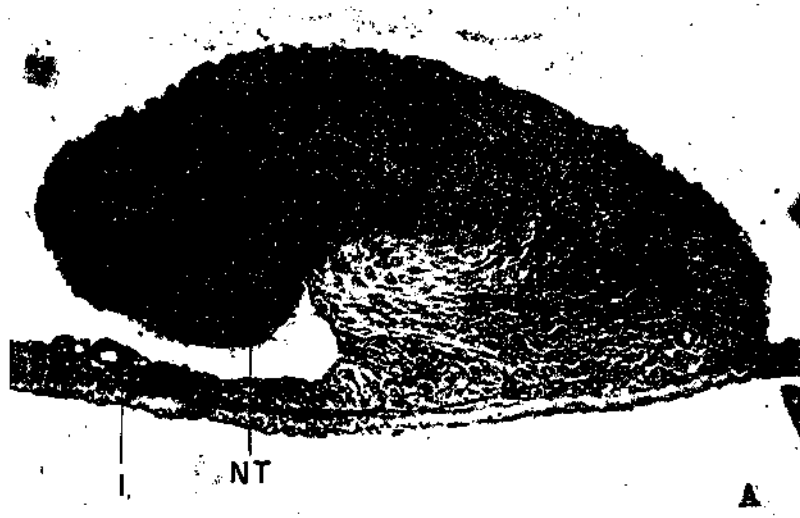
ting direct observation of their intrinsic capacities for migration, proliferation, and pigment synthesis under controlled conditions. Second, by methods of embryonic transplantation one can test reactions between potential pigment cells and their embryonic environment, observing factors extrinsic to the chromatophores themselves but important for pigment synthesis and cell migration. Combining these methods, it is possible first to localize the seat of gene action in the embryo and then to attack the problem of analyzing the nature of this action.

The genes selected for this investigation are those responsible for the pigment patterns characteristic of the white (*dd*) and black (*DD*) strains of the Mexican axolotl *Siredon mexicanum*. It has long been known that these patterns of pigmentation are associated with a single pair of alleles showing Mendelian segregation, the white condition being recessive. Embryonic transplantation experiments described in the literature have suggested that gene action in this case is mediated through the epidermis, which in the white genotype fails to provide some diffusible substance necessary for melanin synthesis. The present investigation was undertaken to determine more precisely the nature of this epidermis effect on pigment development.

Tissue-culture experiments. Before concluding that the effect of gene *d* is limited to the epidermis, it is necessary to exclude the possibility that the gene has some direct action intrinsic to the chromatophores. Several series of tissue cultures were prepared from embryos of both genotypes, involving in all over 300 explantations from the posterior trunk region of early neurulae to hanging drops in Holtfrcter's solution. All tissue cultures were maintained in a constant-temperature room



Proximal piece of tail from n-day *Ki* embryo, from mating *KixKi*; 4 days in host. *NT*, neural tube; *No*, notochord; *S*, somite; *G*, tailgut; *I*, iris. X 200.



A. Tail bud of 9-day embryo from mating normal X *Ki*. Dorsal piece of tail bud only, mostly neural plate. 4 days in host. X200.



B. Proximal piece of tail from normal 11-day embryo from mating *KixKi*. Ventral piece of tail, containing right somite and piece of notochord. 5 days in host. X 200.

Abbreviations as in plate 3

at 20° C. Melanophores of both genotypes are capable of pigment production in vitro, with *no* discernible differences between the two groups in rate or degree of pigmentation of the cells. This result was unexpected, since some diffusible substance from the epidermis was thought to be essential to melanin synthesis in the axolotl, and the melanophores on the cover glasses were not in association with epidermis. The same result was obtained in explants

to distinguish between mesenchyme cells and pro-pigment cells, some of the counts may have represented the former cell type, although it could be seen in the older cultures that the population of migrating cells on the glass was composed almost exclusively of chromatophores. The mitotic rates of the cells observed exhibited a decline from about 6 per cent at 3 days to almost zero at 8 days, mitoses being practically negligible after that time (table

TABLE 6
MITOSES IN TISSUE CULTURES FROM AXOLOTL NEURAL CREST

AGE (days)	WHITE AXOLOTL			BLACK AXOLOTL		
	Total cells	Mitotic cells	Per cent mitosis	Total cells	Mitotic cells	Per cent mitosis
3.....	1263	84	6.6	626	25	4.0
4.....	991	58	5.9	494	13	2.6
5.....	912	39	4.3	580	20	3.5
6.....	1951	63	3.2	980	17	1.7
7.....	1134	28	2.5	763	5	0.7
8.....	1208	6	0.5	203	0	0.0
10.....	732	4	0.5	550	4	0.7
14.....	1674	4	0.2	706	2	0.3
18.....	1347	8	0.6	84	0	0.0
21.....	645	0	0.0	51	0	0.0

of later series from different batches of eggs.

The studies were extended to include observations on mitotic rates and on cell migration. Samples from a series of 63 tissue cultures were fixed and stained in aceto-orcein on successive days, beginning with the third day after explanation, in order to determine whether there is any difference in inherent capacity for proliferation of cells from the two strains. The counts included only such cells of the explants as migrated out onto the cover glasses, exclusive of sheets of epithelial cells that occurred occasionally among the cultures. Since it is impossible

6). The figures for cultures of the black strain are slightly lower than those for the white strain, but not significantly so ($P < 0.20, > 0.10$). In relating these findings to conditions in the embryo, it must be remembered that the tissue-culture medium is composed only of inorganic salts and provides no nutrients to the cells, which consequently are limited to the food reserves present within them at the time of explanation. When the yolk platelets are exhausted, growth and proliferation must necessarily cease. Since, however, the larval pigment pattern is established in control embryos during the same time in which cells of the tissue cultures migrate

and produce pigment in apparently healthy condition, it seems reasonable to expect that if intrinsic differences in capacity for mitosis existed between chromatophores of the two genotypes, and were important in the development of the genetic patterns, they would show up in the mitotic rates observed. The conclusion is that potential and differentiating chromatophores of the two genotypes do not differ in mitotic capacity when isolated from the embryo.

To obtain a measure of migratory activity, the following procedure was applied to the same series of tissue cultures stained

emerge as salient features: Considerable variance was exhibited by all groups into which the data were broken down. As a summary of the genotypic group characteristics, the mean values of tissue-culture measurements of area per culture, number of cells per culture, and cell density are presented in table 7. The average values for the white group are higher than the corresponding figures for the black group. The group mean difference between area measurements is not significant, but there appears to be little doubt that there is a real difference in number of cells per cul-

TABLE 7

MEAN VALUES OF TISSUE-CULTURE MEASUREMENTS

	White (35 cultures)	Black (23 cultures)	<i>t</i>	P*
mm. ² /culture.	2.211± 0.131	1.824± 0.121	2.18	>0.05
Cells/culture.	333±21.7	215±25.3	3.58	<0.01
Cells/mm.*.157± 9.13	126± 11.5	2.13	<0.05

* Approximation to 5 per cent and 1 per cent level computed as mean of *t* values for d.f. 35 and 23 weighted by the two variances.

with aceto-orcein. Camera lucida drawings were made of the areas occupied by migrating cells on the cover glass. These were measured with a planimeter, and the figures converted to square millimeters. The cell density of each culture was computed by dividing the total cell counts made in the mitosis survey by the area measurements. Interpretation of results must take into account the fact that chromatophores in the cultures frequently migrated on both the glass surface and the drop surface, but that all measurements of areas occupied include only the former because cells on the drop surface floated away during fixation. All figures for the black and white groups of tissue cultures were subjected to extensive statistical analysis, from which the following points

emerge as salient features: Considerable variance was exhibited by all groups into which the data were broken down. As a summary of the genotypic group characteristics, the mean values of tissue-culture measurements of area per culture, number of cells per culture, and cell density are presented in table 7. The average values for the white group are higher than the corresponding figures for the black group. The group mean difference between area measurements is not significant, but there appears to be little doubt that there is a real difference in number of cells per cul-

ture, since $P < 0.01$. The difference in density measurements is probably also significant ($P < 0.05$), but this might be expected, since these figures are derived from the cell counts, and the number of cells per culture is correlated with the cell density ($r = 0.736$ and 0.555 , for the black and white series respectively). It was possible to check on the significance of two operative variables, size of explant and age of donor, which might conceivably affect the group comparisons, because the black series contained some explants of small size (one-half and one-third of posterior trunk neural fold) as well as pieces of entire posterior trunk fold such as were used exclusively in the white series, whereas the white series contained some explants from donors with open neural folds (stage

16) as well as some from donors with closed neural folds (stage 20-21), which were used exclusively in the black series. When the data were broken down within a single genotype into two groups, on the basis of either size of explant or age of donor, there was no significant difference between the groups, a fact which indicates that the black and white genotype group differences were not due to these two experimental variables.

Further information on the history of migrating cells in the tissue cultures was obtained by averaging the figures for each day of age and plotting the values against time. The number of cells per culture and the cell density, in both black and white groups, became less with time. The area per culture, however, diminished in the black but increased in the white group. This difference may indicate a greater capacity for migration in cells from white explants as compared with black, but it may also reflect the significantly larger number of cells in the white cultures, since number of cells is correlated with area occupied ($r=0.554$ for black and white respectively). Since the group mean differences between black and white with respect to area occupied are not significant, the evidence at present does not justify the conclusion that the two genotypes exhibit any real difference in migratory capacity of the chromatophores in vitro.

Samples from another series of 63 tissue cultures were fixed in Carnoy's fluid on successive days after the third day following explantation, for detailed study of the morphology of chromatophores and of the structure and history of pigment granules. This study is still in progress, but observations so far completed fail to show any differences that might be correlated with the two genes under investigation.

In summarizing the tissue-culture ex-

periments, it may be said that the study of white and black axolotl chromatophores in vitro has shown that the genes *D* and *d* do not affect the chromatophores themselves in such a way that intrinsic differences in capacity for proliferation, migration, or pigment synthesis are demonstrable in tissue cultures of the two strains. The effects of the genes in question, therefore, appear to be mediated through factors extrinsic to the chromatophores. Evidence about the nature of this extrinsic effect on pigment development has been obtained by embryonic transplantation experiments.

Transplantation experiments. Relations between developing chromatophores and their surrounding tissues were tested by embryonic transplantations, which arranged these components of the two genotypes in different combinations. Early experiments were aimed at demonstrating differences in development of chromatophores of the same origin when in association with tissues of the black strain and of the white strain. Neural folds from the posterior trunk region of early neurulae were transplanted to the mid-ventral belly region of late neurula or early tail-bud hosts. This transplantation site was chosen to eliminate any confusion of graft chromatophores with those of the host, since the ventral belly region does not become pigmented in either strain during the period of observation of this experiment. Each donor provided one transplant in a black host and one in a white host, allowing subsequent comparison of graft chromatophores of identical genotype and age developing in the two environments. Melanophores from these grafts of both genotypes exhibited no disadvantages for melanin synthesis in association with tissues of white embryos. Both types of host permitted the differentiation of completely black melanophores. The same result was obtained when neural crest from tail-bud

embryos of both genotypes was transplanted reciprocally to the normal topographic position for this tissue. On the basis of the mechanism of gene action suggested in the literature, one would expect melanin synthesis to be inhibited in the white hosts. The evidence of these experiments, therefore, does not support this view, since there appeared to be no difference in rate or extent of pigment formation by grafted cells in the two strains.

Comparison of the extent of migration of melanophores in the two types of host, on the other hand, revealed a striking difference. Melanophores of both genotypes appeared to migrate freely beneath epidermis of the black strain but not beneath epidermis of the white strain. Evidence of this inhibitory effect on migration by white tissues appeared in both heterotopic and homotopic grafts. The neural-fold pieces grafted heterotopically into the belly region adhered both to the skin and to the gut surface, and in all cases melanophores migrated into both these regions. The areas occupied by graft chromatophores in both skin and gut were measured by means of camera lucida drawings and a planimeter. When total counts of melanophores from each graft were made and divided by the area measurements, it was apparent that the pigment cells of both genotypes developing in white hosts not only occupied much less area than cells from the same donors developing in black hosts, but also were much more closely crowded together, as if they had failed to spread to a comparable degree.

When neural crest is grafted homotopically from black to white tail-bud embryos, the epidermis lying dorsal to the neural crest is necessarily included in the graft. On the white hosts this bit of dorsal epidermis formed later a section of the dorsal fin above the grafted neural crest.

In all cases melanophores migrated extensively into this section of the fin but not into adjacent areas of host fin. Another set of operations was made in two steps, in order to obtain graft melanophores of black origin growing in the complete absence of any donor epidermis. First, pieces of dorsal epidermis overlying the neural crest of black embryos were replaced by pieces of flank epidermis from white embryos. Flank, rather than dorsal, epidermis was chosen to prevent the possibility of transplanting any adhering neural-crest cells. On the following day, the black neural crests, covered now by white epidermis, were transplanted to white embryos. In these cases, melanophores did not migrate into the skin dorsal to the grafts, but dense black lines of melanophores appeared beneath the epidermis at the edge of the graft regions. Corresponding results of grafting neural crest of white embryos to black hosts under similar conditions also suggest that the mobility of pro-pigment cells is greater in an environment of *DD* (black) tissue than in one of *dd* (white) tissue.

The view that a diffusible substance necessary for melanin synthesis is provided by black epidermis but lacking in white epidermis was based on the assumption that pro-pigment cells in white embryos migrate extensively onto the flank but do not form pigment. The following experiment was designed to investigate the validity of this assumption. Triangular pieces of epidermis were transplanted from black to white embryos, some transplants being oriented with the base dorsal and some with the apex dorsal. If epidermis from a pigmented strain permits pigment formation on a white axolotl by furnishing favorable conditions for melanization to cells normally occurring on the host flank but not normally pigmented under white epidermis, then the orientation of the

grafted triangles, provided they covered the same level of flank, would make no difference in the number of melanophores appearing beneath them. If, on the other hand, the pigment cells observed under grafted black epidermis owe their position on the flank to the removal of migratory inhibition normally encountered with the host epidermis, then triangles with the base up would be available for migration to pro-pigment cells from the length of neural crest covered by the base of the triangle, whereas triangles with the apex up would be available for migration to cells from a much more restricted length of neural crest. These conditions would be reflected in different densities of melanophores beneath grafts of the two types. The process of graft healing and subsequent growth of the embryos rotated some of the triangles, so that some were later not precisely oriented with base or apex up. As a measure of the avenue open to migrating cells moving into the graft area, the ratio of the top length of the graft to the area of the graft was chosen. Graft areas, as indicated later by the distribution of melanophores, were measured by means of camera lucida drawings and planimeter, and melanophores appearing beneath the entire epidermis of the graft were counted. When the density of melanophores was plotted against the ratio of top length to area of the epidermis grafts, there was indicated an obvious correlation, as shown in figure 5.

The results of all experiments support the idea that in the white and black strains of the axolotl, the genetic differences in pigmentation are mediated through differences in the tissue environment and not in the chromatophores themselves, the white pattern resulting from an inhibitory effect concerned, not with conditions for melanin synthesis, but with the migration of pro-pigment cells.

INVESTIGATIONS ON AN ENZYME SYSTEM OXIDIZING TRYPTOPHANE TO KYNURENIN

In *Ephestia*, the gene *a* inhibits the oxidation of tryptophane to kynurenin. Attempts to demonstrate an enzyme catalyzing this activity in *Ephestia* homogenates have been essentially negative. It was therefore decided to study this enzyme system in mouse liver.

Homogenates of mouse liver were prepared by grinding in 0.1 molar phosphate

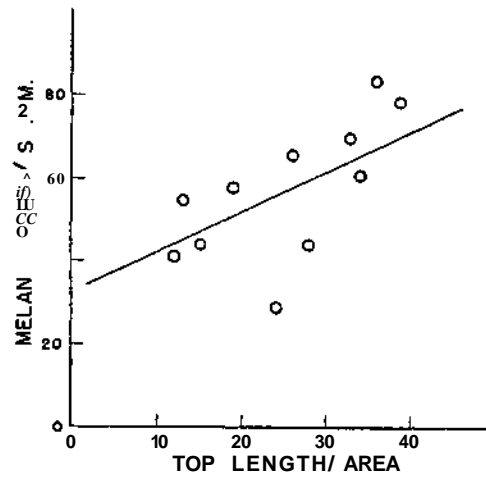


FIG. 5. Regression of melanophore density on ratio of top length to area in epidermis grafts.

buffer, pH 6.8, at 0° C. in Potter Elvehjem homogenizes. The oxygen uptake of homogenates in the presence and absence of tryptophane was determined in a Warburg apparatus at 37° C. At the end of each experiment, tryptophane was determined by the method of Bates, and kynurenin by the method of Otani and Nishino. For each experiment, therefore, there are three sets of data to establish the activity of the enzyme.

With this method it has been shown that oxidation of tryptophane to kynurenin does occur in mouse-liver homogenates. The enzyme concerned seems to be present in

low concentration, since 50 per cent homogenates are necessary to obtain strong activity. Twenty per cent homogenates gave only 3 per cent of the activity of 50 per cent homogenates, and no activity could be detected in 10 per cent homogenates.

Some properties of the enzyme system were studied in 50 per cent homogenates. Its optimum pH is between 6.7 and 7.1. It loses activity on standing, so that after two days at 0° C. only 65 to 70 per cent activity is left. Dialysis against water at 0° C. for 16 hours does not affect the activity. The enzyme system was found in liver only; 50 per cent homogenates of mouse lung, kidney, spleen, and testicles were inactive.

When liver homogenate is centrifuged, activity is found both in the sediment and in the supernatant. After centrifuging for one hour at 18,000 r.p.m., 55 per cent of the original activity is found in the supernatant. After fractionated precipitation with (NHU⁺SCX and subsequent dialysis against water for 16 hours at 0° C, all activity is found in the fraction precipitated at one-third saturation with (NEUJaSO*. Globulins prepared in this way oxidize tryptophane to kynurenin at an optimum pH of 6.8 to 7.0. The oxygen-consumption curve is characterized by a lag period after addition of tryptophane, during which no increased oxygen uptake occurs. This lag period lasts from 20 to 40 minutes. The need for cofactors is demonstrated by the fact that the activity of these preparations is considerably increased by addition of Mg⁺⁺ ions and of a Kochsaft prepared from mouse liver. During the reaction, CO₂ is released.

These **experiments** have suggested two possible reasons for the failure of *Ephestia* homogenates to show oxidation of tryptophane to **kynurenin**. An inhibitory effect of *Ephestia* homogenates on mouse-liver

homogenates was described in Year Book No. 47. Evidence obtained recently indicates that slight activity may be present in the globulin fraction obtained from *Ephestia* pupae, but not in that obtained from larvae. This would indicate that the enzyme system may be more concentrated in pupae than in larvae, in agreement with the fact that most of the pigment production takes place in the pupal stage.

Serological differences between a and a Ephestia.* Evidence was reported last year that the gene *a*, besides affecting pigment metabolism, also causes the formation of qualitatively different proteins. This assumption has been further investigated by serological methods. Rabbits were immunized by five subcutaneous injections at 6-day intervals with 0.9 per cent NaCl extracts from homogenized *Ephestia* larvae. Three rabbits were immunized against *a⁺a⁺* and two received extracts from larvae of an *aa* strain that had been made isogenic with the *etc?* strain by outcrossing for 8 to 9 generations. Seven days after the last injection, the rabbits were bled and the serum obtained.

The sera were tested against antigens from both strains by means of the precipitin reaction. The antigens used in this case were cleared by centrifugation at 12,000 r.p.m. for 1 hour. The supernatant was of a clear greenish color, but had a tendency to form a black precipitate on standing. The antigens were therefore used immediately after being prepared, and compared on the basis of their nitrogen content, which was determined by a micro-Kjeldahl procedure.

No differences in the reaction of the sera against homologous and against heterologous antigens were found by simple dilution. Three of the sera that had relatively high titers (between 1/10,000 and 1/100,000) were more thoroughly tested by the optimum **flocculation** method. **Dif-**

ferent dilutions of antigen were mixed with a constant dilution of serum, and the time of the first appearance of visible flocculation was determined for each tube. The ratio of serum to antigen in the tube showing fastest flocculation was designated as optimal proportion. In table 8 the optimum proportion for the sera tested is expressed as milligrams of antigen nitrogen per cubic centimeter of antiserum. The numbers in parentheses indicate the number of determinations performed.

TABLE 8

OPTIMUM PROPORTIONS FOR THREE ANTI-EPHESTIA RABBIT SERA WITH THE HOMOLOGOUS AND HETEROLOGOUS ANTIGENS
(Optimum proportion expressed as milligrams antigen N reacting optimally with 1 cc. of serum)

Serum	Antigen	
	a ⁺ a ⁺	aa
1 (anti-a ⁺ a ⁺)	0.282 (3)	0.379 (4)
3 (anti- $\&^+\&^+$)	0.280 (4)	0.379 (3)
6 (anti-flo)	0.206 (3)	0.138 (2)

The table indicates that in every case the sera showed a higher titer when tested with the homologous than when tested with the heterologous antigen. In repeated determinations, some variability of the optimum proportion values, expressed in milligrams N, was found; but in not a single case did the ranges for the heterologous and the homologous antigens overlap.

The sera were absorbed with the heterologous antigen by incubating antigen and serum at optimum proportions for 1 hour at 37⁰ C. and leaving in the cold for 24 hours. After centrifugation, the supernatant sera were tested against the homologous and heterologous antigens. Sera 1 and 6 still gave reactions with both anti-

gens after absorption, but higher titers with the homologous than with the heterologous antigen. After a second absorption, these two sera did not react with either antigen.

Serum 3, on the other hand, lost its ability to form a precipitate with the heterologous aa antigen after one absorption at optimum proportions. It still reacted with the homologous a⁺a⁺ antigen in a 1:25 dilution (0.0055 mg-N). This absorbed serum, which formed a specific precipitate with *etc?* antigen, was used to determine the chemical nature of the active antigen. Antigen fractions were obtained from both *etc?* and aa saline extracts, by half and full saturation with (NH⁺SCX and subsequent dialysis against H₂O. The serum gave a precipitate only with the fraction obtained by half saturation of a⁺a* extract with (NHU⁺SO*. All other preparations failed to react.

These experiments demonstrate that antigenic differences exist between a⁺a* and aa *Ephestia*. They furthermore indicate that at least one of the substances responsible for these differences is a protein belonging to the globulin fraction.

SEROLOGICAL STUDIES WITH THE BRACHYURY MOUSE

The positive finding of serological differences between tf⁺ and a *Ephestia* suggested the possibility that similar serological differences might be found for mouse genes. The gene *T* (Brachyury) was used. Testicles and spleens from freshly killed T/H- mice were ground up in physiological saline and extracted three times with saline in the cold. The pooled extracts were injected into rabbits. Five subcutaneous injections were made at 6-day intervals. Sera were obtained on the tenth day after the last injection. The sera were then tested against antigens obtained from

males of the genetic constitutions $T/+$ and $+/+$, which had been made isogenic by outcrossing for 18 generations. Two different antigens were used: the first saline extract, and a second saline extract obtained by treatment with saline of the sediment from the first extraction. It is assumed that the first extract contained easily soluble proteins, including the serum proteins from the blood left in the organs, whereas the second extract contained substances that dissolved with more difficulty, probably including small particles. These two types of antigen were prepared from both testes and spleens of both strains, and kept at 0° C. after preservation with merthiolate 1/10,000. Their nitrogen content was determined, and they were adjusted to contain equal amounts of nitrogen.

In precipitation tests the sera did not show any differences in titer when tested with the homologous and heterologous antigens. Absorption experiments with the first antigen of the $+/+$ strain were unsuccessful, except in the case of one anti-spleen serum, which reacted with the homologous antigen. After absorption with the second antigen of the heterologous strain, three sera (two against testicles, one against spleen) turned out to give precipitates with the homologous but not with the heterologous antigen. Serological differences between $T/+$ and $+/-f$ animals are therefore clearly indicated.

Serological differences between $T/+$ and $+/\sim f$ testes were also demonstrated by complement fixation. The same sera were used as for the precipitation tests, after inactivation for 30 minutes at 56° C. The antigens were adjusted to contain 0.05 mg. N. Different dilutions of sera were used, and incubated in the presence of complement (2 units) for 30 hours at 37° C, and for 30 minutes at room temperature. At the end of this period, the

indicator system, consisting of 2.5 per cent sheep erythrocytes and 2 units of hemolysin, was added, the tubes were incubated for 30 minutes at 37° C, and the degree of hemolysis was recorded. The tubes were then kept for 24 hours in the refrigerator. At the end of this period, the tubes were centrifuged, the supernatant discarded, and the sedimented blood corpuscles suspended in 5 cc. of 0.9 per cent saline. The saline suspension was then read in the Klett-Summerson colorimeter with a blue filter. The degree of lysis was recorded in terms of the percentage of red blood corpuscles found as compared with the saline control.

The results of one of these experiments are recorded in figure 6. The curves obtained in this way for the second antigen from $+/+$ and from $T/+$ are obviously different, the serum reacting more strongly with the homologous than with the heterologous antigen. This is apparent not only from the titer for complete lysis, but particularly from the general shape of the curves. More complement appears consistently to be fixed by $T/+$ antigen than by $+/+$ antigen at the same serum dilutions. The same result has been obtained with two other anti-T testicle sera, tested with the second antigen. On the other hand, no differences were found in the same sera when they were treated with the first saline extracts of $T/+$ and $+/+$ testicles.

The anti-T testicle sera were also tested against antigens obtained from $Ki/+$ animals. Ki is a gene situated close to T on the same chromosome, having similar though not identical phenotypic effects. It appears from the curve of figure 6 that $Ki/+$ is intermediate between $T/+$ and $\bullet f/+$. This result is also evident in the other two anti- $T/+$ testicle sera tested. It therefore appears that connected with the gene Ki there is an antigenic structure similar to but not identical with X. The

results of the experiments with *Ki* antigens seem to indicate a physiological relation between the closely linked genes *T* and *Ki*.

Absorbed sera proved to be highly anti-complementary when tested by means of the complement-fixation method.

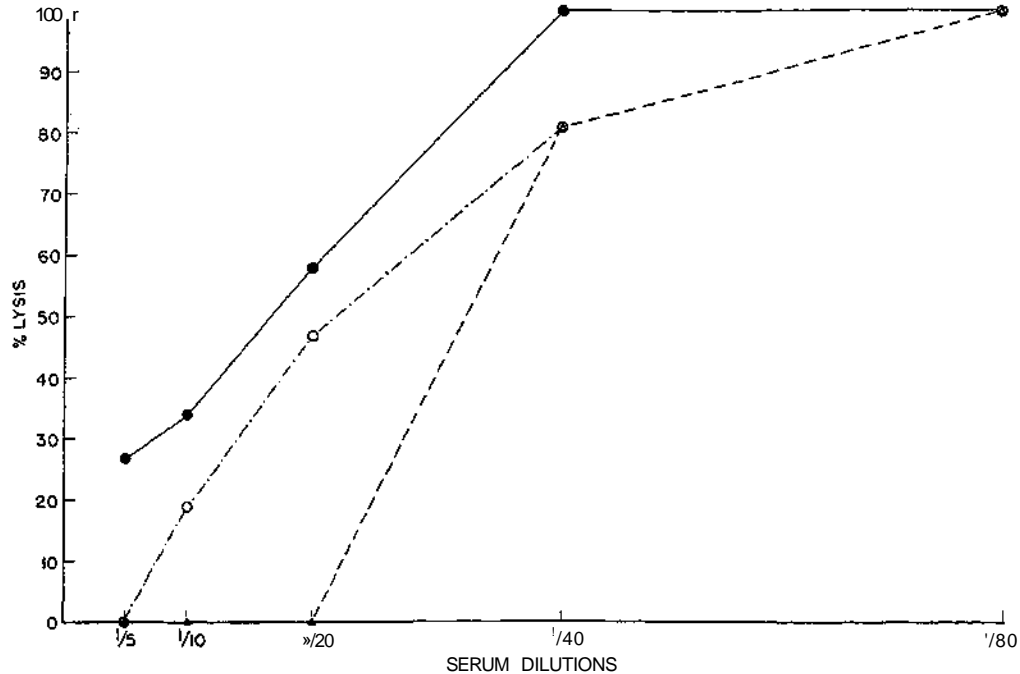


FIG. 6. Complement fixation of an anti-T testicle serum with "second" antigens from *T*, -f, and *Ki* testicle antigens, adjusted to contain 0.05 mg. N each. Abscissa: serum dilutions. Ordinate: per cent hemolysis, determined by nephelometric measurement of nonhemolyzed corpuscles. \bullet — \bullet = *T*/+; \circ — \circ = *Ki*/+.

GENETIC STRUCTURE OF NATURAL POPULATIONS

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Most biologists agree that adaptation to the environment is the principal driving force of organic evolution. But modern genetic thought visualizes the relations between organism and environment in a way quite different from environmentalistic theories of the nineteenth century, which have recently become a topic of renewed discussion because of the polemics aroused by Lysenko and his partisans. Living beings are not passively molded by physical agencies, as mechano-Lamarckists believed. Nor can a species change by exertion of its

will, as supposed by psycho-Lamarckists and finalists. Moreover, organisms are not altered by a kind of sympathetic magic, which makes them able to "select" useful and to reject useless materials from changed environments, as imagined by Lysenko. It is the view of a majority of evolutionists that mutation and Mendelian recombination continually produce innumerable genetic materials, some of which are more and others less suitable for perpetuation in various environments. The available genotypes are then adjusted by

natural selection to the opportunities available in the world.

To say that evolution is brought about because organisms are changed by environment is inexact. Organisms change in the process of becoming better able to survive and reproduce in the environments in which they live. Evolution is a response of the organism to the challenge of the environment. And this challenge does not arise from physical conditions alone, but also from interactions with other organisms that share the same physical environment. It is probable, at least in higher organisms, that the biotic environment is more important in evolution than are physical conditions in the narrow sense.

CO-OPERATIVE STUDIES ON TROPICAL DROSOPHILAS

The above conception of the relations between environment and evolution suggests new types of study. Genetic experiments are done mostly with domestic animals, cultivated plants, or species otherwise associated with man, such as commensals, weeds, or pests. Many organisms domesticated by or living with man have important technical advantages for use as experimental materials. But to understand the evolutionary process as a whole, work on such species is not sufficient. We must examine the causal links connecting the evolutionary patterns of different organisms with the biota of which these organisms are constituent parts. Interrelationships between living beings and the main types of environment that exist on our planet are to be studied.

Comparison of genetic population structure in related species living in temperate and in tropical climates seems especially promising. In any temperate or cold climate, sharp seasonal changes in the environment occur every year. Any popula-

tion of organisms living under such conditions must evidently be adapted to cope with a succession of sharply different environments. Tropical climates in general permit the environment to remain relatively more uniform throughout the year, and may seem to demand less adaptive versatility from the inhabitants. Tropical biota, however, include greater numbers of animal and plant species than is the case in temperate or cold lands. Thus members of tropical biotic communities meet a great variety of challenges, to which they may respond by adaptive evolutionary changes. The evolutionary process in the tropics, taken as a whole, may have a faster tempo and a greater creativeness than in temperate and cold climates.

A program of comparative studies on population genetics of species of *Drosophila* in temperate and in tropical climates was initiated some seven years ago (see Year Books Nos. 43, 1943-1944, and 47, 1947-1948). In 1943, the writer, in cooperation with Dr. C. Pavan, of the University of São Paulo, made an exploratory survey of species of *Drosophila* that occur in the state of São Paulo and near Belem in the state of Pará in Brazil. Two species, *Drosophila willistoni* and *Drosophila pro saltans*, were chosen as materials for further and more intensive study. The former is the commonest species in most of Brazil; it is also ecologically most versatile, since it occurs in a great variety of environments and feeds on many species of fruits. In contrast, *D. pro saltans* is a rare form, ecologically specialized; thus far it has been found in large numbers only in a few localities, in the state of Maranhão and on the island of Marajó. Preliminary genetical work on these species, necessary to make them available for experimental studies of the genetic population structure, has been carried out at Columbia University by Mr. B. Spassky, Mr. S. Zimmering, Pro-

fessor A. G. L. Cavalcanti, and the writer.

From August 1948 to June 1949, inclusive, a group of investigators, assembled at the laboratory of Professor André Dreyfus, at the University of São Paulo, carried out a program of orientation studies on population genetics and ecology of tropical species of *Drosophila*, especially *D. willis-toni* and *D. prosaltans*. The group included, besides Professor Dreyfus, Drs. C. Pavan, A. Brito da Cunha, and E. Nascimento Pereira, of the University of São Paulo; Professor A. G. L. Calvalcanti and Miss Ch. Malogolowkin, of the University of Brazil, Rio de Janeiro; Dr. A. R» Cordeiro, of the University of Porto Alegre, Rio Grande do Sul; Ing. Agr. M. Wedel, of the University of Buenos Aires, Argentina; Dr. Hans Burla, of the University of Zürich, Switzerland; Mrs. N. Dobzhansky, and the writer. Mr. B. Spassky remained at Columbia University in New York, but participated in the cooperative program by furnishing certain mutant strains and carrying out many experiments. The work of the above group was made possible by financial assistance extended by the University of São Paulo, the Carnegie Institution of Washington, and the Rockefeller Foundation. Brazilian military and civil authorities, in particular General Newton Cavalcanti, Brigadier General Eduardo Gomes, the governors and their aides in the states of Goyaz, Amazonas, Paraná, Rio Grande do Sul and the territories of Rio Branco, Acre, and Guaporé, Dr. Felisberto de Camargo, director of the Instituto Agronomico do Norte at Belem do Pará, and other officials and private persons too numerous to be named, greatly facilitated the field work by providing airplane and other transportation and by extending many valuable courtesies, which made the travel and collection in remote parts of Brazil a success as well as a pleasure.

COLLECTING JOURNEYS

The first task was to collect material for cytological studies and for genetic experimentation and to make field observations on ecology of *Drosophilas* that inhabit the principal climatic and vegetational zones of Brazil. The regions in which collections were made are shown by black circles on the accompanying map (fig-7)-

Three stations were established in the state of São Paulo, at which collections were made at approximately bimonthly intervals. One of them, in coastal rain forest near Vila Atlantica, is sufficiently warm and humid throughout the year to permit the maintenance of flourishing *Drosophila* populations. The second, near Mogi das Cruzes on the plateau, has a cool and fairly dry winter season; and the third, near Pirassununga in the interior of the state, is hot and humid in summer but dry in winter. These three stations formed a climatic gradient, whose influence on *Drosophila* populations was studied.

The collecting expeditions to localities more remote from the laboratory in São Paulo were as follows. From October 29 to November 10, 1948, Dr. Pavan and the writer visited the central part of the state of Goyaz, and made collections at Monjolinho (near Anapolis) and at Palma (Paraná, see map). This is a zone of *crrado* savanna and of gallery forest vegetation, warm throughout the year but with pronounced wet and dry seasons, the latter causing reduction of the abundance of most species of *Drosophila*. From January 5 to 26, Professor Cavalcanti and the writer collected in the vicinities of Cruzeiro do Sul and Palraares, territory of Acre, and at Porto Velho, territory of Guaporé. This is a zone of exuberant forest vegetation of Amazonian type (*hylaesa amazonku*); despite variations in the amount of precipi-

tation at different seasons, temperature and relative humidity remain continually favorable for *Drosophila* breeding. Between February 22 and March 5, Dr. Pavan and

most of the year there is almost no precipitation and the vegetation dries out and loses its foliage, as it does in winter in temperate climates. These conditions are

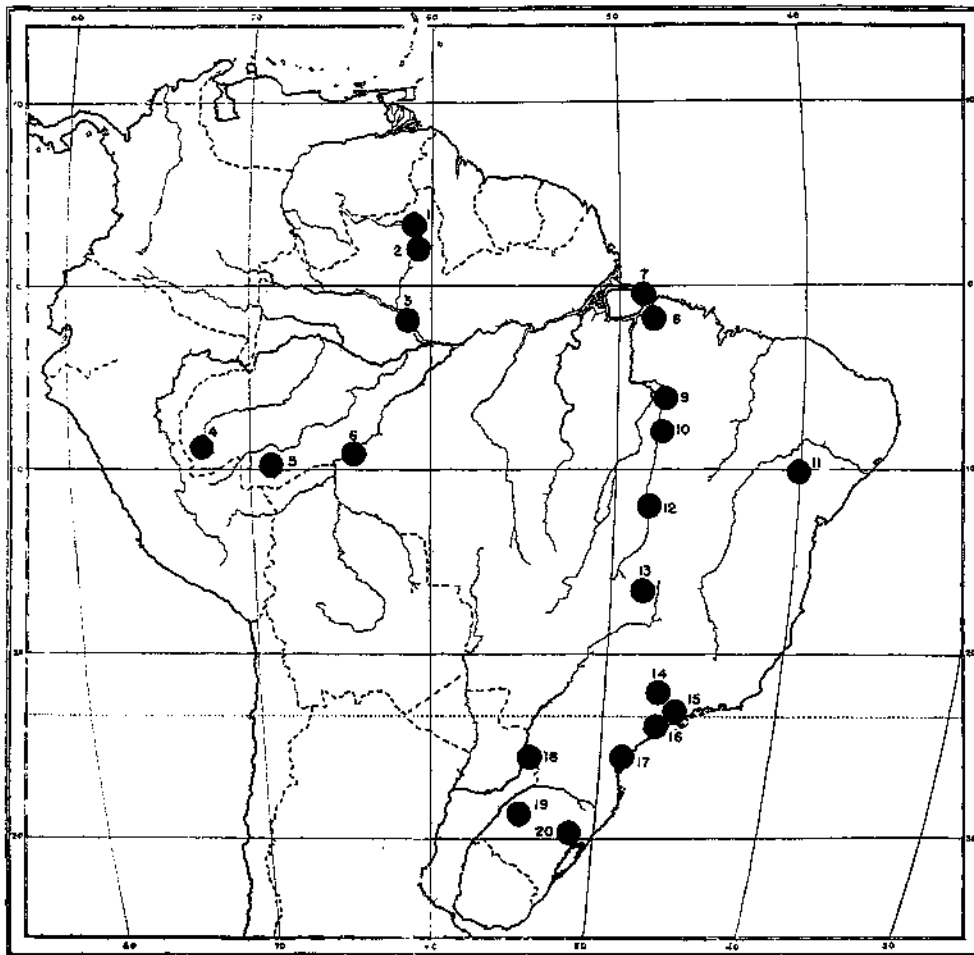


FIG. 7. Map of South America, showing the location of the places where samples of *Drosophila* populations were taken. 1, Savanna of Rio Branco; 2, Rio Mucajaf, territory of Rio Branco; 3, Rio Negro, Aniazonas; 4, Cruzeiro do Sul, Acre; 5, Palmares, Acre; 6, Porto Velho, territory of Guaporé; 7, Marajó Island; 8, Belem, Pará; 9, Imperatriz, Maranhão; 10, Carolina, Maranhão; 11, Catunf, Bahia; 12, Paraná (Palma), Goyaz; 13, Monjolinho, Goyaz; 14, Pirassununga, São Paulo; 15, Mogi das Crazes, São Paulo; 16, Vila Atlantica, São Paulo; 17, Paranaguá, Paraná; 18, Iguassu National Park* Paraná; 19, Santo Angelo, Rio Grande do Sul; 20, Reuter, Rio Grande do Sul.

the writer made an excursion to Rio Salitre and to Catuni, near Bomfim in the *caatingas* in the state of Bahia. The *caatingas* are characterized by the sharpest seasonal climatic chintzes possible in the tropics;

close to the limit of endurance for *Drosophila*, and only a few species are able to withstand the rigors of the *caatingas*.

Dr. A. da Cunha, Mrs. Dobzhansky, and the writer visited the state of Paraná, and

made collections at Paranaguá on the Atlantic coast, and at the Iguassu National Park at the boundary with Argentina and Paraguay, between March 22 and April 2. The conditions at Paranaguá are, on the whole, similar to those at Vila Atlantica (see above). The Iguassu region has a forest of tropical aspect, but the temperatures are low in winter and frosts occur. A relatively small variety of species of *Drosophila* was encountered in the region. From May 18 to 23, Dr. Cordeiro and the writer visited the state of Rio Grande do Sul and collected at Santo Angelo in the western part of the state. Dr. Cordeiro made a collection at Reuter in the south central part at an earlier date, in January. The state of Rio Grande do Sul is outside the tropical zone and has cool winters. A rather large number of species of *Drosophila* was nevertheless encountered in the collections made in that state.

Between April 12 and May 10, Dr. Pavan and the writer collected in the savannas of the territory of Rio Branco and at the margin of the forest zone at Rio Mucajai in the same territory, on the lower Rio Negro in the state of Amazonas, and near Belem in the state of Pará. The savanna of Rio Branco has a long dry season, and tree vegetation occurs there chiefly in the form of gallery forests along streams and near marshes. Rio Negro has exuberant rain forests and an equable hot and humid climate. Mucajai is probably intermediate climatically. The Belem region, despite its having a drier and a wetter season, is probably as favorable for the maintenance of large *Drosophila* populations as is the Rio Negro region; and a large variety of species, many of them new to science, was encountered there. Collections were made near Belem also in September and October 1948 by Dr. Pavan and the writer, in June 1948 by Dr. Pavan, and in July-September 1949 by Dr. Pavan and the writer, so that

information was obtained about the status of *Drosophila* populations at different seasons.

From July 29 to August 13, Dr. Pavan and the writer traveled in the state of Maranhão, and collected in the vicinities of Carolina and Imperatriz. This region is transitional between the savannas (*campos cerrados*) of central Brazil and the Amazonian rain forests of Pará. Pronounced wet and dry seasons occur yearly. A gradual change in the composition of the *Drosophila* fauna was observed, from that previously found in the state of Goyaz to that living near Belem. Between August 27 and September 5, Dr. Pavan and the writer visited the estates belonging to the family Tocantins Penna in the northeastern part of Marajó Island, state of Pará. Despite its relative proximity to Belem, this part of Marajó has extensive grass-covered swamps, alternating with rather open forests growing on higher ground; wet and dry seasons are clearly differentiated; and the *Drosophila* fauna proved to be unique because of a high frequency of *D. prosaltans*, which is rare in most other parts of Brazil. Finally, Dr. Warwick Kerr very kindly collected and sent to us a sample of the *Drosophila* population from the vicinity of Santa Cruz de la Sierra, in Bolivia, in July 1949.

The collecting journeys made from August 1948 to September 1949 in the Brazilian territory entailed between 27,000 and 28,000 kilometers of airplane travel alone. The number of *Drosophilas* of various species collected and classified during this time was close to 110,000 individuals.

DIVERSITY OF SPECIES IN TROPICAL ENVIRONMENTS

Perhaps the most striking and significant difference between *Drosophila* communities in tropical and in temperate countries

is that the former, as a rule, contain more species than the latter. For example, at Chitina, Alaska, the writer collected at least a thousand *Drosophila*s, all of which belonged to a single species, *D. athabasca*. Collection with the aid of fermenting banana bait in a favorable locality in California mountains is likely to yield, among some hundreds of individuals, only 5 to 10 species. Localities are common in which only 2 or 3 species, or even a single species, are found. Furthermore, regardless of the number of species caught, one of them, *D. pseudoobscura*, is in many localities more common than all others combined. By way of contrast, collection on banana bait in Brazilian forests usually yields more than 10 species, and may reveal more than 30 in a single neighborhood less than one square kilometer in area. Moreover, several species may be common in such collections, none of which is dominant. Only in those tropical environments that are intrinsically unfavorable for *Drosophila* does the number of species become reduced and one or two species become dominant. This occurs, for example, in the peculiar desert regions of northeastern Brazil called *caatingas*, where the heat and aridity of a prolonged dry season appear to be beyond the toleration limit of all except a few species. *Drosophila nebulosa* is the unchallenged dominant in the *caatingas*, as it is also during the dry season of the year in the savanna (*campo cerrado*) of central Goyaz.

Drosophila species that are attracted to banana bait subsist in nature mainly on fermenting fruits, tree sap, and fallen flowers, which in tropical forests often ferment on the humid and well shaded ground. For insects that feed on substances of this kind, tropical forests evidently offer more diversified and favorable conditions than do tropical savannas, or temperate—particularly cold—environments. It may

be noted, however, that many other groups of organisms likewise show greatest proliferation of species in tropical lands. Thus the astounding diversity of species of trees found growing in small areas in Brazilian forests was noticed and commented upon by the early explorers. In co-operation with Dr. George Black, of the Instituto Agronomico do Norte, and Dr. Pavan, counts were made of trees with diameters of more than 10 cm. at chest height on two one-hectare (100 X100 m.) plots near Belem do Pará. One of these plots in a periodically inundated forest (*igapó*) had 60 species among 564 individual trees. The second, on higher land (*terra firme*), had 87 species among 422 trees.

Tropical environments thus contain a greater variety of ecological niches (biological opportunities) for living beings than do temperate-zone environments. To occupy an ecological niche, however, an organism must be adapted for it, that is, must possess a combination of physiological and morphological traits that make maintenance and perpetuation of life possible. Different combinations of traits are needed for efficient exploitation of different ecological niches. To conquer the ecological niches available in a given region, life must accordingly evolve a variety of genetic constitutions proportionate to the variety of biological opportunities. This can occur in two ways. First, numerous species may be formed, each adjusted to a single ecological niche or to a few similar ones. Second, a relatively small number of species may become polymorphic, that is, each composed of a variety of adaptively different genetic types. It appears that both ways have been followed in the evolution of *Drosophila*, and that a greater variety of species, and a greater polymorphism within some of these species, have evolved in the tropics than in temperate countries.

LOCAL AND SEASONAL VARIATIONS IN
RELATIVE FREQUENCIES OF
DROSOPHILA SPECIES

A community of many related species, each adapted to a slightly different set of environmental conditions, represents a sensitive system, which can be expected to react to even slight changes in the environment. *Drosophila* populations of tropical forests show this lability in several different ways. In experiments made by Burla, Brito da Cunha, Cavalcanti, Pavan, and the writer, similar bait was placed at 10-m. intervals along 200-m. transects in what appeared to be a reasonably uniform forest at Horto Florestal near São Paulo. *Drosophila* flies that came to each bait were collected separately and classified as to species. The results showed that the different species are far from being uniformly distributed over the territory sampled. Instead, nuclei of high population density are scattered through the forest, separated by areas where flies are rare or even absent. Furthermore, the localization of these nuclei is by no means the same for different species. A neighborhood that is evidently attractive for *D. willistoni* may contain few individuals of *D. griseolinata*, and vice versa. This microterritorial differentiation breaks up a community consisting of many competing species into tiny colonies with a much smaller number of competitors.

The formation of nuclei of population density for each species is caused by the fact that different species are attracted preferentially to different food substances. This can be shown if, instead of collecting *Drosophila*s on uniform bait placed at different points, one finds them on different natural baits located as close together as possible. On the ground in tropical forests one often finds fallen and fermenting fruits or flowers of one species of tree, and a short distance away fruits or flowers of

another species. Collections made on such natural baits by Pavan, da Cunha, and the writer disclosed two interesting facts. First, different species of *Drosophila* show preferences for different foods. Within a distance of some ten meters, hundreds of individuals of a *Drosophila* species may congregate on one and largely ignore another natural bait. Second, such preferences do not reach the stage of rigid dietary specialization, and a kind of fruit avoided by a species of *Drosophila* in one place may be relatively well attended in another place. Just what causes these variations in attractiveness of the same kind of fruit is not clear. Possibly some microclimatic differences between places in which the fruits happen to be located are effective, and the yeasts and other microorganisms that make the fruits ferment may well be different and may attract or repel different *Drosophilas*. However that may be, most or all species of *Drosophila* (except the fungus feeders, which we did not attempt to include in our studies) can utilize a great variety of foods, and hence are competitors in nature. The competition is alleviated, however, by different food preferences, which may permit some species to be temporary monopolists in some parts of the environment.

Seasonal changes in the environment occur in tropical forests, even in regions such as the coast of São Paulo or the equatorial rain forests near Belem do Pari, where temperature and humidity are favorable at all times for the development of plants as well as of *Drosophila*. For example, different species of trees come into flowering and fruiting at different seasons. Although such seasonal changes may seem small compared with those in temperate climates, they profoundly affect the *Drosophila* populations.

As indicated above, *Drosophilas* were

collected at approximately bimonthly intervals, chiefly by Pavan, da Cunha, Burla, and the writer, at three stations in the state of São Paulo. At each of these stations, about a dozen places were marked, so that banana bait was always placed in the same positions. The flies collected were classified as to species. The relative frequencies of various species at each collecting station changed greatly from season to season. Thus, *D. willistoni* and its sibling species *D. paulistorum* were common at all stations during the summer, at times even reaching the status of dominants; but during the cooler part of the year they became relatively less common than certain other species (*D. simulans* at Pirassununga, *D. mediostriata* at Mogi das Cruzes, *D. capricorni* at Vila Atlantica). Distinct seasonal changes in relative frequencies of *Drosophila* species were observed also at Belem do Pará in the equatorial zone.

A problem of much interest is whether these seasonal changes are cyclic; that is, whether the same relative frequencies of the different species will recur in each locality year after year. One can well imagine that a system composed of some twenty species, ecologically rather similar and hence competing, may be so sensitive to environmental variation that climatic differences between years will make repetition of the same set of relative frequencies improbable. This problem must be settled by future observations. Thus far we possess only some data collected by Dr. Pavan in 1946 and 1947 at Mogi das Cruzes. Comparison of these earlier collections with those made in 1948-1949 at similar seasons indicates quite considerable differences in the composition of the populations. Unfortunately, the earlier collections were not made at precisely the same neighborhoods as the later ones, and hence the disturbing element of territorial variation is not excluded*

DIFFUSION RATE AND POPULATION DENSITY

The fact that uniform bait may attract quite different collections of species of *Drosophila* when placed at points only some 10 m. apart suggests that the flies do not travel over long distances, but come to bait chiefly in the immediate vicinity. This inference was checked by experiments on *D. willistoni* carried out by Burla, da Cunha, Cavalcanti, Pavan, and the writer in the vicinity of São Paulo.

The technique of such experiments, worked out previously for *D. pseudoobscura* (see Year Books Nos. 39-47), consists in releasing at some point in a natural environment a known number of flies marked by an easily recognizable but innocuous mutant trait, and then recording the numbers of marked and wild flies captured on bait placed at regular intervals at different distances from the point of release. Mathematical methods applicable to the resulting data were developed by Professor Sewall Wright, of the University of Chicago, for the experiments on *D. pseudoobscura*. Professor Wright has very kindly checked also the calculations made for *D. willistoni*.

The diffusion rates of flies in a two-dimensional environment are measured by the variance of the distribution of the marked flies in the territory around the point of their release at different times after their liberation. In *D. willistoni*, as well as in *D. pseudoobscura*, this variance increases with time much faster at high than at low temperatures. Temperatures of about 15° C. are limiting, since at lower temperatures the flies remain about stationary. But above this limit, *D. willistoni* shows migration rates of a lower order of magnitude than does *D. pseudoobscura*. In other words, *D. willistoni* is even more inclined to form semi-isolated local populations or colonies than is *D. pseudoobscura*.

The same experiments used to study the migration rates of the flies yield data from which the population densities of the wild flies in the experimental field can, granting certain assumptions, be deduced. Such deductions indicate population densities of wild *D. willistoni* between 10 and 28 flies per 100 sq. km. in the experimental fields near São Paulo. Population densities of all species of *Drosophila* in the same fields can be estimated, with less assurance than for *D. willistoni* alone, at between 60 and 139 flies per 100 sq. m. Population densities of *D. pseudoobscura* and related sibling species in California mountain forests have been estimated, during the most favorable season of the year, at from 0.4 to 10.0 flies per 100 sq. m.; and this species and its relatives are usually more abundant than all other species in the same localities. The conclusion is justified that the aggregate population densities, of *Drosophila* are appreciably greater in tropical forests than in the forests of California.

CONCEALED GENETIC VARIABILITY IN THE
CHROMOSOMES OF *DROSOPHILA WILLISTONI*
AND *DROSOPHILA PROSALTANS*

Representatives of a species of *Drosophila* collected in nature are usually rather uniform in their externally visible traits. The morphology of each species appears to be fairly well stabilized, and deviations from the norm are adaptively undesirable. An exception to this rule is the fairly common and widespread Brazilian species *D. polymorpha*, which shows a very considerable variation in the color pattern of the abdomen. This case, studied in detail by da Cunha, proved to be a clear instance of balanced adaptive polymorphism. The forms having very light and very dark abdomens are homozygotes, whereas the intermediates are heterozygous for a pair of alleles of a single

gene. The heterozygotes are adaptively superior to both homozygotes, (cf. discussion of chromosomal variability in the next section).

Studies made by several investigators, chiefly on European and American *D. melanogaster* and *D. pseudoobscura*, have disclosed that flies of these species found in nature are often heterozygous for various recessive mutant genes. These genes, when homozygous, cause the death of all or a part of their carriers (lethals, semi-lethals, subvitals), as well as sterility, modifications of the development rate, and various morphological abnormalities. The heterozygotes for these recessive genetic variants, however, are "normal" flies in every respect. These variants are thus concealed in heterozygous condition, and form a store of potential, rather than actually expressed, variability.

We have extended studies of the above type to the Brazilian *D. willistoni* and *D. prosaltans*. Strains have been synthesized in the laboratory, chiefly by Mr. Spassky, which have the second or third chromosomes (autosomes) "marked*" by various mutant genes and inverted sections. By making appropriate crosses of flies collected in nature to these "marked" strains, it is possible to obtain, in the third filial generation, flies that carry the same wild chromosome twice (are homozygous for it). If the chromosome in question causes, when homozygous, a reduction of the viability of its carriers, certain deviations from normal Mendelian segregation ratios result in the cultures. Inspection of the homozygotes makes possible the detection of morphological abnormalities; and breeding tests show whether these homozygotes are fertile or sterile.

Drs. Pavan, Malogolowkin, Cordeiro, and Wedel, Mrs. Dobzhansky, Mr. Spassky assisted by Mrs. Spassky, and the writer carried out analyses of the second and

third chromosomes in population samples of *D. willistoni* collected in various parts of Brazil. Professor Cavalcanti took charge of experiments of a similar nature with *D. prosaltans*, which, being a rare species, was found mostly as isolated individuals. The results of these rather large-scale and very laborious experiments cannot be reported yet. One can state, however, that the store of concealed genetic variability found in the natural populations of the two above-named species in Brazil is at least as great as it is in the temperate-zone species studied previously. Genetic variants of diverse kinds have been encountered.

CHROMOSOMAL VARIABILITY IN *DROSOPHILA WILLISTONI*

Contrasted with the constancy of external traits in natural populations of most species of *Drosophila* is the great variability of structure of their chromosomes. This variability consists chiefly in inversions of chromosome segments. Individuals having two chromosomes of a pair with like gene arrangements (inversion homozygotes) and with unlike arrangements (inversion heterozygotes) are encountered in nature. Flies of the same species carrying chromosomes of different types interbreed at random. Experiments and observations made on *D. pseudoobscura* have shown that the chromosomal polymorphism is adaptive and that it is balanced (see Year Books Nos. 40-47). Inversion heterozygotes, with a single known exception, have adaptive values higher than homozygotes. This being the case, natural selection maintains the polymorphism in natural populations, and yet permits these populations to react by rapid alteration of their genetic composition to even small and temporary changes in the environment. Chromosomal polymor-

phism is, consequently, a part of the actual rather than of potential variability, even though this variability happens to find its expression, in *Drosophila*, in physiological rather than in visible morphological traits.

Samples of natural populations of *D. willistoni* from different parts of Brazil were brought to the laboratory at São Paulo, and the larval salivary-gland chromosomes were studied in their offspring by da Cunha, Burla, and the writer. A chromosomal polymorphism more extensive than that known in any other species of *Drosophila* was disclosed. Inversions were found in all the chromosomes—the autosomes as well as the sex chromosomes. At least 34 different inversions were encountered (as compared with 20 in *D. pseudoobscura*, chromosomally the most variable species hitherto known, in which 15 of the inversions are concentrated in a single chromosome, the third). Taking the Brazilian populations as a whole, individuals that carry no inversions (inversion homozygotes) are definitely less common in nature than are inversion heterozygotes. One of the larvae examined was heterozygous for 16 inversions, which seems to be the highest degree of inversion heterozygosity found thus far in any organism.

Although there is no direct proof that the chromosome polymorphism in *D. willistoni* is balanced and adaptive, this may be accepted as the most likely working hypothesis, by analogy with other species studied in this respect. Chromosomal polymorphism may be regarded, then, as a means whereby the species becomes capable of occupying and exploiting efficiently a variety of ecological niches in the environment in which it lives. The amount of this polymorphism in populations that inhabit different climatic regions of Brazil becomes an interesting problem for study, and efforts have been made to approach the problem from several angles. The re-

suits are not ready to be reported in detail. One fact is clear enough, however; namely, that inversion heterozygosis is more frequent in some parts of Brazil than in others. Thus far, the minimal value was observed in a sample from the *caatingas* in the state of Bahia, where the average number of inversions carried by an individual in the heterozygous state is about 0.8. The maximal value, about 9 heterozygous inversions per individual, occurred in samples from the savanna-gallery forest region of central Goyaz. A high degree of inversion heterozygosis was found also in samples from the tropical rain forests of Acre, Guaporé, and Rio Negro, whereas the rain forests near Belem do Pará have relatively few inversion heterozygotes.

COMPARISON OF CHROMOSOMAL VARIABILITY IN DIFFERENT SPECIES

Drosophila willistoni is the commonest species of the genus *Drosophila* in many parts of Brazil. Collection on different natural baits (see above) showed that this species is also very versatile ecologically, in the sense that it is found on a great variety of foods. It is tempting to correlate these facts with the great amount of chromosomal polymorphism present in the species; and in this connection it is logical to inquire whether, in other species of *Drosophila* that are ecologically less versatile and less common than *D. willistoni*, chromosomal polymorphism is proportionately less. A study of chromosomes was accordingly undertaken, by Professor Dreyfus on *D. nebtidosa*, by Professor Cavalcanti on *D. prosaltans*, by Miss Pereira on *D. sturtevantii* by Dr. Burla on *D. annulimana* and related forms, and by da Cunha, Burla, Pavan, and the writer on *D. paitlistorum*, *D. equinoxialis*, *D. tropicalis*, and other species. The work has not been completed yet, but it is safe to say that no other species so far studied approaches *D. willistoni* in frequency of inversion heterozygosis.

Comparison of *D. willistoni*, *D. pauUstorum*, *D. equinoxialis*, and *D. tropicalis* is particularly instructive. These species are very closely related and are morphologically almost indistinguishable (see below). *D. willistoni* has the widest geographical distribution: it extends from central Mexico and the southern tip of Florida, southward to at least the Rio Grande do Sul in Brazil and the territory of Misiones in Argentina. As stated above, it is in many places the commonest species of *Drosophila*, and it shows the greatest chromosomal polymorphism. *D. pauUstorum* has a somewhat narrower distribution: it is known from equatorial Brazil (territory of Rio Branco, state of Para) down to the coast of Sao Paulo and Parana. Within this distribution region it competes in commonness with *D. willistoni* and is, in fact, more numerous than the latter in some places (Belem do Para) or in some seasons (in summer on the coast of Sao Paulo). Its chromosomes show a variety of inversions second only to that in *D. willistoni*; the average number of heterozygous inversions per individual, although varying from region to region, is fairly high. *Drosophila equinoxialis* and *D. tropicalis* are thus far known to occur only in the basins of the Amazon and the Tocantins—from Belern do Pará to Rio Branco and Rio Negro, and from central Goyaz to Guaporé and Acre. Not only are the distribution regions of these species included among those of *D. willistoni* and *D. pauUstorum*, but even where *D. equinoxialis* and *D. tropicalis* do occur they have rarely been found to be as abundant in their rivals. Only a few Inversions have been observed in *D. eqtti-noxialis* and *D. tropicalis*, and the fre-

quency of heterozygous inversions per individual is distinctly low.

SIBLING SPECIES

Mass collecting of *Drosophila* in different parts of Brazil led, as was to be expected in a tropical land whose *Drosophila* fauna had been only superficially studied, to the finding of many new species. Unfortunately, our program of research left little time for strictly taxonomic work. Some taxonomic situations had to be straightened out, however, before other types of work could proceed. One such situation arose with the discovery that the flies originally classified as *D. willistoni* actually belong to four distinct species, which are very similar in morphological traits. Following Mayr, such morphologically similar species are called sibling species. Burla, da Cunha, Cordeiro, Malogolowkin, Pavan, and the writer submitted the four sibling species of the *willistoni* group to a comparative morphological, genetic, and cytological analysis.

The four species, *D. willistoni*, *D. paulistorum*, *D. equinoxialis*, and *D. tropicalis*, are reproductively isolated populations, which cannot exchange genes with one another. If females of any one of these species are kept with males of any of the other three, few or none of the females are inseminated. This sexual isolation is relatively weaker when *D. paulistorum* males are used; that is, *D. paulistorum* males are accepted more readily by females of the other species than are other heterospecific males. Regardless of whether or not some interspecific matings occur, however, no hybrid offspring are produced.

Whether this is due to early death of the hybrid progeny or to failure of the heterospecific sperm to consummate fertilization is not known. In any case, the reproductive isolation is complete.

Apart from crossing experiments, a safe method of identification of the four species is examination of their salivary-gland chromosomes. Five chromosome strands are present in the salivary-gland nuclei of each of the four species. Four of these strands have disk patterns sufficiently similar in all species so that they can easily be recognized and homologized. The fifth strand, which corresponds to the third genetic linkage group (the third chromosome), is rather similar in *D. paulistorum* and *D. equinoxialis*, but quite differently built in *D. willistoni* and *D. tropicalis*. Examination of the third chromosome is therefore sufficient for identification of the species, except that *D. paulistorum* and *D. equinoxialis* have to be distinguished by less striking differences in other chromosomes.

A detailed comparison by Burla of the external morphological traits of the four species, and a comparison of the genitalia made by Malogolowkin, disclosed several small differences which, after some practice, suffice to distinguish the species. These distinguishing traits are subject to some geographic variation within species, however, which somewhat complicates the situation. Thus, whereas *D. willistoni* from southern Brazil always differs from *D. tropicalis* in the position of the anterior scutellar bristles, *D. willistoni* from equatorial Brazil shows a variation that makes this trait no longer reliable.

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DIVISION OF HISTORICAL RESEARCH

Cambridge, Massachusetts

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In archaeology it is always difficult, often impossible, to adhere to a prearranged schedule of field work. The archaeologist never knows what lies underground: its quantity, the state of its preservation, or the often entirely unexpected leads and problems it may open up. Also, he must be prepared temporarily to abandon any given undertaking because of some reported discovery which, for one reason or another, must at once be exploited. This is particularly true of work in the Maya area, in which so little actual excavation has been done that one can seldom predict what or how much or how little one is going to find; there is so great an amount of unexplored country that at any time may come word of a ruin of such obvious importance that it must immediately be given at least preliminary investigation.

The finding of the unique wall paintings at Bonampak, described in the last two Year Book reports, was a case in point. Another was the discovery, in 1948, of the extraordinarily rich tomb of the Miraflores phase at Kaminaljuyu, in the outskirts of Guatemala City. The clearing of this tomb not only took the time of two staff members for many weeks and further required many months for the study of the specimens recovered, but also proved that native Middle American culture had reached so high a state of both technological and social advancement at what had formerly been believed to be a still formative period that future investigation of the origin of Middle American civilization will have to be rather radically reoriented.

The Division's troubles at Kaminaljuyu did not end with 1948. The upper benches of the tomb, which lay several meters down

from the top of a 20-m. mound, could not be entirely excavated during that year without burrowing too deeply for safety into a tremendous overburden of hard-packed earth. So, with labor kindly supplied by the Government of Guatemala, the off season was employed in cutting it away, with the result that a second tomb, set somewhat higher, came to light. The extremely difficult task of its excavation and the recovery of its many mortuary offerings was carried out by Mr. Shook.

To Mr. Shook also belongs the credit for identification of a hitherto unrecognized stage of culture older than Miraflores but younger than one we have called Las Charcas. The original find, a burial accompanied by much pottery, was made in the side of a road-cut west of Guatemala City in the Department of Sacatepequez; this name has been assigned to the phase. Subsequently, excavation for the storage tank of a gasoline station near Chimaltenango yielded a further large Sacatepequez collection.

Study of the enormous amount of Miraflores material from Kaminaljuyu, of the Sacatepequez specimens, and of a rich new Las Charcas find has shown that these three phases were sequent stages in the (we believe very long) development of an important pre-Classic highland culture that culminated in Miraflores. The forerunners of that culture, even in Las Charcas times far from primitive, are still unknown, and we cannot as yet explain the apparently abrupt and certainly very great change marked by the opening of the Classic Esperanza phase at some time during the early centuries of the Christian Era.

Establishment of the Las Charcas-

Sacatepequez-Miraflores sequence contributes significantly to knowledge of Guatemala highland prehistory. From the broader view of the history of art and technology, its potential value is great, for it provides a large and fully documented body of data to supplement our scanty knowledge concerning such aspects of cultural growth as developments in the techniques of pottery making and the life histories of decorative styles. Much of our theorizing on these matters has been highly speculative, because based on series of specimens whose relative ages are uncertain. The only weakness of the present sequence—and it is of course a very serious one—is due to our ignorance of the amount of time that elapsed between earliest Las Charcas and latest Miraflores. For all we now know, it may have been two hundred or a thousand years. But there is hope that further perfection of methods for employment of radioactive elements for determination of the age of archaeological specimens may eventually permit reasonably accurate dating.

A second unplanned investigation was made necessary by road work at Asuncion Mita in eastern Guatemala, where in the elimination of a curve some large mounds were being cut down. In one of these, previous digging by pot hunters had exposed an earlier structure containing vaulted chambers. Mr. Strömsvik accordingly went to Asuncion Mita in April to make record of such parts of the building as had been exposed. Both architecture and pottery seem to indicate connections with Copan during the so-called Acropolis Period. During his visit, Mr. Strömsvik took opportunity to reconnoiter the region, mapping and collecting potsherds from a number of other sites.

The final season of Mr. A. L. Smith's survey of the Guatemala highlands was devoted to the Departments of Quiche,

Alta Verapaz, and Chimaltenango. Mr. Smith revisited Nebaj, Quiche, where he made remarkable finds in 1946 and 1947. This year a tomb, located in 1947, was excavated. Among the mortuary offerings of pottery and jade was a vessel, apparently a trade piece from Alta Verapaz, which is believed to date from the early years of the Late Classic Period. If this is confirmed, it will show that part at least of what we have considered the Early Classic Period of Nebaj was contemporaneous with the beginning of the Late Classic of Peten; in other words, that we must recognize a certain amount of cultural lag in the highlands.

After a survey of the San Andres Sajcabaja region of Quiche, where a number of large sites were mapped, Mr. A. L. Smith, accompanied by Mr. R. E. Smith and Dr. Stephen Borhegyi, proceeded to Alta Verapaz. Sites in the neighborhood of Coban and in the upper and middle drainage of the Rio Polochic were examined. Some of these contain remarkably fine construction in which very large and accurately cut stone slabs were used. Potsherds were scarce, but those that were recovered and certain vessels from a tomb make it evident that the ruins in question date from the Late Classic Period.

Finally, A. L. Smith visited the ruins of Mixco Viejo in the drainage of the Rio Grande. From this extensive site, known to have been occupied at the time of the Spanish conquest, he recovered a large collection of potsherds illustrating the hitherto little-known wares of the sixteenth century.

Mr. E. M. Shook, as already stated, excavated a second rich tomb in the great Miraflores phase mound at Kaminaljuyu. The specimens from the tomb, hundreds of thousands of potsherds from the fill of the mound, more thousands from various other mounds at Kaminaljuyu that are

being cut down for brickmaking, and still others from road operations to the west, are being classified and studied by Mr. Shook and the Chairman.

The most important field operation of the period under review was the Campeche expedition of Dr. George W. Brainerd and Mr. Karl Ruppert. Past work of the Division had developed a sound framework of knowledge relative to much of the southern area of the Maya and to northern Yucatan. There existed, however, an intervening area that was but sketchily known and little understood, our lack of knowledge being particularly acute in respect to ceramics. Although this little-known area stretches across the peninsula from coast to coast, it seemed of particular importance to gain knowledge of the so-called Rio Bee and Chenes areas of eastern Campeche. By making a survey of the pottery of these areas it was hoped that relationships might be established with the Peten region to the south and Yucatan to the north, and that the cultural development of Yucatan might thus be anchored more firmly to the relatively well dated cultures of the south. Such linking up of southern and northern areas seemed of particular importance before embarking upon any new operations in Yucatan.

By arrangement with the University of California at Los Angeles and the Southwest Museum, Dr. Brainerd obtained leave from those institutions for a period of six months to carry on this work for the Division. He was assisted by Mr. Ruppert. During the winter and spring they conducted excavations at the Chenes sites of Santa Rosa Xtampak and Dzibilnocac, and at a number of Rio Bee locations centering about the site of Xpuhil. At the time of writing this report we record that the materials collected are being analyzed; the study has progressed far enough, however, to indicate that the Chenes-Rio Bee archi-

tectural styles and the associated pottery are in part contemporaneous with the Puuc remains in Yucatan and with the Late Classic (Tepeu) in the Peten. The Peten association should provide approximate dating, in terms of the Maya calendar, of Chenes-Rio Bee and Puuc remains, a dating that has at times been hotly debated. There is every reason to hope that the work of Brainerd and Ruppert has made an important advance toward solving the relative chronology of the northern, intermediate, and southern areas, and that the work also will throw light on cultural influences between areas.

Laboratory and desk work again occupied a large proportion of the efforts of the staff. Miss Shepard has continued her work in ceramic technology. A special project dealing with pottery of the southwestern United States and designed to acquaint archaeologists more generally with the applications and significance of ceramic technological data is nearing completion. Miss Shepard has also given time to the preparation of a ceramic handbook for the use of archaeologists. Miss Proskouriakoff has brought to completion her initial studies of Maya sculpture. It is anticipated that this work will go to press by the end of this year. During the period under review Mr. Thompson completed and submitted the manuscript of the introductory volume of his studies of Maya hieroglyphic writing. This volume is now in press. Similarly completed and in press is Mr. A. L. Smith's work on the excavations at Uaxactun. Prior to entering the field with Dr. Brainerd, Mr. Ruppert continued his study, preparation, and arrangement of unpublished materials dealing with earlier activities of the Institution at Chichén Itza. Dr. Morris made further progress toward the publication of his researches in Southwestern archaeology. Although by

far the greater portion of his efforts was devoted to administrative duties, Dr. Pollock gave some time to his study of Yucatan architecture.

Dr. Norman A. McQuown, by arrangement with the Department of Anthropology of the University of Chicago, again gave part of his time to the Institution for researches in Maya linguistics. Dr. McQuown spent five months in Guatemala, working mainly on the Mam language, but giving some attention to other highland Maya languages, to Xinca, and to arranging for collaboration with a number of individuals in the field. Dr. McQuown's field trip marks the end of active participation by the Institution in the field of Maya linguistics. It is hoped, however, that the work will continue under the auspices of the Department of Anthropology of the University of Chicago.

Mr. Roys spent two months in Yucatan in continuance of his studies of native Maya literature and the history of the Maya area. As in the past, he devoted much of his time to field research bearing upon the political geography of the region at the time of the Spanish conquest. He also gave special attention to the search for conquest-period sites that might merit particular attention under the Division's proposed program of operations in Yucatan.

Most of the above-noted activities have been devoted to winding up the over-all study of the Maya and to clearing the deck for intensive research on the archaeology and history of northern Yucatan. In focusing its attention on this field, the Division is returning to the area in which, over forty years ago* opened the long and fruitful career of Sylvanus Griswold Morley as a Mayanist.

With Morley's death on September 2, 1948, the Maya, ancient and modern, lost

their most tireless and effective advocate. His whole adult life was dedicated to the furtherance of Maya research. That he was able to accomplish so much was due to a unique combination of scholarly ability, skill as a promoter, unbounded energy, and limitless persistence. His firsthand contributions as explorer, recorder of texts, and student of the hieroglyphs were outstanding. His driving enthusiasm resulted in the entrance of Carnegie Institution into the Maya field. He lost no opportunity to induce agencies to co-operate or to undertake independent investigations. He was thus largely responsible for the fact that so many persons have been able to devote themselves to Maya studies.

Morley early realized that in the last analysis any branch of research is made possible only by popular understanding of its aims and appreciation of its value. He was therefore tireless in publicizing the Maya by lectures and writings. With the same end in view, and also to preserve for the future the finest examples of Maya architecture and sculpture, he inaugurated at Chichen Itza and continued at Quirigua and Copan the custom of stabilizing and repairing excavated buildings and re-erecting fallen monuments. This has greatly helped to stimulate interest in their antiquities on the part of the governments of the countries in which these ruins are situated, and has led to the preservation of much that might otherwise have been lost.

Morley's scientific, practical, and promotional accomplishments were many. But, in the long run, undoubtedly the greatest was his success in inculcating confidence in the good faith of American scientific agencies and in bringing about the close and cordial relations, both personal and intellectual, that now exist between those scholars of the United States

and of Latin America whose common interest is in the prehistoric past of the New World.

Shortly after the close of the period under review, Dr. George Sarton will retire from the staff of the Division. His retirement will terminate an association of thirty-one years. Dr. Sarton's unceasing labors in the history of science have resulted in transforming a previously unorganized and largely unrecognized field of study into a recognized discipline. Though his retirement will mark the end of the Institution's activities in the history of science, his efforts have assured a continuing interest, both in this country and abroad, in this field of learning.

On June 11, 1949, the Chairman received the honorary degree of Doctor of Science from the University of Michigan.

GUATEMALA HIGHLANDS

EDWIN M. SHOOK

The cutting away of Structure E-III-3, Kaminaljuyu, to obtain material for bricks continued throughout the past year. The great mound, as previously reported (Year Book No. 47, pp. 215-217), is the largest individual structure of some two hundred which make up the ruins on the southwest edge of Guatemala City. The salvaging of the invaluable archaeological material and data from the excavations was possible through the co-operation of Sr. Moises de Leon, superintendent of the government brick factory. He not only placed several laborers under our supervision, but permitted his workmen to be shifted here and there on the mound to facilitate the recording of the exposed remains.

Information was gathered on the architectural development of Structure E-III-3 from a small semicircular adobe unit about 2 m. high (no. 1) through six successive major additions. The final stage (no. 7)

was a massive, flat-topped, rectangular pyramid over 20 m. in height and measuring 70 by 90 m. at the base. Ceramic material obtained from the fill of each unit indicated that the long sequence of architectural activities took place during the Early and Middle Miraflores phases of the Pre-Classic Period. The lavishly stocked Tomb 1, reported in Year Book No. 47, had been cut through the top of Structure 5. A similarly constructed and equipped tomb, no. 2, was found during the past field season. It had been dug through the top of Structure 6, the next pyramid, which completely encased Structure 5 and Tomb 1.

Tomb 2 occupied a position just west of and higher than Tomb 1, its east wall actually penetrating the upper west bench of the older tomb. Although less carefully constructed and less richly furnished, Tomb 2 provided more information because it had not been disturbed by the brick-factory workmen as had Tomb 1. Prior to the roofing of the tomb with timbers, the principal individual, a mature adult, had been laid horizontally, head to the south, on a low wooden table or platform resting on the tomb floor. The body was completely painted red and probably dressed or wrapped. Two children, about eight years of age, had been sacrificed and placed in an extended position on the tomb floor just west of the principal body. Another skeleton, that of a young adult, lay extended, face downward, on one of the east benches above the roof of the tomb. Over one hundred pottery vessels, some containing ash and charcoal, had been placed on the surrounding benches after the timber roof was laid.

In contrast with Tomb 1, only a few pottery vessels were found on the tomb floor. Here, however, were most of the nonpottery objects: mica sheets, sting-ray

tails, fossil fish teeth, stuccoed containers (possibly gourds), quartz crystals, water-worn stone pebbles, basalt implements, bone spatulas and ornaments, mosaic mirrors of pyrite and of crystalline hematite, obsidian pellets and flake-blades, tubular jade beads, mosaic sets, and a mask or headdress heavily incrustated with jade elements. The last was found face down off the southeast corner of the wood platform that had supported the principal body. The decay of the platform, or a fragment of falling roof timber, may have rolled the object to the tomb floor. The backing of wood and leather, we believe, was still firm when this occurred, and the heavy jade elements remained in position until falling material settled around and covered the whole. This earth fortunately maintained the original form of the object fairly well after the backing rotted, and the recording and removal of the jade elements more or less in their original positions were possible. The headdress or mask has been reconstructed by the artists of the Archaeological Museum of Guatemala.

After Tomb 2 was roofed, additional offerings were placed on the benches and the large space was filled with earth from the roof level to the floor of Structure 6. An adobe floor then covered the entire tomb area, permitting the use of the pyramid top again. At the same time, we think, the last great increment to **E-III-3** was started. Prior to the completion of Structure 7, which added some 4 m. to the *total* height of the mound, Tomb 2 was re-entered *unit* partially rifled. The looters disturbed only the principal skeleton, from the pelvic region to the head; we found none of those bones in place. We did find scattered human bones and teeth in earth mixed with red paint, well above the tomb floor, apparently from the main skeleton *set* aside by the looters. The jade ornaments necklace, *and* pendants

which normally would adorn a person of such obviously high rank were entirely missing. The jade-incrusted headdress and beads lay oil to the sides of the wood platform and were overlooked by the looters, perhaps because they were covered by debris.

The evidence strongly suggests that only one generation (twenty-five years or less) elapsed between tombs. The occupant of Tomb 2 may have been the immediate successor of the individual buried in Tomb 1. Some striking changes had taken place in Kaminaljuyu in those few years. A higher percentage of Usulután ware vessels was found in Tomb 2, many of them similar in shape to fine, incised red ware bowls, with everted rims and three sharply pointed nubbins. The latter vessels, although among the most common in Tomb 2, did not occur in Tomb 1. They apparently developed during the interim for specialized ceremonial uses, and often are found containing ashes and charcoal. As a result of the burning, the bowls' interiors frequently are blackened. Tomb 2 contained no "mud" pots or frog effigy vessels of fine red ware, as did Tomb 1, no stone vessels, mortars, or "mushroom" stones. The less care shown in the construction of Tomb 2, the fewer offerings, and the evidence of looting may indicate that the principal individual buried there had not been so successful a ruler or priest as his predecessor, or that economic conditions at the time of his death were more severe than at the beginning of his rule.

Structure E-III-3 is only one of a number of ancient structures being dismantled in Kaminaljuyu. Periodic visits to the various excavations helped to gather much information **that** otherwise would have been lost. Fortunately, one of the principal mound groups has been set aside through the civic interest of the owner,

Sr. Arturo Samayoa, to be preserved as a national monument. The National Institute of Anthropology and History has appointed a caretaker, whom we have trained, to maintain the monument and to collect archaeological material as it is excavated elsewhere in Kaminaljuyu.

Modern road construction and trenching for pipe lines led to more discoveries in the past year that amplified knowledge of the oldest known ceramic phase in the Guatemala highlands, the Las Charcas, and of the subsequent Sacatepequez phase. Both of these predate the long Miraflores phase. An ancient pit, containing a rich deposit of apparently pure Las Charcas material, was found in Colonia Progreso, southeast of Mound B, Kaminaljuyu. The material, sealed by a heavy layer of obsidian flakes, included a wide range of ceramic types illustrating many specialized features. Among the latter were spouted and shoe-shaped vessels, effigy monkey heads broken from vessel walls, small solid crudely hand-modeled monkey figurines, two cylindrical and two stemmed flat seals or stamps. Also found were many burned adobe fragments bearing impressions of leaves, corncobs, and other vegetable objects, carbonized seeds of several fruits, and ashy remains of woven fibrous material. The Colonia Progreso find represents the first large lot of Las Charcas material recovered by a controlled excavation.

Further search along the newly constructed Roosevelt Highway west of Guatemala (see Year Book No. 47) disclosed more deposits of the Sacatepequez phase, usually in deep rectangular or circular bottle-shaped pits. The pits occur sporadically along the road from San Bartolome Milpas Altas to Chimaltenango. Archaeological sites with mounds were located in the vicinity of the pits, but direct associations were difficult to ascertain, because the surface material collected from

these sites shows that occupancy continued into later periods. The ruins, in order along the highway, are Xaraxong, Chacaya, Santa Maria Cauque, Manzanales, Los Pinos, San Roberto, Rio Sumpango, Tejar, Tejarcito, Santa Fe, El Rancho, and Santa Sofia. The last six sites are in the Chimaltenango Valley; the others, in the Rio Sacatepequez Valley.

Advantage was taken of an opportunity offered by Mr. Hugh Craggs, of Guatemala City, to accompany him on an automobile trip through southeast Guatemala, Salvador, and Honduras. A brief reconnaissance was made of eastern Salvador, the coastal plain of Honduras north of the Gulf of Fonseca, and the highland valleys of Zamorano, Tegucigalpa, and Comayagua. The archaeological museums of Comayagua and Tegucigalpa and the ruins of Tenampua and other sites were visited.

Firsthand observation of the topography and climatic conditions of eastern Salvador and of southern and central Honduras directs attention to the importance of the natural intercoastal corridor running from the Pacific coast via the Rio Goascoran Valley to the upper Comayagua, thence to the Ulua Valley and north coast of Honduras. Through this natural funnel must have flowed not only aboriginal commerce, but forces affecting the cultural development of much of southern Mesoamerica. Eastern Salvador and the upper Comayagua Valley have a concentration of ancient remains showing occupancy over the known range of pre-Columbian history. It is most desirable that a study be made of the Rio Goascoran drainage from the Pacific lowlands to the upper Comayagua Valley.

Shook also visited the archaeological zone of Chalchuapa, where the Salvador Government for several years has carried on intensive excavation and repair of the

principal structures, under the able direction of Mr. Stanley H. Boggs.

In southeast Guatemala a record was made of the small, hitherto unreported site of San Juan Las Minas, 1 km. west of Asuncion Mita. The extensive, well known ruins of Asuncion Mita, south of the modern town, have been noted for their excellent state of preservation, the quality of the thin-slab masonry, and the corbeled arch used by the builders to roof their structures. This is the most southern site on record where the Maya-type vault was utilized. Despite the importance of these ancient buildings, they were being destroyed to obtain stone for road construction. Shook submitted a report to the Institute of Anthropology and History which curtailed the quarrying of the site except for the main structure, already beyond saving. Strömsvik later visited Asuncion Mita and obtained as full a record as possible of what remained (see p. 231).

In 1945 the Guatemala Government began an agricultural colonization project in the southeastern corner of the Department of Peten. Poptun, a small village near the headwaters of the Rio Machaquila, was selected for the permanent site of the colony and renamed Poptun. The operation entailed intensive land clearing for agricultural experimentation, surveys, building of the physical plant, and opening of a highway to Cardenas on the Rio Sarstoon. The road, to be completed in 1949, is being constructed from Poptun south through San Luis and north from Cardenas. Since 1945, reports have been received repeatedly from individuals and through the local press of archaeological discoveries made in Poptun, Cardenas, and along the highway. This past year the governor of the colony, Col Ernesto Alvarez, invited the Institute of Anthropology and History and Carnegie Institution

to visit Poptun and report on the finds. Lie. Adolfo Molina, then Director of the Institute, R. E. Smith, and E. M. Shook went to Poptun in November and spent four days recording the cultural material, and mapping and photographing the archaeological sites.

Five ancient sites—Los Cimientos, Hortaliza, Sabana, Poptun, and Petensuc—were studied briefly during the visit. They lie roughly in an east-west line, north of the modern settlement, between the drainage of the San Pedro and Machaquila Rivers. The remains of Poptun are the best preserved architecturally. They undoubtedly represent what was the region's civic and religious center. There are three known groups, South, Central, and North, connected by a broad artificial causeway with a low masonry parapet on each side.

The North Group consists of five rectangular platforms of fairly well cut stone masonry on top of a leveled and terraced limestone hill. The southern base of the hill also was terraced, but no masonry stairway connected the lower with the upper terraces. Apparently a pathway zig-zagged up the steep hillside to the structures on top. The causeway from the South and Central Groups joined the great basal terrace of the North Group. Here a single plain stela of limestone was erected.

No evidence was found in Poptun or in the small outlying sites that the Maya here used the typical vault or even masonry buildings. All the masonry noted in the area was for substructures, terraces, and low walls bordering courts, terraces, and causeways. The substructures were low rectangular platforms to support houses and temples constructed of perishable materials.

Poptun evidently was never a major center. It may have served the civic and religious needs of a comparatively small

population scattered widely throughout the area. Although there is evidence from the cultural material of occupation from Pre-Classic (Chicanel) to Late Post-Classic time, the period of greatest activity was during the Late Classic (Tepeu).

Engineers of several American oil companies have for the past two years been making an intensive topographical survey of the Department of Peten. They have discovered many hitherto unreported archaeological sites and caves containing ancient cultural material, and deposited their data, maps, and specimens with the Division. Dr. Barnum Brown, of the Sohio Oil Company, made important discoveries along the Rio de la Pasion from the junction of the Santa Amelia to the confluence of the Rio Salinas or Chixoy. He recovered quantities of petrified bones of Upper Pleistocene fauna on bars and barrier reefs along the Pasion. They had been washed out from deposits somewhere on an eastern tributary of the stream, possibly the Rio Santa Amelia. One bone fragment, as yet unidentified but thought to be that of a sloth, has three sharp V-shaped cuts on the unbroken external surface. These cuts, according to Dr. Brown, were made in living tissue. If the cuts were made by man, as Dr. Brown thinks, this is the first evidence found in Guatemala of man associated with now extinct fauna.

Dr. Brown also discovered an artificially built island in the Rio de la Pasion, above the ruins of Seibal, and a previously unreported sculptured stele at the latter site. He collected large samples of pottery from various points along the river, including Seibal and Altar de Sacrificios. The ceramic material shows a range of occupancy in southern Peten from the Chicanel phase of the Pre-Classic through the Early and Late Classic periods.

Two efforts were made during the spring

of 1949 to locate the conquest town of Nito, an important commercial center of the Indians. The town was conquered by Gil Gonzalez in 1524 and renamed San Gil de Buena Vista. Apparently the site proved unsuitable to the Spaniards and they soon abandoned it for a new location on the Rio Duke near the sea. The Spanish name, often reduced to San Gil, as well as the Indian name Nito, continued to be applied synonymously to the new town site where, in April or May 1525, Cortes arrived after his extraordinary march overland from Mexico to Honduras. Cortes found his countrymen starving at San Gil and shortly after his arrival he moved them to Honduras. Since the abandonment in 1525, both sites, San Gil near the sea and Nito the original Indian trading town, have been unoccupied and their exact locations lost. It is of historical significance to place these sites and especially to obtain from Nito data on cultural material anchored firmly in Christian chronology.

A launch and other facilities were generously provided by the United Fruit Company through the courtesy of Mr. William Taillon and Mr. Edward Farnsworth. The latter accompanied Shook on the second expedition. Two areas were intensively investigated. The first was the south side of the Golfete, the body of water connecting Lake Izabal with the Rio Dulce, which existing information suggested as the most likely area in which to find Nito. Several days were spent along this shore, penetrating the dense tropical jungle wherever possible and pushing a dugout canoe up the tributary streams. Finally an elderly native of unusual intelligence was encountered. He knew the area well, including a navigable stream, an abandoned property, and a site with mounds, all still known as San Gil. He had discovered and removed a frag-

mentary bronze bell from the site about eighteen years ago. The bell was later seen and recorded by Shook, and efforts were made to have it sent to the Guatemala Museum.

We followed the Rio San Gil upstream for approximately 3 km. from the Golfete. Only the first kilometer was navigable in a dugout canoe, the next 2 km. having been blocked by fallen trees and by sand bars, but the entire distance would be navigable by canoe if the stream channel were cleared. At the 3-km. point two shallow, swift-running branches join to form the Rio San Gil. Here the land is level, fertile, and at present heavily overgrown with dense tropical rain forest. This latter condition so obscured the area that, though we searched over a wide zone, we were unable to locate the mounds where the bell had been found. The general locality, Shook nevertheless believes, must have been the site of Nito. Strong evidence for it is the survival of the name San Gil for the place, the river, and a high mountain rising immediately behind, as well as availability of a fine water supply and *access* by canoe to the Golfete.

The second area investigated extends about 4 km. along the south bank of the Rio Duke, from its mouth opposite Livingstone to the foot of the mountain range paralleling the coast. Here undoubtedly was the later settlement of San Gil de Rueea Vista, where Cortes found the Spaniards. Most of the 4 km. may be eliminated as unfit for settlement because of swamps and lack of fresh water. Two places were found to offer adequate facilities to sustain a small colony. One, on elevated and well drained ground 2 km. southwest and upstream from Livingstone, still is known as Buena Vista. An excellent spring of fresh water issues from the base of the hills only about 50 m. back from the Rio Duke. No ruins were visible,

but reports from the local people tell of ancient artifacts being found when the land is cleared for corn planting. The other possible location is on a high bluff at the outlet of the Rio Duke, known today as Herreria Point. A fresh water supply from two small streams is available and there is a certain amount of cultivable land. A small preconquest Indian site (Herreria) was found overlooking the Caribbean Sea 500 m. south of the Rio Duke. Broken china, crockery, and glass bottles gave evidence of occupation during Spanish colonial and modern times, but nothing definitely assignable to the early colonial period was found.

While awaiting transportation in Puerto Barrios, Shook mapped and recorded a previously unreported site called San Manuel. The ruins are situated on the south bank of Rio Cacao and are cut by the auto road to Santo Tomas. The principal structures are *so* grouped around a plaza as to form an oval, in contrast with the normal rectangular arrangement. Some forty or more mounds, the majority low house platforms, compose the site. All are substructures built of earth. The largest structure, about 5 m. in height, has its surface partially faced with water-rounded boulders. No ball court, stone sculpture, or surface pottery was found to suggest the occupation period of the site.

GUATEMALA HIGHLANDS

A. L. SMITH

The 1948-1949 field season was the final of four seasons devoted to archaeological reconnaissance in the Guatemala highlands. As in previous years, the main purpose of the expeditions was to obtain as much information as possible in a minimum period of time and with little excavation. In most cases sites were roughly mapped, detailed measurements were taken

of individual buildings, a photographic record was made, and pottery was collected. In some instances perspective sketches were made of whole sites or of individual groups. The season included expeditions to sites in five areas: Sacapulas, Nebaj, San Andres Sajcabaja, all in the Department of Quiche; various sites in the Department of Alta Verapaz; Mixco Viejo in the Department of Chimaltenango.

Mr. A. L. Smith, assisted by Sr. Gustavo Espinoza, began the season at Sacapulas and visited three sites: Chutixtiox, Xolpacol, and Chuchun. At Chutixtiox, a late hilltop site, a tomb was found associated with an early structure. Unfortunately it had been robbed in ancient times, but the excavation yielded a few objects from the tomb and from the entrance.

Xolpacol, also a late hilltop site, proved, although small, to be extremely interesting. Among its ten structures were a well preserved late-type ball court, a small round platform supporting a round superstructure with four doorways, and a low platform upon which rested twin temple mounds. The main temple mound stood in the center of a small plaza. It had been dug into and a tomb exposed (see Year Book No. 46, 1946-1947, p. 185). A very good sample of pottery was recovered from the surrounding fields and down the steep slopes which had been used as refuse dumps.

Chuchun is a small valley site. Of the eight structures noted, the most important were an open-end ball court and two fairly large temple mounds. A small surface collection of pottery was recovered*

Leaving the Sacapulas area, Smith, accompanied by Sr. Cesar Tcjeda, assistant archaeologist, and by Mrs. Smith, moved to Nebaj. For the remainder of the field season Mrs. Smith took charge of the food and the gathering of surface pottery collec-

tions. While in the Nebaj area time was found to visit Finca San Francisco, belonging to the Brol family; from here the ruins of Chikal were visited.

The major effort at Nebaj was the further investigation of the large mound excavated during an earlier field season (Year Book No. 46, pp. 185, 186). A tomb was found that proved to be of the same ceramic period as the earliest tomb previously discovered, late Early Classic or early Late Classic. The grave was relatively rich in furniture, and additional material was found in the dirt that had caved in from the sides of our original excavation. Before leaving the site, we completely filled the hole.

Two small groups of mounds just north of the main ruins of Nebaj were mapped. Both groups have open-end ball courts leading into small plazas with an altar mound in the center, the ball court being the principal structure in each group.

The site of Chikal, which is of the Huil type, namely, open-end ball court and adjoining plaza with an altar in the center of the latter, is in a very poor state of preservation. The ruins are used as a corral, and stones from the structures have been removed to build walls. No map of the ruins was made, as Burkitt had published one. No pottery was found at the site.

From the Nebaj area the expedition moved to San Andres Sajcabaja. This area had previously been reconnoitered from the air (Year Book No. 46, p. 187). Eleven sites were visited: La Lagunita, Xcpom, Xolja Alto, Xolja Bajo, Pantzac, Patzac, Llano Grande, Los Cimientos, La Iglcsia, Chuscab, and Xabaj. These were located on a tracing of a map of the municipality.

La Lagunita, a large site, consists of two groups separated by a deep ravine. Group A has fourteen structures, an enclosed court, and several plazas. In the

main plaza is a plain stela, beneath which someone had dug. Six of the mounds are large, but no traces of superstructures were found. Group B, with thirteen structures, has two possible ball courts, one with open ends, the other closed but with no apparent end zones. The rest of the structures are platforms surrounding courts or small plazas. Both groups are surrounded by deep ravines and lend themselves to defense. Pottery from the site seems to place it in the Late Classic Period.

Xepom, Xolja Alto, and Xolja Bajo are small sites. Xepom and Xolja Alto are on the tops of hills overlooking the Rio Agua Caliente; Xolja Bajo is in the river valley. Very few architectural details of interest were found and only a small amount of pottery was collected. From the sherds recovered, Xolja Alto and Xolja Bajo appear to have been occupied in Late Classic times.

The largest site visited in this area was Pantzac. The ruins are not unknown, having been mapped by Sapper and briefly described by Maudslay. Sapper's map is extremely accurate and needs few changes. There are thirty-eight mounds still in existence, but Sapper shows others which have been razed; many of the architectural details that he indicates have long since disappeared. The masonry is of well cut and faced blocks which the inhabitants now living in the ruins use to build their houses and fences. The site divides itself into about five groups containing temple mounds and long structures surrounding plazas and courts, altar mounds, platforms, and a sunken court. There are two ball courts, one with open end zones and an adjoining plaza not unlike the Huil type, another with end zones probably closed with low walls. Pottery, found in quantities in the surrounding fields, places the occupation of the site in plumbate times. Although probably not built as a defense

site, Pantzac lies *on* a plain surrounded by deep ravines which would afford easy defense.

Patzac, like Pantzac, is surrounded by deep ravines. It is a large site having thirty-four structures. These are mostly in the two main groups, the rest being in scattered groups of two and three mounds. Each of the main groups has a ball court with closed end zones. Other structures take the form of temple pyramids, platforms, and altars. Several of the structures support low walls of superstructures. The pottery collected shows that this site was occupied at the time of the conquest.

Llano Grande lies in a plain, and the site definitely was not built with any idea of defense. The ruins consist of a large rectangular platform, reached by a stairway on its south side, and supporting four mounds and a sunken court. The last, which is at the west end of the platform, is probably a ball court. To judge by the pottery recovered, the site was probably occupied during the Late Classic Period.

The best-fortified site visited in the area was Los Cimientos, completely surrounded by steep ravines, the only entrance being over a causeway. Nine structures at the highest point comprise the ceremonial center. Eight of these follow the edges of terraces and form the sides of a small plaza; the ninth structure, a temple mound, is in the plaza. Two of the nine structures are temple mounds, seven are low platforms, most showing signs that they once supported superstructures. The main feature at the site is the terracing on its east, south, and west sides. The terrace walls are built against natural levels running in all directions and varying in height. Reaching to the side of the ravine, the terraces are quite extensive and may once have been used as sites for houses of perishable material. Surface pottery is

scarce, but a small collection places Los Cimientos in the conquest period.

La Iglesia, the main structure of which has been turned into a crude modern church, has two groups. Group A lies on a large oval hill which rises about 50 m. above the surrounding plain. It consists of a rectangular court surrounded by a low wall with an entrance in the south side. All but one of four platforms in the court carried stone superstructures. The superstructure of the largest was of great interest, as the walls stood to considerable height in some places, and the method of construction could be examined in detail. A good sample of pottery of the plumbate period was obtained from this group. Group B is on the level of the plain. It is made up of a plaza with a large rectangular platform on each of three sides. No masonry was showing, and there was no evidence of superstructures.

Chuscap was visited because of a reported subterranean passage that had never been explored. It turned out to be a bottle-shaped hole in the center of a small field on a hill near the Rio Agua Caliente. In shape it resembles a *chultun*, having a narrow neck for an entrance, with stones around the top, which was covered with a stone lid. Nothing was found in it and its purpose could not be determined. No structures of any kind were found in the vicinity.

The last site visited in the San Andres Sajcabaja area was Xabaj. It is a small group of three platforms strung out along the top of a hill high above the plain. The platforms, which are almost square, still support the remains of superstructures. Two of the structures had each a small modern shrine on top, and the third had six. Several of the shrines contained small carved monuments. No pottery was recovered. According to the local inhabi-

tants, Xabaj is an important place of worship today.

The fourth expedition was to Alta Verapaz, the personnel being Mr. and Mrs. Smith, Mr. R. E. Smith, and Dr. Stephen Borhegyi. Ceramic studies were in the charge of R. E. Smith and are reported by him below. Perspective sketches of ruins were made by Borhegyi. The following sites were visited: Esperancita, Tampoma, Omaxa, Canchunac, Las Tinajas, Pueblo Viejo, Seacal, Chijolom, Chichen, Santa Elena, Chicuxab, Valparaiso, and Chin-chilla.

The expedition's first base was Finca Mocca, belonging to Mrs. Robert Hempstead. The finca lies northwest of La Tinta, a village on the Rio Polochic. The first ruin visited was Esperancita, a small site consisting of eight mounds and several terraces on top of a sloping ridge. The ridge rises in natural terraces, the upper three having been artificially leveled and faced with rough stone slabs. Large stairways lead from one terrace to another. The lower terrace supports the main group. Here six long low platforms surround a small plaza with a rectangular platform in the center. The second terrace supports a long low platform. There were no structures in the third. The platforms are not oriented to one another, as they follow the contour of the ridge. A sketch was made of this site.

Tampoma has five small groups spread out on the flat valley floor. A small river, the Tampoma, flows through the ruins to join the Polochic. The largest group has what appears to be a ball court with an adjoining plaza with long low platforms on three sides. Off to one side is a low mound with a plain stela and altar in front of it. The other groups had three or four mounds each, surrounding small plazas.

At Omaxa, close to Tampoma, are several small mounds and one large one. Un-

fortunately the site was so overgrown that no work could be done. Canchunac, west of Finca Mocca, consists of a series of terraces and one mound.

After leaving Finca Mocca the expedition moved to Finca Las Tinajas on the south side of the Rio Polochic, where two ruins, Las Tinajas and Pueblo Viejo, were visited.

The ruins of Las Tinajas, also called Tzesac and located on Sapper's map as Chacujal, are close to the Rio Tinaja, which flows into the Polochic. It is possible that this may be the Chacujal mentioned by Cortes in his fifth letter to the Emperor Charles V. It is a large site, at present badly overgrown. Twenty-six structures were found, a number of which supported the remains of stone superstructures. Two temple platforms stood in the center of a large plaza. The buildings were constructed of a hearting of clay and water-rolled stones faced with thin stone slabs laid in clay. It is worthy of note that no clay or thin stone slabs seem to occur in the vicinity of the ruins. A sketch was made of the site.

Pueblo Viejo is an extensive ruin not more than a kilometer west of the ruins of Las Tinajas. The mounds are piles of water-rolled stones with only a few instances of thin slabs around the base. It is very possible that these mounds formed a part of Las Tinajas that was never completed. No map was made of Pueblo Viejo because of its overgrown condition.

The expedition next proceeded to Finca Scamay and thence to Finca Arena!, both of which belong to Mr. George Kocster, and which lie north of the Rio Polochic not far from the village of Senahu. At Arenal two sites, Seacal and Chijolom, were visited.

Seacal lies on top of a steep hill covered with outcroppings of rock of the type used in the masonry at the site. The main

feature of this small ceremonial group is a rectangular sunken court with sides of large, beautifully cut and faced stone blocks. The court is surrounded by rectangular platforms on the ground level above it. A sketch was made of the site.

Chijolom, a small group in which the ball court is the main feature, lies in the saddle between two hills. The ball court is the open-end type and has its sides built against the two hills. On one side terraces and stairways rise farther up the hill. Beyond a rectangular platform at either end of the ball court, terraces extend down into ravines. The masonry, the most beautifully cut and fitted seen in any part of Guatemala, was laid without the use of mortar. A sketch was made of the group, showing details of stonework.

After leaving Arenal the expedition moved to Coban, once more as guests of Mrs. Hempstead. Three sites were visited: Chichen, Santa Elena, and Chicuxab.

The ruins of Chichen are about 30 m. above the north side of the Rio Chichen. Almost all the seventeen structures are oriented to one another and are grouped around three plazas. There are rectangular platforms, altar platforms, a temple mound, and two open-end ball courts. The last are very similar to the ball court at Chijolom. No evidence of superstructures was found. A sketch was made showing the entire site.

Santa Elena, south of Coban, is a small valley site of four platform mounds, three of which are oriented around a small plaza. The fourth mound lies to the north of the plaza group. Chicuxab, a three-mound site, lies in a hollow in the hills. The three platforms are almost shapeless from having been plowed over.

After leaving Coban the expedition returned to Guatemala City via Salama. Between Coban and Salama two sites, Valparaiso and Chinchilla, were examined.

Valparaiso, a small site lying north of the Rio Coban, had been visited by Shook and Smith some years ago, when a collection of surface pottery was made and the site was roughly mapped. We now attempted to obtain more pottery, but the owner was away and the resident Indian family would not permit any work.

The two groups which make up the ruins of Chinchilla are on the slopes of two adjacent hills on the north side of the Rio Frio. The site was previously seen by Dr. Kidder and at that time was in a much better state of preservation. The main group consists of five structures: four platform mounds are oriented around the four sides of a small plaza, in the center of which is a large temple platform. The other group consists of three small mounds.

The final trip of the season was by Mr. and Mrs. Smith and Borhegyi to Finca Las Pilas, belonging to Sr. Francisco Martinez del Rosal, in order to examine the ruins of Mixco Viejo, a conquest site mentioned in early accounts. The ruins have been mapped by Sapper. Mixco Viejo consists of twelve groups spread along the flat top of a ridge completely surrounded by steep ravines. It is a well fortified place which can be entered only by a steep narrow trail, easily defended. The larger* groups consist of structures surrounding, in each case, a plaza, often with a temple mound or platform in the center. The structures are not oriented to one another because they follow the edges of the ravines. In many cases the upper edges of the ravines are faced with stone. On the south side there are natural terraces where the abundance of refuse indicates that this may have been an area where houses of perishable material once stood.

The sixty-eight or more structures at the site consisted of temple platforms, long platforms with three or more stairways on

one side, altar mounds, long low rectangular platforms, twin temple platforms on a single platform, and two ball courts. The last were of the conquest type with closed end zones and a stairway at the center of each end. Although many of the structures must have had superstructures, no evidence of them was found. A sketch was made of Group B, one of the largest groups, that contained the greatest variety of structures. A large surface collection of pottery was recovered, but nothing was found in the several pits that were dug in platforms. The surface pottery contains some Early Classic, a good deal of Late Classic, and an overwhelming amount of conquest period wares. No plumbate period pottery was found.

GUATEMALA HIGHLANDS

ROBERT E. SMITH

The pottery findings of the Alta Verapaz archaeological survey were quantitatively small but typologically significant. The hilltop sites, Seacal, Esperancita, Chijolom, Chinchilla, and Samac, were especially lacking in potsherds with the exception of Seacal, which contributed about a hundred. These sites, including the ball-court site of Chijolom, probably served as shrines, and therefore ceremonial pottery normally associated with tombs and caches was mostly used. In their function the Alta Verapaz hilltop sites differ markedly from those of other sections of the country, which appear to have been fortified retreats and where quantities of utilitarian as well as more elaborate pottery are found.

A tomb with skeleton, six pottery vessels, and bone ornaments was uncovered at Seacal. Two of the vessels equate with Tcpeu 3 types. A cache in Mound 1 at Esperancita contained two pottery vessels suggesting a Tcpeu 1, 2-Chipoc-Chama 3,

4-Alta Verapaz II horizon. In addition, some sixty sherds were recovered from three sizable trenches. None were found on the surface. At Chijolom a trench was dug across the center of the ball court and a pit was sunk into the center of the northeast platform mound, but the only place from which potsherds were recovered was a low terrace off the southwest end of the ball court, where there was a vegetable garden. Here some thirty sherds were gathered from the surface. These indicate a Tepeu 3 to Tohil, or even later, horizon. The pottery picked up from the surface of the larger group at Chinchilla included two faceted, red ware, Z-angled sherds of Miraflores-Chicanel type. The other eight fragments recovered were too small and weathered for identification. A tomb, excavated on Finca Samac many years ago, included a skeleton and five pottery vessels. One of the latter is a Tohil plumbate specimen which dates this grave as Post-Classic-Tohil phase according to Dr. Wauchope, or as Alta Verapaz III according to Dr. Butler. On top of the hill upon which the tomb was discovered we were fortunate in finding a ready-made cut, exposing a section filled with potsherds ranging from Chipoc (Tepeu 1, 2-Chama 3, 4-Alta Verapaz II) to Yaqui, the latest pre-conquest ceramic phase.

The valley sites, Las Tinajas and Tampoma in the Polochic valley, Chichen, Chicuxab, Santa Elena, and Valparaiso in the Coban area, as a rule were well supplied with potsherds. The exception was Las Tinajas, a large site where we dug a deep pit into the center of a long low platform mound and found a handful of unidentifiable sherds. Pits were dug throughout the main plaza, from which a total of one potsherd and an effigy-head foot resulted. At Tampoma two pits were dug, one into a rock-pile mound, the other under an unmarked stela. Most of the

identifiable sherds from the former belong to the Chipoc phase, as do those from the latter pit, which also contained jade (two tubular beads and three very thin flat perforated ornaments) and a pyrite mosaic mirror fragment. Most of the sherds from this site were picked up from the surface, an ancient river bed. Because of the water-worn or generally weathered condition of these fragments, much of the material is unidentifiable, but a quantity of Chipoc types were recognized. It is likely that further study will bring to light later horizons.

In the Coban area the largest site observed was Chichen. Here we dug pits into three mounds, trenched across the center of the main ball court, and collected surface pottery. Mound III contained the earliest (Chkanel) as well as the latest recognized (Tohil) pottery. Undoubtedly the site was occupied up to the conquest, and further study presumably will prove this. Chichen is a present-day pottery-manufacturing center. Chicuxab is a small site, but the surface is literally bristling with potsherds. We did no digging here, but picked up a carefully selected collection, containing largely Chipoc types besides a few Tzakol-type and Tohil pieces. Another small site, Santa Elena, close to Chicuxab, had an abundance of pottery on the surface and inside small platform mounds, two of which we trenched. The only Chicanel-type sherds were found on the surface. The mounds and surface yielded Tzakol and Chipoc types in considerable quantity and probably later types. At Valparaiso a surface gathering contained a few Tzakol and Chipoc types and an abundance of unidentified fragments which probably form part of later cultures.

From this survey we find that in the Alta Verapaz we can recognize various phases which closely parallel those suggested by Dr. Butler *{The Maya and their*

neighbors, pp. 250-267, New York, 1940). Dr. Butler's work, however, deals for the most part with the Chixoy drainage area and includes sites in the western part of the Alta Verapaz or farther west in the Department of Quiche, an area not touched in this survey. In order to keep the ceramics of the two areas separate, we are proposing new ceramic phase names for central and eastern Alta Verapaz: Chipoc (Tepeu 1, 2; Pokom; Chama 3, 4; Alta Verapaz II; Late Classic), Seacal (Tepeu 3), Samac (Tohil; Chipal 2; Alta Verapaz III; Post-Classic). The Pre-Classic and Early Classic, as well as the later phase corresponding to Yaqui, will be named when further knowledge as to the distribution of the wares of these periods in the Alta Verapaz is available.

HONDURAS AND GUATEMALA

GUSTAV STRÖMSVIK

During the past season no archaeological excavation took place at Copan, but an experiment in preserving archaeological sculpture should be recorded. Sculpture that has lain in the ground for some time is subject to minute plant growth, such as lichen and fungus, whose roots loosen the tiny particles of the surface of the sculpture; rain and wind continue the erosion. The sandstone at Quirigua and the tuff at Copan are particularly liable to this action. Some years ago S. G. Morley sprayed Stela K at Quirigua with a hardening agent, but the monument is again covered with lichen and fungus. Early this season Mr. R. E. Smith sent a new hardening agent to Copan for experimentation. The six lowest steps of the Hieroglyphic Stairway were carefully cleaned and dried; the viscous fluid was then applied with a brush. It will be some years before results can be seen.

Progress was made toward completing

a report on the three superimposed ball courts at Copan (see Year Book No. 36, 1936-1937, p. 138). Considerable work also was done in amplifying, checking, and correcting the specimen catalogue at the Copan Museum as a preliminary step in the preparation of a report on the cultural material in the Copan Valley.

In March, Strömsvik went to Guatemala for consultation with the Chairman of the Division. About this time, near the town of Asuncion Mita, Department of Jutiapa, several mounds of ruins were in danger of being razed by the construction of the Roosevelt Highway, whose route lay directly across the biggest and most important mound, locally called Cerro de Laja. Strömsvik consequently went there at the beginning of April to obtain all possible information before the mounds were destroyed and to co-operate with the Institute of Anthropology and History of Guatemala, which defrayed half the costs and supplied the services of Sr. Gustavo Espinoza, who assisted most ably in the excavations and explorations.

At the Cerro de Laja, which was our chief concern, a room filled with debris was completely excavated in order to examine the structural and architectural details, particularly the vaulting. This is the southernmost occurrence of the vault so far found in the Maya area. There can be no doubt, moreover, that the structure is of Mayaoid origin, for numerous fragments of Maya Polychrome and Copador pottery were found in the debris, thereby placing the remains in the Classic Period.

There undoubtedly are other rooms in this large mound, which will soon be removed completely by the highway work. At that time the details of construction should be recorded as they become apparent.

The countryside surrounding Asuncion Mita was given a hasty reconnaissance;

Brunton compass survey maps were made of four main ruins and some minor ones. The main groups were designated A, B, C, and D (Cerro de Laja is Mound i in Group A). In Group A lies the best-preserved ball court, from which were removed two parrot-head sculptures now to be seen in the entranceway of a house in town. These heads, like those at Copan, were cut to stand vertically in the benches. Another less well preserved ball court is located in Group C, west of the road and uphill from Group A. Groups A, B, and C are located south of town, between it and the bridge of Tamazulapa. All appear to be more or less of the Late Classic Period, in part contemporaneous with the Acropolis at Copan. These groups consist mainly of small courts each dominated by a larger structure. All constructions, of mud and slate on lava-block foundations, are oriented roughly to the cardinal points. Another type of construction seen in these groups is the tombs, of which there seem to be a great many, both sacked and untouched. All are vaulted.

Group D is located about 1 km. due north of town. It consists of a number of round, much eroded mounds, irregularly placed, built of mud with very little admixture of river boulders and no slate. Wherever a road cut through a mound, potsherds were gathered. These proved to be of Usulután ware and decoration, very similar to ceramics of the Miraflores phase in the Guatemala highlands. No polychrome pottery was recovered at Group D.

There apparently was a pre-Columbian settlement just where the town of Asunción Mita is now located. We were told by inhabitants that wherever one digs, artifacts turn up. A typical stone "yoke" and some pottery showing accentuated phallic symbols have been found here. Mexican affinity is suggested, and one wonders if the remains may be Pipil.

In reviewing the findings at Asunción Mita and vicinity, we seem to see three phases of occupation: (1) A population of Mexican stock apparently resided here at the time of the Spanish conquest, how long is unknown. (2) There was a Classic Maya phase of unknown duration, contemporaneous with one phase at Copan, when Copador pottery spread over parts of Honduras and El Salvador. That was the high point in the Maya culture. (3) There apparently also had existed an earlier settlement, characterized by earth mounds and Usulután pottery, and probably contemporaneous with the Miraflores phase in the Guatemala highlands.

CAMPECHE

GEORGE W. BRAINERD

The purpose of the field work undertaken in Campeche this year was the linking of the ceramic sequences of Yucatan and the Peten region of Guatemala by excavation at a series of intermediate points in the area lying between them. The work was in charge of Dr. George W. Brainerd, who took six months' leave of absence from the University of California at Los Angeles and from the Southwest Museum for this purpose. Mr. Karl Ruppert, who was thoroughly familiar with the southern part of the area to be worked, was the other member of the expedition.

Previous architectural exploration of this intermediate territory between the northern and central Maya ruin areas had been done by Ruppert (*Archaeological reconnaissance in Campeche, Quintana Roo, and Peten*, Carnegie Inst. Wash. Pub. 543), and by Dr. H. E. D. Pollock (Year Book No. 35, 1935-1936, pp. 122-125). The standing ruins in the Chenes, which is the northern part of this area, are distinctive and are similar in many particulars of style to those of the southern part of the area,

which has been called Rio Bee after the site of that name. These two architectural styles (or, perhaps better, this joint style) are in turn easily distinguishable from that to the north in the adjoining Puuc area of Yucatan, and from that of the central or Peten Maya area which adjoins the Rio Bee area to the south. The explored Chenes and Rio Bee sites are separated by about 100 km. of unexplored area which presumably also contains ruins of Chenes-Rio Bee architectural style.

Three sites were tested ceramically this season: Santa Rosa Xtampak and Dzibilnocac in the Chenes region, Xpuhil in the Rio Bee area. Collections were also made at other sites within 30 km. of Xpuhil. Approximately three months were spent in excavation. The next three months were spent in Merida in recording the collections, to be followed by a month at Los Angeles in analysis and preparation of a report. During the time in Merida Mr. R. E. Smith gave two weeks to the identification of Peten influence and trade in the collections. This procedure was of great benefit in establishing approximate chronological equivalence between the Campeche ceramics and the sequence at Uaxactun in the Peten, where stratigraphic phases have been fixed in time by Maya dates on accompanying monuments.

The determination of the correct time relations between the ruins of the Peten and Yucatan areas is of importance both in the telling of Maya history and in the dating of the Maya culture in terms of the Christian calendar. The Chenes-Rio Bee area, intermediate between the two, is the likeliest place to look for datable connections between them. Its study is also important in the determination of the nature and direction of cultural influence at all periods between Yucatan and Peten.

All three sites dug showed evidence of occupation ranging from prc-stela (Forma-

tive) times until somewhat before the period of Toltec influence at Chichen Itza. In each site, however, one period predominated in the pottery, presumably that of the standing architecture of the site. This period in all cases was characterized by a predominance of the Medium Paste Slateware which also predominates in the Puuc ruins and others of the Classic Period in Yucatan. The occurrence of this pottery at Xtampak in quantity in the terrace underlying stelae, two of which bear Maya dates of approximately 9.16.0.0.0, gives a contemporaneous date for this ceramic horizon in the Maya calendar. This dating by sherd material was reinforced by the finding of cache pottery of Classic ware in the stela platform. The terminal dates of the period are harder to work out, but some information should appear from the detailed analysis not yet begun. At present there is a suggestion that the occupation of the Puuc sites was somewhat later than, though overlapping in time with, that of the Chenes sites. The beginning date of this pottery period is also not closely fixed as yet. The absence in all three sites of clearly defined deposits between the Classic and the underlying Formative suggests either a period of abandonment or a longer use of the pottery we call Classic. At various sites in Yucatan a series of ceramic phases between Formative and Classic have been found and designated by the general term Developmental. Yucatan Developmental pottery occurs very sparingly in the Chenes sites tested.

Dating In the Maya calendar of Chenes architecture at Xtampak is given confirmatory evidence by Smith's classification of the Peten tradewares. The majority of polychrome tradeware sherds from each site tested falls into the Uaxactun subphase Tpcu 2, which Smith dates 9.13.0.0.0-9.19.0.0.0. Thus the dating of the Chenes and Rio Bee architectural styles falls well

within the Maya Initial Series Period. The coming detailed analysis of these collections should limit more closely the dating of the Yucatan periods in reference to the period of these sites.

The degree of cultural similarity among the material of the Puuc, Chenes, Rio Bee, and Peten areas is useful in evaluating Maya cultural development. Both Chenes and Rio Bee pottery of the Classic Period show preponderant percentages of Medium Paste Slateware, the chief Yucatan ware of this period. This ware is distinctive from that of the Peten. The pottery of each of these three Slateware areas, however, is easily distinguishable from that of the others by differences in detail of vessel forms. The finer monochromes of the Rio Bee area are very similar to certain wares of their corresponding horizons in the Peten, whereas the Chenes fine monochromes resemble those of the Puuc area. These degrees of relationship seem at least roughly to parallel the architectural relationships which have been observed.

An additional finding of interest is that of a new Formative phase which seems to precede the Late Formative apparent in nearly all Yucatan sites thus far sampled. The Yucatan Late Formative, as well as that found in the Campeche sites this year, bears certain marked similarities to types of pottery found in the Chicanel phase at Uaxactun, according to the findings of R. E. Smith. There are also sporadic resemblances to the earlier Mamom Uaxactun phase. At Dzibilnocac and Xtampak were found deposits of a phase seemingly earlier than Late Formative and showing ware and form resemblances to the early deposit found at Maul under a late Formative deposit (see Year Book No. 41, 1941-1942, p. 255). At Dzibilnocac the deposit of this new phase includes some Late Formative pottery; at Xtampak the diagnostic Late Formative wares are absent.

This period may be provisionally called Middle Formative; the Mani deposits, Early Formative. The Middle Formative phase shows less similarity to Chicanel than does the Late, but no similarity to Mamom. The Early Formative seems to show no resemblances to any pottery of other areas thus far known.

The dating of the Early and Middle Formative periods of the northern Maya area is not at present a profitable subject even for conjecture. The Middle Formative collections are small; the Early Formative, although larger, are limited almost exclusively to water jars. The distinctive character of these periods is striking, but further excavation is imperative before they can be properly placed. The study of the cultures sampled this season has scarcely been initiated.

CERAMIC TECHNOLOGY

ANNA O. SHEPARD

Work in ceramic technology has been concentrated on two principal projects during the current year: the preparation of a general book on ceramics for archaeologists, and a special study in the Southwestern field. The purpose and plan of the ceramics book has been stated in an earlier report (Year Book No. 46, 1946-1947, p. 190). The Southwestern study, also mentioned previously (Year Book No. 47, pp. 219-220), deals with the brown wares of the southern part of the area, the so-called Mogollon pottery.

The age and the place in Southwestern culture history of the Mogollon people has been a central theme of controversy among archaeologists for a number of years. One school has maintained that the Mogollon constitutes a distinct basic culture which influenced the better-known Pueblo development to the north; the other school regards the Mogollon as merely a variant

Pueblo culture. This question of basic cultures is one of definition and does not directly concern the technologist, whereas the record of contacts between peoples and their interactions is one of primary interest to him because it is preserved in no small measure by pottery. Furthermore, the interpretation of ceramic characteristics is one of his direct concerns and responsibilities. Brown pottery is an important diagnostic of the southern culture, and the contrast of its color with that of Pueblo pottery in the north, which is whitish or gray, is generally considered indicative of a fundamental difference in preferences and practices of the Mogollon potters. Quite aside from the question of how much weight should be given pottery color in evaluating a culture, there is need of reviewing the factors which affect the color of fired clay. Composition of clay and method of firing, or, stated in other terms, natural resources and a trait of material culture, are both primary factors. Stressing the latter to the neglect of the former leads to erroneous conclusions. If the same kinds of clay had been available throughout the Southwest, the color difference in the pottery of the two regions would reflect the potters' choice of clay and method of firing and could be considered an independent culture trait. But this is not the case. In much of the northern part of the territory there are extensive formations of Cretaceous age which abound in good-quality, buff-firing clay. These formations are absent throughout most of the southern part of the area, where the common clays are red-firing. To overlook this fact magnifies cultural differences. Likewise the idea that brown wares are earlier and more primitive than grays and that similarity of color indicates relationship arises from failure to recognize that the most widely distributed and abundant clays are red-firing. The Pueblos

of the north were favored in their ceramic resources and in consequence developed a nearly unique class of primitive pottery.

Evidences of contact between Mogollon and Pueblo peoples consist largely in trade pottery: black-on-white in southern sites, and red, brown, and smudged in northern. The latter have been identified as Mogollon only by surface appearance. One of the purposes of the Southwestern technological project has been to determine as far as possible the source of all supposed Mogollon intrusives and to consider the bearing of technological features on the question of the influences of the two peoples on each other. It has been necessary first to define the range of paste variations in the Mogollon area as far as collection and survey permit. This work has in itself direct archaeological applications. Since the two principal types of Mogollon pottery, red and brown, are undecorated and lack distinct, variable features of workmanship, varieties peculiar to certain localities or periods cannot be recognized by simple inspection, and consequently pottery does not serve as fully as in some areas to correlate occupations and to establish time sequences. This is a serious disadvantage, because the pottery was made over a large area and during a considerable time span. If it is to be broken down into spatial and temporal varieties, this must be done primarily on the basis of body composition. Pottery from the Petrified Forest region illustrates how paste analysis may add detail to the picture and alter interpretations. Sherds from twenty-one sites surveyed by the Laboratory of Anthropology included a brown ware defined as a homogeneous type which upon petrographic analysis was found to include four different pastes identified by temper. Of the four tempers—sand, sherd, altered feldspar, and volcanic rock—the last named occurred in only one specimen, which was

doubtless an intrusive from the south. The principal pastes, sand- and sherd-tempered, show sufficient difference in distribution to suggest that they were made by different peoples or at slightly different times, a possibility considered by the Museum of Northern Arizona in selecting sites for excavation in the region this summer. A clear-cut and readily recognized temporal difference in paste occurs in the Forestdale region, where most of the pottery is sand-tempered, but an early variety from Bluff Ruin is tempered with crushed diabase.

Collections studied to date, in addition to those mentioned, are from Dr. Emil Haury's excavation at Point of Pines, Dr. Paul Martin's from the SU site, the major excavated sites of the Mimbres region, and San Simon to the southwest. Collections from some sixty sites covered by Peabody Museum's survey of the north central and northeastern part of the area are now in process of analysis.

The project has been a venture *in* cooperation, since we have depended for pottery on a half-dozen institutions, and many of the archaeologists representing them hold sharply opposed views. Both samples and data on provenience and frequency of occurrence have in every case been supplied freely and generously, and there has been no reluctance to loan even rare sherds. The study has also been greatly facilitated by a gift of 333 analyzed petrographic sections from Mr. Harold Gladwin, of Gila Pueblo, and Mrs. Nora Gladwin Fairbanks. The collection was prepared and studied by Mrs. Fairbanks for Gila Pueblo and includes a large series from Magellan sites.

A short study of Mesa Verde pottery from the excavations of Dr. Deric O'Bryan was undertaken because of its close similarities to La Plata pottery analyzed for Dr. Morris some years ago. The study clarified the relationship of pottery types

and showed distinct fluctuations in classes of paste which, when correlated with other archaeological data, may have a bearing on the shifts of peoples within the region. One interesting result was the discovery that a considerable proportion of the pottery, including nearly all the earliest, was tempered with crushed andesite, a source of which was found in an early gravel sheet on the Mesa. That Basket Maker III potters should search out this deposit and select from it the igneous rock would indicate that they came to the Mesa from a region, such as the La Plata Valley, where it was the custom to temper with crushed rock instead of sand, the common temper of this period.

LINGUISTIC RESEARCH

NORMAN A. MCQUOWN

Dr. McQuown's primary purpose in his trip to Guatemala in the summer and autumn of 1948 was to educate himself with respect to the phonemics and a good part of the morphology and syntax of the Mam language. Among his secondary purposes were: to familiarize himself with the phonemics of the other Guatemalan languages in which Andrade made phonograph recordings (Quiche, Kanjobal, Quekchi); to do the phonemics of Cakchiquel, for which Andrade gathered extensive written texts (transcribed by Juan Resales); to take a look at Xinca, in order to get an outside check on Maya-non-Maya cultural contacts; and to make arrangements (both in Guatemala and in Mexico) for utilizing, so far as possible, the Maya materials already collected, or in process of being collected, by missionaries working with these languages in the field, and to ensure their collaboration and aid in further work on the languages of the family. A certain measure of success was attained in all these undertakings.

Five months were spent in Guatemala and Mexico, and the items listed were accomplished:

(1) Three and a half months were spent in Guatemala City, working on Mam, with an informant generously furnished by the Instituto Indigenista; and one week was spent with Mr. and Mrs. H. D. Peck, at Txol Be, San Juan Ostuncalco. (a) A Mam grammar and dictionary file of some 7500 entries was accumulated, (b) The phonemics and a considerable portion of the grammar of Mam was worked out. (c) Some ten Mam texts were collected incidentally, in the process of teaching the informant to write with a phonemic alphabet, (d) The Instituto Indigenista was provided with a Mam alphabet, (e) Mutual orientation sessions were held with the Pecks, in which the problems of Mam phonemics and grammar were discussed. (f) Arrangements were made with the Pecks, with the informant, and indirectly with Mr. and Mrs. Edward Sywulka, to collaborate in the preparation of a Mam dictionary, (g) Arrangements were made with the Pecks to complete the transcription and translation of Andrade's 300 Mam recordings (all material to be phonemically transcribed according to a system worked out on this trip); they have already made great progress, and should be able to complete the work in another year, (h) Arrangements have been made with Mr. Edward Sywulka to have access to his master's dissertation, ^UA morphology of the Mam language,¹¹ recently completed at the University of Oklahoma. The continued collaboration of Mr. Sywulka and of the Pecks in working out unsolved problems of Mam grammar will probably make further actual field work on Dr. McQuown's part unnecessary for completing the descriptive phase of the work on Mam.

(2) Two weeks were spent in becoming familiar with the phonemics of the Quiche,

Kanjobal (Jacalteco), and Quekchi languages, in each of which Andrade made a fair number of recordings (55, 37, and 23, respectively), (a) Two days were spent in Chichicastenango, working on Quiche, with three informants from Chichicastenango and one from Totonicapan. A vocabulary of 1000 items was taken and a tentative sketch of the phonemics was worked out (subsequent work in the Instituto Indigenista with another informant from Totonicapan verified the results of the first sketch and, in addition, established beyond question the phonemic status of the short and long vowels). Brief checking was done on a preliminary transcription of the Quiche recorded materials prepared by Andrade (the newly discovered feature of vowel length will make retranscription of all materials necessary). The Instituto Indigenista was provided with a Quiche alphabet; on the previous trip to visit the Pecks, arrangements were also made with Mr. and Mrs. Stanley Wick, working with the Quiche of San Cristobal, for future collaboration in all aspects of the work on Quiche, (b) Two days were spent in Huehuetenango, working on Kanjobal (Andrade's Jacalteco) of Santa Eulalia, with the informant of Mr. and Mrs. Newberry Cox, at present in San Miguel Acatan. A vocabulary of some 1500 items, a text, and some paradigmatic materials were recorded; a tentative sketch of the phonemics was worked out; vowel length was not found, but subsequent work with other Guatemalan Maya languages (Quiche, Cakchiquel, and Quekchi) which have it makes a recheck desirable (although there is evidence that some of the Chiapas languages do not have phonemic vowel length and that Kanjobal may belong with them rather than with the other languages in Guatemala). The collaboration of the Coxes will make this recheck possible without further field

work; they can also be counted on for help in the transcription and translation of that part of Andrade's recorded material which is as yet unworked. The Instituto Indigenista was provided with a Kanjobal alphabet, (c) Two days were spent in San Juan Chamelco, working on Quekchi, with the informant of Mr. and Mrs. William Sedat. A vocabulary of some 1000 items was recorded; a tentative sketch of the phonemics was worked out (phonemic vowel length was definitely established). The collaboration of the Sedats can be counted on in the future, for the working out of the grammar, and the transcription and translation of that part of Andrade's materials which is as yet unworked. The rechecking of the already transcribed materials will in the case of the Quekchi involve a minimum of effort, since Andrade indicated in these materials a fair portion of the long vowels. The Instituto Indigenista was provided with a Quekchi alphabet.

(3) One week was spent on the phonemics of Cakchiquel; one day was spent in San Pedro Yepocapa, working on the local dialect. A vocabulary of some 750 items was recorded, and a beginning was made toward teaching the informant to write; a tentative phonemic sketch was worked out. Subsequently, three days were spent in Panajachel, working on a slightly divergent dialect, with five informants. A vocabulary of some 1300 items was recorded; a tentative phonemic sketch was worked out (subsequent work in the Instituto Indigenista with an informant from Comalapa, and in Mexico with Juan Rosalcs of Panajachel, confirmed the sketch, and established both short and long vowels as phonemic). The Instituto Indigenista was provided with a Cakchiquel alphabet. Juan Rosales, in view of the establishment of vowel length as phonemic, will have to recheck the texts

written by him, indicating long vowels where they occur.

(4) The extra-familial check was accomplished in two days, which were spent in Chiquimulilla, working on Xinca, with an informant from Santiago and one from San Sebastian. A vocabulary of some 750 items was recorded and a tentative sketch of the phonemics was worked out. The Instituto Indigenista was provided with a Xinca alphabet. Subsequent inspection of the Xinca vocabulary showed some dozen loan words, some from Nahuatlan languages, some from Maya; consideration of the Maya loans ("bean," "mat," "custard apple," "witch doctor," "ladino") shows that they were borrowed from Mam or from Kanjobal (or from one of the Chiapas languages), indicating a previous more northerly location for the Xinca; comparison of "bean" (from Maya) with "corn" (either a loan from Lenca or a loan into both Xinca and Lenca from a third language) would indicate that "bean" was acquired considerably later than "corn," and was introduced to the Xinca (and apparently to the Lenca and other Middle American groups) by the Maya. These "explanations" are only conjectures, of course, but they indicate how extremely fruitful further work might be. Xinca is rapidly going out; someone should do a thorough study before it becomes extinct.

(5) The fifth purpose was accomplished, by and large, in the personal meetings with individual missionaries. Collaboration has been secured with the Pecks and the Sywulkas for Mam; with the Coxes for Kanjobal; with the Wicks for Quiche; with the Oughtens for Ixil; with the Sedats for Quekchi; with Antonio Goubaud and the workers of the Instituto Indigenista for Cakchiquel, Quiche, and Mam; with the workers of the Summer Institute of Linguistics in Mexico for all the Maya languages there. In Mexico, ma-

terials for volume 26 of the University of Chicago's Microfilm Series on Middle American Cultural Anthropology (texts and dictionaries in Choi, Tojolabal, and Tzotzil) were obtained from the Summer Institute and were filmed; other Maya language materials of Summer Institute workers will be added to this series from time to time.

HISTORY OF THE MAYA AREA

RALPH L. ROYS

During the past year *The prophecies of the Maya tuns or years in the Books of Chilam Balam of Tizimin and Mani* has been completed for publication, and some time has been spent in reading the proofs of this Contribution and the *Guide to the Codex Perez*.

The source material for the history of Mayapan has been assembled and translated into English from the Spanish and Maya, where such translations were not already available. The sources quoted are accompanied by commentaries, in which they are compared and discussed and some of the more important implications are noted.

There is fairly good evidence that the area ruled by Mayapan included most of the Yucatan Peninsula north of Campeche, although the evidence for the northeast and east coasts is conflicting. Nothing has been found about Campeche in this connection.

Taken as a whole, the references to Mayapan indicate the need of a new interpretation of the source material. In the past, historical reconstructions have been based principally on the chronology of the Maya Chronicles in the Books of Chilam Balam, but with unsatisfactory results. Uxmal, Chichén Itza, and Mayapan were long considered to have been contemporary capitals in Yucatan, but archaeol-

ogists have found that their more important building activities occurred at three different periods in the order named. J. E. S. Thompson has noted the weakness of this chronology, and a comparison of all the historical sources suggests that the accounts by Gaspar Antonio Chi offer a framework in which Landa's narrative and the episodes related in the Chronicles could more easily be reconciled with the archaeological findings. This compilation also presents evidence that the important Xiu family became a political factor in northern Yucatan only in the late fourteenth or early fifteenth century, and not in the eleventh century as previously believed.

The late winter and spring were spent in Yucatan continuing previous studies of the political geography at the time of the Spanish conquest (Year Book No. 36, *Wlfi-Wdfl** PP. 23²4; No. 38, 1938-1939, pp. 252-253). One purpose of the present survey was to see what indications exist that the modern towns are situated at conquest sites of the same names; another was to locate historic sites that are no longer shown on the maps.

A search was also made for conquest sites which would be likely sources for the recovery of late preconquest and early colonial pottery. Although ceramic sequences have been established from very early times down to the fall of Mayapan, about the middle of the fifteenth century, it has been difficult to find sherds which could be identified as belonging to the period following that time. It seems possible that this is partly due to a Spanish policy of replanning the Indian towns, by which it is known that the houses were concentrated nearer the church and central plaza and for the first time arranged along regular streets with smaller yards and gardens than previously. Several centuries of occupation in colonial and modern times

have further aggravated this disturbance of the topsoil containing the sherds which had accumulated during the last pre-Spanish period and that of the conquest. For this reason particular attention was paid to historic sites from which the people had been removed at a fairly early period. A few of these—Hunacti, Calotmul, and Cauich—had been located during previous visits to Yucatan. This year four more were visited and identified: Kanchunup near Sotuta, Tecoh near Izamal, Tuchicaan near Maxcanu, and Quizil near Uman. Hunacti and Kanchunup were subject to European influence for the shortest time, since they were left deserted prior to 1582, and very possibly as early as the 1560's. The churches of these two towns, as well as at Tecoh and Quizil, were of an early type. Only the chancel, sacristy, and priests' quarters were built of masonry. There was no nave, and the congregation evidently occupied an open structure of pole and thatch, called a *rarnada*, of the sort described in various sixteenth-century Spanish reports. It seems probable that all four sites were abandoned at an early date. Since there are cenotes at three of them and a pond of potable water at Tecoh, they would appear to be promising sources of late preconquest and early colonial pottery. Tuchicaan and Tecoh are of especial interest, since both played an important part during the Spanish invasion of Yucatan. There are still extensive Maya ruins at all seven sites mentioned above except Kanchunup and Cauich.

Indications exist that a very large number of the colonial churches in Yucatan were built partly of hewn blocks taken from ancient structures in the immediate vicinity, although such material apparently was sometimes supplemented, as at Kinchil and Kaua, by hewn stone from other sites as far as 6 km. distant. Two of the oldest convents, at Merida and Izamal, were

built on the summits of fairly high substructures, and in the northwestern and the northern parts of the state, as far east as the vicinity of Izamal, a great many churches are set in spacious patios which are raised from 1 to 2 m. above the level of the town. In such cases this platform appears to have been constructed from the rubble fill of a large Maya substructure. Possibly it consisted partly of the flattened base of a pyramid and was completed from other neighboring mounds. There is some evidence of this at Hunucma and still more at Chuburna. Raised platforms on which the churches stand are much rarer in the Sotuta and Valladolid areas farther east, and in the former Xiu Province of Mani to the south.

These raised church patios are frequently enormous structures, although they probably average only about 1.5 m. in height. Roughly paced off, those at Hunucma, Kinchil, Hocaba, and Humun have an average surface of about 8000 sq. m. The platform at Humun is 3 or 4 m. in height, but here it is partly formed of a low rocky knoll. Nowhere did there appear to be a depression from which this material might have been excavated. There are still many large Maya ruins in northern and northwestern Yucatan, but this use of the substructures for church platforms probably explains why they are not so numerous today as would appear from the sixteenth-century Spanish reports. In spite of the important fisheries and salt industry in former times, agricultural conditions in northwestern Yucatan are such that it is difficult to explain the great building activities which evidently existed. Apparently there was a highly developed social organization over a very long period of time, and in this connection it seems relevant to note that some sherds gathered at Cuzco, when submitted to Dr. G. W. Rainerd, were identified as corresponding

in age to the Chicanel phase at Uaxactun.

In a number of towns, where the site of the church is raised but little or not at all above the surrounding area, some of the old pyramids or other substructures were left standing in the immediate vicinity. This has been recorded of the cathedral at Merida and the church at Motul. It is still the case at Ucu and Cauceh, both of which might be considered sites of some promise for recovering sherds of the conquest period. Other instances can be cited at Yaxkukul, Acanceh, Sotuta, Dzan, Chapab, and Teabo. At Sotuta a large substructure near the church is surmounted by a fortified colonial building. Similar remains are also to be seen at Chicxulub, Ticul, Oxkutzcab, and near Tekax, but, like many others reported from Yucatan towns, they lie at some distance from the church and the center of town.

Visits were made to the ruins at Ake, Acanceh, Izamal, Uxmal, Chichen Itza, and Mayapan, and to the town of Champoton, all of which figure in the historical legends of the Books of Chilam Balam. At the time of the Spanish conquest Champoton was a large and commercially important town with many stone buildings. Although the architectural remains are scanty, there are still a number of scattered drums of cylindrical columns. They are rather roughly hewn and are reminiscent of those at Mayapan. Surface sherds are abundant in the streets, and the gullies which intersect the town seem to offer some promise of stratified deposits.

HISTORY OF SCIENCE

GEORGE SARTON

As my connection with Carnegie Institution ends August 31, 1949, it seems proper to devote this, my final, report not only to the last year **but** to the whole duration of my service, which began July 1, 1918.

Rereading my yearly reports, the first of which appeared in Year Book No. 18 (1918-1919), I find that my main work was not announced until the third report (Year Book No. 20) and that I began the writing of my *Introduction to the history of science* only on January 12, 1921. When I started that undertaking, I did not, and could not, realize its size, complexity, and difficulties; I thought that it would occupy only part of my time and that I would be able to complete two other projects, each of which was of lifetime size. These two projects were, first, a history of modern physics (physics in all its ramifications in the nineteenth and twentieth centuries); second, a full account of the life and achievements of Leonardo da Vinci.

As this second project is not unrelated to the main undertaking, I may be permitted to say a few words about it. In 1916, I had delivered six lectures on Leonardo da Vinci at the Lowell Institute in Boston. After the completion of that course of lectures, it dawned upon me that my knowledge of the subject was very insufficient. Leonardo, sometimes called the father of modern science, was the child of the Middle Ages. In order to appreciate his thoughts correctly it was necessary to have a deeper knowledge of medieval science than I could boast at that time. It was probably then that I resolved to make a systematic and thorough survey of the progress of science, century by century; I sincerely thought that I would be able to reach our time, or at least the **beginning** of this century, within ten or twenty years. In reality, so many were the obstacles that I did not even reach Leonardo's time, but had to stop my survey about the year 1400.

The main cause of delay was the necessity, unsuspected at first, of studying the Arabic language. This was a heavy task in itself. At first, I had **been** helped by my **kind** friend the Rev. Duncan Black

Macdonald (1863-1943), of Hartford, Connecticut, but in spite of his willingness he was not always able to help me, nor could I appeal to him as often as would have been necessary, and the obligation to study Arabic could not be eschewed any longer.

It is proved in great detail in my *Introduction* that for three centuries at least (the ninth to the eleventh), Arabic was the international language of science, and that in the following two centuries (the twelfth and thirteenth) the study of it remained the shortest cut to up-to-date knowledge. We often speak of the iron curtain separating eastern from western Europe; another curtain began to separate them (that is, to separate the Orthodox, Greek East from the Latin, Catholic West) as early as the fifth century, and three centuries later it had become almost impenetrable. The Latin doctors refused to read Greek; therefore, they were finally obliged to read Arabic, a language entirely unrelated to theirs, the language not of Christians but of Muslims. That is one of the paradoxes of history. It is because of it that medieval science and medieval culture cannot be understood without a sufficient knowledge of the Arabic writings.

While I was engaged in the survey of ancient and medieval science which would bring me back within a few years (so I thought) to Leonardo, the Carnegie Institution commissioned Professor J. Playfair McMurrich (1859-1939), of Toronto, to investigate Leonardo's anatomical drawings and notes. Indeed, it is clear that Leonardo's main source in this field could not be medieval knowledge, but only his own dissections, and the value of his anatomical drawings could not be appreciated except by a man with a long anatomical experience. The results of Professor McMurrich's investigations were published by the Institution in 1930 (J. Playfair McMurrich,

Leonardo da Vinci the anatomist (1452-75/9). Carnegie Inst. Wash. Pub. 411. xx+265 pp., frontispiece, 89 figs. *Isis*, vol. 15>PP- 342-344)-

In the meanwhile, volume 1 of the *Introduction to the history of science*, dealing with the period from Homer to Omar Khayyam, a period of two thousand years, had appeared in 1927, and volume 2, in two parts, devoted to the twelfth and thirteenth centuries, appeared in 1931. The effort made to bring these volumes to relative perfection had been so long-drawn and intense that it left the author exhausted. Thanks to the wisdom and generosity of the Institution, I was permitted to spend a sabbatical year abroad. I resided half a year in Syria, where I was a guest of the American University of Beirut and was able to extend my knowledge of the Arabic language, the Arabic people, Eastern Christianity, and Islam. Shorter times were spent in other countries which were (or had been) parts of the Arabic or Islamic world:- Egypt, Palestine, Turkey, Rhodes, Cyprus, Tunis, Algeria, Morocco, Spain, and Sicily. After my return to Cambridge my work was resumed and centered upon the fourteenth century. This again took far more time than had been expected—volume 3 (in two parts) appeared only in 1948—partly because my standards of scholarship had become more severe as my experience increased, partly because the amount of accumulated materials was so much greater. Materials had been accumulating for the whole work from the beginning; the accumulation had lasted about 9 years for volume 1, 13 years for volume 2, 27 years for volume 3. Many of the documents had been published, or at least listed, in *Isis*. By the time of publication of volume 1, 27 numbers (almost 8 volumes) of *Isis* had appeared, including 18 critical bibliographies; by the time of publication of volume 2, 46 numbers (al-

most 15 volumes), including 30 bibliographies; by the time of publication of volume 3, 103 numbers (35 volumes), including 67 bibliographies, plus 7 volumes of *Osiris*. The materials contained in the *Introduction*, *Isis*, and *Osiris* are integrated by means of thousands of cross references. Thus we may say that volume 1 was built on a foundation of 8 volumes; volume 2, on a foundation of 15; volume 3, on a foundation of 42.

Reference to *Isis* suggests that the mass of information included in the three published volumes of the *Introduction* is much larger than appears at the surface. These three volumes include 4334 pages, but there is scarcely a page which does not refer to *Isis* or *Osiris*, where more information can be obtained immediately. Moreover, additions and corrections to the published volumes are included periodically in the critical bibliographies of *Isis*, the 75th of which is now in process of preparation, to appear in volume 41.

The author is keenly aware of the need of correction and amplification, but such as it is, the *Introduction* is the most elaborate work of its kind, and by far, in world literature. This statement can be made without falling under the suspicion of boasting, for it is objective, controllable, and obviously correct.

At the end of my thirty years of service, I wish to express my deep gratitude to the Institution which made it possible for me to do the work which I loved best and for which I was most fit. Thanks to its patronage, Rooms 185 and 189 of the Widener Library, Harvard University, became an international center and clearing house for the history of science. These rooms were never called an "institute," but they deserved the name far more than many of the "institutes" attached to European universities.

It is certain that every student of the

history of science, the world over, shares my gratefulness to the Carnegie Institution for having allowed this fundamental work to be undertaken and partly completed. The history of science is like any other discipline in the field of science or the humanities, in that the fundamental work is slow and difficult, and the results austere. It is also expensive, or at least seems to be. It requires the most expensive of all scientific instruments, far more expensive than the greatest telescopes or cyclotrons—a large library, the larger the better (try to evaluate the total cost of such libraries as the Library of Congress or the Harvard College Library). To this one may answer that though the historian of science needs such a library more than any other scholar, he is not by any means alone in using it. Each library is used simultaneously by many thousands of people. In the second place, genuine scientific work is always expensive, at any rate as compared with secondhand work which requires only enough literary ability to exploit the investigations of other people or rephrase their reports; scientific work is apparently expensive, but it alone has any chance of permanence. The popular books, however successful (and their success is often in inverse ratio to their scientific value), are ephemeral; one can never really depend upon them; it is hardly worth while to refer to them. The writing of such books is a Sisyphian labor without value, except perhaps a monetary one. It must be done over and over again. There is no cheapness in them, at least no financial cheapness. For a longer explanation of my views than there is space for here, the reader is referred to my article "The scientific basis of the history of science," published by the Institution in the volume dedicated to the late President Merriam, *Cooperation in Research* (Carnegie Inst. Wash. Pub. 501, pp. 465-471, 1938).

PUBLICATIONS

MARGARET W. HARRISON

Volume X of Contributions to American Anthropology and History (Publication 585), now in page proof, contains four papers: *The Maya Chronicles* (no. 48), by Alfredo Barrera Vasquez and Sylvanus G. Morley; *Guide to the Codex Perez* (no. 49), by Ralph L. Roys; *The Pendleton ruin, Hidalgo County, New Mexico* (no. 50), by A. V. Kidder and H. S. and C. B. Cosgrove; and *The prophecies for the Maya tuns or years in the Books of Chilam Balam of Tizimin and Mani* (no. 51), by Ralph L. Roys. This group completes the volume, which will be issued in the fall of 1949.

The Maya Chontal Indians of Acalan-Tixchel: a contribution to the history and ethnography of the Yucatan Peninsula (Publication 560), under the joint authorship of France V. Scholes, Vice-President of the University of New Mexico, and Ralph L. Roys, with the assistance of Robert S. Chamberlain and Eleanor B. Adams, was published early in the winter of 1949.

A. Ledyard Smith has completed his part of the general survey of the Division's work at Uaxactun. His monograph, entitled *Uaxactun, Guatemala: excavations of 1931-1937* (Publication 588), is now in press; it will be followed shortly by Robert

E. Smith's companion report on the pottery at this site. Dr. Kidder's introduction to the first book gives the archaeological background for both studies.

J. Eric S. Thompson's *Maya hieroglyphic writing: introduction* (Publication 589) is in galley proof. It forms the first volume of Mr. Thompson's projected series on this subject and will be published by the end of 1949.

Two compilations were mimeographed and issued by the Division this year: *Historical source material for the history of Mayapan*, by Ralph L. Roys; and *Selected references on the Maya area*, brought up to date as of December 1948, by Margaret W. Harrison.

The fourth volume of Notes on Middle American Archaeology and Ethnology was begun with three papers: the first two by Dr. Kidder, listed in the bibliography following this report; the third, *Some new discoveries at Coba* (no. 93), by William R. and Michael D. Coe.

Several manuscripts soon to be presented by the Division for publication await completion of editorial work: *The conquest of Honduras and Higuera*, by Robert S. Chamberlain; *Co pan ceramics: a study of southeastern Maya pottery*, by John M. Longyear III, of Colgate University; and *A study of Classic Maya sculpture*, by Tatiana Proskouriakoff.

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SPECIAL PROJECTS: HISTORICAL RESEARCH

E. A. LOWE, The Institute for Advanced Study, Princeton, New Jersey. *Collection and study of paleographical material.* (For previous reports see Year Books Nos. 9 to 35, 37 to 40, and 47-)

The four volumes of *Codices latini antiquiores* so far published have dealt with the oldest Latin manuscripts in the Vatican City, in Great Britain and Ireland, and in Italy outside the Vatican City. The next two volumes deal with the manuscripts preserved in France: volume V deals with the Paris manuscripts, volume VI with the manuscripts in French libraries outside of Paris. The Paris manuscripts are nearly all in the Bibliothèque Nationale; a few are in the Arsenal and Ste Geneviève libraries, and some papyri are in the Louvre. Together they comprise a round two hundred items, making volume V almost twice the size of volume I. The fifth volume is nearly all set up in type. The lay-

out of the facsimiles was completed last October, and work on the collotypes is to begin shortly. Barring unforeseen obstacles, the volume may see the light by the end of 1949. One hurdle, however, still remains to be cleared: the papyri in the Louvre Museum have so far been inaccessible. It is greatly to be hoped that it will soon be possible to photograph them.

Field work on volume VI has commenced. Preparations are now being made for taking the necessary photographs of items scattered in a rather large number of libraries. It is gratifying to report that the editor can count on the helpful co-operation of the French libraries.

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- Year Book No. 47, 1947-1948. Octavo, xxxvi-f'5 + 235 pages, 5 plates, 9 figures.
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In memoriam: Richard Chace Tolman, 1881-1948. *Science*, vol. 109, no. 2819, pp. 20-21 (Jan. 7, 1949).

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Remarks at the Centennial Observance of the Lawrence Scientific School of Harvard University (see Year Book No. 47). *Excerpt reprinted* (under the title "The hallmark of a profession") in: *Nieman Reports*, vol. 2, no. 3, p. 2 (July 1948); *Texas A. & M. Engineer*, vol. 7, no. 1, pp. 10, 32, 34 (Oct. 1948).

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t Deceased May 22, 1949.

X Resigned September 15, 1949.
§ Deceased November 18, 1949.

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f Resigned in 1949.
% Retired in 1949.

§Term of appointment completed in 1949.
‡Deceased August 31, 1949.

CARNEGIE INSTITUTION OF WASHINGTON

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Central Laboratory, Stanford, California

Desert Laboratory, opened in 1903, became headquarters of Department of Botanical Research in 1905. Name changed to Laboratory for Plant Physiology in 1923; reorganized in 1928 as Division of Plant Biology, including Ecology. Herman A. Spoehr, Chairman 1927-1930 and 1931-1947, Chairman Emeritus 1947--.

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Cold Spring Harbor, Long Island, New York

Station for Experimental Evolution opened in 1904; name changed to Department of Experimental Evolution in 1906; combined with Eugenics Record Office in 1921 to form Department of Genetics. Charles B. Davenport, Director 1904-1934; Albert F. Blakeslee, Director 1935-1941.

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DIVISION OF HISTORICAL RESEARCH

10 Frisbie Place, Cambridge 38, Massachusetts

Department of Historical Research organized in 1903; Andrew C. McLaughlin, Director 1903-1905; J. Franklin Jameson, Director 1905-1928. In 1930 this Department was incorporated as a section of United States history in a new Division of Historical Research.

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ORGANIZATION, PLAN, AND SCOPE

The Carnegie Institution of Washington was founded by Andrew Carnegie, January 28, 1902, when he gave to a board of trustees an endowment of registered bonds of the par value of ten million dollars. To this fund an addition of two million dollars was made by Mr. Carnegie on December 10, 1907, and a further addition of ten million dollars was made by him on January 19, 1911. Furthermore, the income of a reserve fund of about three million dollars, accumulated in accordance with the founder's specifications in 1911, is now available for general use, and in recent years a total of ten million dollars has been paid by the Carnegie Corporation of New York as increase to the Endowment Fund of the Institution. The Institution was originally organized under the laws of the District of Columbia and incorporated as the *Carnegie Institution*, articles of incorporation having been executed on January 4, 1902. The Institution was reincorporated, however, by an act of the Congress of the United States, approved April 28, 1904, under the title of the *Carnegie Institution of Washington*. (See existing Articles of Incorporation on following pages.)

Organization under the new Articles of Incorporation was effected May 18, 1904, and the Institution was placed under the control of a board of twenty-four trustees, all of whom had been members of the original corporation. The trustees meet annually in December to consider the affairs of the Institution in general, the progress of work already undertaken, and the initiation of new projects, and to make the necessary appropriations for the ensuing year. During the intervals between the meetings of the trustees the affairs of the Institution are conducted by an Executive Committee chosen by and from the Board of Trustees and acting through the President of the Institution as chief executive officer.

The Articles of Incorporation of the Institution declare in general "that the objects of the corporation shall be to encourage, in the broadest and most liberal manner, investigation, research, and discovery, and the application of knowledge to the improvement of mankind."

The Institution is essentially an operating organization. It attempts to advance fundamental research in fields not normally covered by the activities of other agencies, and to concentrate its attention upon specific problems, with the idea of shifting attack from time to time to meet the more pressing needs of research as they develop with increase of knowledge. Some of these problems require the collaboration of several investigators, special equipment, and continuous effort. Many close relations exist among activities of the Institution, and a type of organization representing investigations in astronomy, in terrestrial sciences, in biological sciences, and in historical research has been effected. Conference groups on various subjects have played a part in bringing new vision and new methods to bear upon many problems. Constant efforts are made to facilitate interpretation and application of results of research activities of the Institution, and an Office of Publications and Public Relations provides means for appropriate publication.

ARTICLES OF INCORPORATION

PUBLIC NO. 260. An Act to incorporate the Carnegie Institution of Washington.

Be it enacted by the Senate and House of Representatives of the United States of America in Congress assembled, That the persons following, being persons who are now trustees of the Carnegie Institution, namely, Alexander Agassiz, John S. Billings, John L. Cadwalader, Cleveland H. Dodge, William N. Frew, Lyman J. Gage, Daniel C. Gilman, John Hay, Henry L. Higginson, William Wirt Howe, Charles L. Hutchinson, Samuel P. Langley, William Lindsay, Seth Low, Wayne MacVeagh, Darius O. Mills, S. Weir Mitchell, William W. Morrow, Ethan A. Hitchcock, Elihu Root, John C. Spooner, Andrew D. White, Charles D. Walcott, Carroll D. Wright, their associates and successors, duly chosen, are hereby incorporated and declared to be a body corporate by the name of the Carnegie Institution of Washington and by that name shall be known and have perpetual succession, with the powers, limitations, and restrictions herein contained.

SEC. 2. That the objects of the corporation shall be to encourage, in the broadest and most liberal manner, investigation, research, and discovery, and the application of knowledge to the improvement of mankind; and in particular—

(a) To conduct, endow, and assist investigation in any department of science, literature, or art, and to this end to cooperate with governments, universities, colleges, technical schools, learned societies, and individuals.

(b) To appoint committees of experts to direct special lines of research.

(c) To publish and distribute documents.

(d) To conduct lectures, hold meetings, and acquire and maintain a library.

(e) To purchase such property, real or personal, and construct such building or buildings as may be necessary to carry on the work of the corporation.

(f) In general, to do and perform all things necessary to promote the objects of the institution, with full power, however, to the trustees hereinafter appointed and their successors from time to time to modify the conditions and regulations under which the work shall be carried on, so as to secure the application of the funds in the manner best adapted to the conditions of the time, provided that the objects of the corporation shall at all times be among the foregoing or kindred thereto.

SEC. 3. That the direction and management of the affairs of the corporation and the control and disposal of its property and funds shall be vested in a board of trustees, twenty-two in number, to be composed of the following individuals: Alexander Agassiz, John S. Billings, John L. Cadwalader, Cleveland H. Dodge, William N. Frew, Lyman J. Gage, Daniel C. Gilman, John Hay, Henry L. Higginson, William Wirt Howe, Charles L. Hutchinson, *Samuel P. Langley*, William Lindsay, Seth Low, Wayne MacVeagh, Darius O. Mills, S. Weir Mitchell, William W. Morrow, *Ethan A. Hitchcock*, Elihu Root, John C. Spooner, Andrew D. White, Charles D. Walcott, Carroll D. Wright, who shall constitute the first board of trustees. The board of trustees shall have power from time to time to increase its membership to not more than twenty-seven members. Vacancies occasioned by death, resignation, or otherwise shall be filled by the remaining trustees in such manner as the by-laws

ARTICLES OF INCORPORATION

shall prescribe; and the persons so elected shall thereupon become trustees and also members of the said corporation. The principal place of business of the said corporation shall be the city of Washington, in the District of Columbia.

SEC. 4. That such board of trustees shall be entitled to take, hold, and administer the securities, funds, and property so transferred by said Andrew Carnegie to the trustees of the Carnegie Institution and such other funds or property as may at any time be given, devised, or bequeathed to them, or to such corporation, for the purposes of the trust; and with full power from time to time to adopt a common seal, to appoint such officers, members of the board of trustees or otherwise, and such employees as may be deemed necessary in carrying on the business of the corporation, at such salaries or with such remuneration as they may deem proper; and with full power to adopt by-laws from time to time and such rules or regulations as may be necessary to secure the safe and convenient transaction of the business of the corporation; and with full power and discretion to deal with and expend the income of the corporation in such manner as in their judgment will best promote the objects herein set forth and in general to have and use all powers and authority necessary to promote such objects and carry out the purposes of the donor. The said trustees shall have further power from time to time to hold as investments the securities hereinabove referred to so transferred by Andrew Carnegie, and any property which has been or may be transferred to them or such corporation by Andrew Carnegie or by any other person, persons, or corporation, and to invest any sums or amounts from time to time in such securities and in such form and manner as are permitted to trustees or to charitable or literary corporations for investment, according to the laws of the States of New York, Pennsylvania, or Massachusetts, or in such securities as are authorized for investment by the said deed of trust so executed by Andrew Carnegie, or by any deed of gift or last will and testament to be hereafter made or executed.

SEC. 5. That the said corporation may take and hold any additional donations, grants, devises, or bequests which may be made in further support of the purposes of the said corporation, and may include in the expenses thereof the personal expenses which the trustees may incur in attending meetings or otherwise in carrying out the business of the trust, but the services of the trustees as such shall be gratuitous.

SEC. 6. That as soon as may be possible after the passage of this Act a meeting of the trustees hereinbefore named shall be called by Daniel C. Gilman, John S. Billings, Charles D. Walcott, S. Weir Mitchell, John Hay, Elihu Root, and Carroll D. Wright, or any four of them, at the city of Washington, in the District of Columbia, by notice served in person or by mail addressed to each trustee at his place of residence; and the said trustees, or a majority thereof, being assembled, shall organize and proceed to adopt by-laws, to elect officers and appoint committees, and generally to organize the said corporation; and said trustees herein named, on behalf of the corporation hereby incorporated, shall thereupon receive, take over, and enter into possession, custody, and management of all property, real or personal, of the corporation heretofore known as the Carnegie Institution, incorporated, as hereinbefore set forth under "An Act to establish a Code of Law for the District of Columbia, January fourth, nineteen hundred and two," and to all its rights, contracts, claims,

CARNEGIE INSTITUTION OF WASHINGTON

and property of any kind or nature; and the several officers of such corporation, or any other person having charge of any of the securities, funds, real or personal, books, or property thereof, shall, on demand, deliver the same to the said trustees appointed by this Act or to the persons appointed by them to receive the same; and the trustees of the existing corporation and the trustees herein named shall and may take such other steps as shall be necessary to carry out the purposes of this Act.

SEC. 7. That the rights of the creditors of the said existing corporation known as the Carnegie Institution shall not in any manner be impaired by the passage of this Act, or the transfer of the property hereinbefore mentioned, nor shall any liability or obligation for the payment of any sums due or to become due, or any claim or demand, in any manner or for any cause existing against the said existing corporation, be released or impaired; but such corporation hereby incorporated is declared to succeed to the obligations and liabilities and to be held liable to pay and discharge all of the debts, liabilities, and contracts of the said corporation so existing to the same effect as if such new corporation had itself incurred the obligation or liability to pay such debt or damages, and no such action or proceeding before any court or tribunal shall be deemed to have abated or been discontinued by reason of the passage of this Act.

SEC. 8. That Congress may from time to time alter, repeal, or modify this Act of incorporation, but no contract or individual right made or acquired shall thereby be divested or impaired.

SEC. 9. That this Act shall take effect immediately.

Approved, April 28, 1904.

BY-LAWS OF THE INSTITUTION

Adopted December 13, 1904. Amended December 13, 1910, December 13, 1912, December 10, 1937,
December 15, 1939, December 13, 1940, December 18, 1942, and December 12, 1947

ARTICLE I

THE TRUSTEES

1. The Board of Trustees shall consist of twenty-four members, with power to increase its membership to not more than twenty-seven members. The Trustees shall hold office continuously and not for a stated term.
2. In case any Trustee shall fail to attend three successive annual meetings of the Board he shall thereupon cease to be a Trustee.
3. No Trustee shall receive any compensation for his services as such.
4. All vacancies in the Board of Trustees shall be filled by the Trustees by ballot at an annual meeting, but no person shall be declared elected unless he receives the votes of two-thirds of the Trustees present.

ARTICLE II

OFFICERS OF THE BOARD

1. The officers of the Board shall be a Chairman of the Board, a Vice-Chairman, and a Secretary, who shall be elected by the Trustees, from the members of the Board, by ballot to serve for a term of three years. All vacancies shall be filled by the Board for the unexpired term; provided, however, that the Executive Committee shall have power to fill a vacancy in the office of Secretary to serve until the next meeting of the Board of Trustees.
2. The Chairman shall preside at all meetings and shall have the usual powers of a presiding officer.
3. The Vice-Chairman, in the absence or disability of the Chairman, shall perform the duties of the Chairman.
4. The Secretary shall issue notices of meetings of the Board, record its transactions, and conduct that part of the correspondence relating to the Board and to his duties.

ARTICLE III

EXECUTIVE ADMINISTRATION

The President

1. There shall be a President who shall be elected by ballot by, and hold office during the pleasure of, the Board, who shall be the chief executive officer of the Institution. The President, subject to the control of the Board and the Executive Committee, shall have general charge of all matters of administration and supervision of all arrangements for research and other work undertaken by the Institution or with its funds. He shall prepare and submit to the Board of Trustees and to the Executive

CARNEGIE INSTITUTION OF WASHINGTON

Committee plans and suggestions for the work of the Institution, shall conduct its general correspondence and the correspondence with applicants for grants and with the special advisers of the Committee, and shall present his recommendations in each case to the Executive Committee for decision. AH proposals and requests for grants shall be referred to the President for consideration and report. He shall have power to remove, appoint, and, within the scope of funds made available by the Trustees, provide for compensation of subordinate employees and to fix the compensation of such employees within the limits of a maximum rate of compensation to be established from time to time by the Executive Committee. He shall be *ex officio* a member of the Executive Committee*

2. He shall be the legal custodian of the seal and of all property of the Institution whose custody is not otherwise provided for. He shall sign and execute on behalf of the corporation all contracts and instruments necessary in authorized administrative and research matters and affix the corporate seal thereto when necessary, and may delegate the performance of such acts and other administrative duties in his absence to the Executive Officer. He may execute all other contracts, deeds, and instruments on behalf of the corporation and affix the seal thereto when expressly authorized by the Board of Trustees or Executive Committee. He may, within the limits of his own authorization, delegate to the Executive Officer authority to act as custodian of and affix the corporate seal. He shall be responsible for the expenditure and disbursement of all funds of the Institution in accordance with the directions of the Board and of the Executive Committee, and shall keep accurate accounts of all receipts and disbursements. Following approval by the Executive Committee he shall transmit to the Board of Trustees before its annual meeting in December a written report of the operations and business of the Institution for the preceding fiscal year with his recommendations for work and appropriations for the succeeding calendar year.

3. He shall attend all meetings of the Board of Trustees.

4. There shall be an officer designated Executive Officer who shall be appointed by and hold office at the pleasure of the President, subject to the approval of the Executive Committee. His duties shall be to assist and act for the President as the latter may duly authorize and direct.

5. The President shall retire from office at the end of the calendar year in which he becomes sixty-five years of age.

ARTICLE IV

MEETINGS

1. The annual meeting of the Board of Trustees shall be held in the City of Washington, in the District of Columbia, on the first Friday following the second Thursday of December in each year unless the date and place of meeting are otherwise ordered by the Executive Committee.

2. Special meetings of the Board may be called by the Executive Committee by notice served personally upon, or mailed to the usual address of, each Trustee twenty days prior to the meeting.

3. Special meetings shall, moreover, be called in the same manner by the Chairman upon the written request of seven members of the Board.

BY-LAWS OF THE INSTITUTION

ARTICLE V

COMMITTEES

1. There shall be the following standing Committees, *viz.* an Executive Committee, a Finance Committee, an Auditing Committee, and a Nominating Committee.

2. All vacancies occurring in the Executive Committee, the Finance Committee, the Auditing Committee, and the Nominating Committee shall be filled by the Trustees at the next regular meeting. In case of vacancy in the Finance Committee, the Auditing Committee, or the Nominating Committee, upon request of the remaining members of such committee, the Executive Committee may fill such vacancy by appointment until the next meeting of the Board of Trustees.

3. The terms of all officers and of all members of committees, as provided for herein, shall continue until their successors are elected or appointed.

Executive Committee

4. The Executive Committee shall consist of the Chairman and Secretary of the Board of Trustees and the President of the Institution *ex officio* and, in addition, five trustees to be elected by the Board by ballot for a term of three years, who shall be eligible for re-election. Any member elected to fill a vacancy shall serve for the remainder of his predecessor's term.

5. The Executive Committee shall, when the Board is not in session and has not given specific directions, have general control of the administration of the affairs of the corporation and general supervision of all arrangements for administration, research, and other matters undertaken or promoted by the Institution. It shall also submit to the Board of Trustees a printed or typewritten report of each of its meetings, and at the annual meeting shall submit to the Board a report for publication.

6. The Executive Committee shall have power to authorize the purchase, sale, exchange, or transfer of real estate.

Finance Committee

7. The Finance Committee shall consist of five members to be elected by the Board of Trustees by ballot for a term of three years.

8. The Finance Committee shall have custody of the securities of the corporation and general charge of its investments and invested funds, and shall care for and dispose of the same subject to the directions of the Board of Trustees. It shall have power to authorize the purchase, sale, exchange, or transfer of securities and to delegate this power. It shall consider and recommend to the Board from time to time such measures as in its opinion will promote the financial interests of the Institution, and shall make a report at each meeting of the Board.

Auditing Committee

9. The Auditing Committee shall consist of three members to be elected by the Board of Trustees by ballot for a term of three years.

CARNEGIE INSTITUTION OF WASHINGTON

10. Before each annual meeting of the Board of Trustees, the Auditing Committee shall cause the accounts of the Institution for the preceding fiscal year to be audited by public accountants. The accountants shall report to the Committee, and the Committee shall present said report at the ensuing annual meeting of the Board with such recommendations as the Committee may deem appropriate.

Nominciting Committee

11. The Nominating Committee shall consist of the Chairman of the Board of Trustees *ex officio* and, in addition, three trustees to be elected by the Board by ballot for a term of three years, who shall not be eligible for re-election until after the lapse of one year. Any member elected to fill a vacancy shall serve for the remainder of his predecessor's term, provided that of the Nominating Committee first elected after adoption of this By-Law one member shall serve for one year, one member shall serve for two years, and one member shall serve for three years, the Committee to determine the respective terms by lot.

12. Sixty days prior to an annual meeting of the Board the Nominating Committee shall notify the Trustees by mail of the vacancies to be filled in membership of the Board. Each Trustee may submit nominations for such vacancies. Nominations so submitted shall be considered by the Nominating Committee, and ten days prior to the annual meeting the Nominating Committee shall submit to members of the Board by mail a list of the persons so nominated, with its recommendations for filling existing vacancies on the Board and its Standing Committees. No other nominations shall be received by the Board at the annual meeting except with the unanimous consent of the Trustees present.

ARTICLE VI

FINANCIAL ADMINISTRATION

1. No expenditure shall be authorized or made except in pursuance of a previous appropriation by the Board of Trustees, or as provided In Article V, paragraph 8, hereof.

2. The fiscal year of the Institution shall commence on the first day of July in each year.

3. The Executive Committee shall submit to the annual meeting of the Board a full statement of the finances and work of the Institution for the preceding fiscal year and a detailed estimate of the expenditures of the succeeding calendar year.

4. The Board of Trustees, at the annual meeting in each year, shall make general appropriations for the ensuing calendar year; but nothing contained herein shall prevent the Board of Trustees from making special appropriations at any meeting.

5. The Executive Committee shall have general charge and control of all appropriations made by the Board. Following the annual meeting each year, the Executive Committee may make allotment of funds for the period from January 1 to termination of the fiscal year on June 30. It may also make allotment of funds for the period from July 1 to December 31 in advance of July 1. The Committee shall, however, have full authority for allotment of available funds to meet necessary

BY-LAWS OF THE INSTITUTION

expenditures by other methods, if desirable, and for transfer of balances to meet special needs. It shall make provision for outstanding obligations and for reversion of unexpended balances at termination of the fiscal year.

6. The securities of the Institution and evidences of property, and funds invested and to be invested, shall be deposited in such safe depository or in the custody of such trust company and under such safeguards as the Finance Committee shall designate, subject to directions of the Board of Trustees. Income of the Institution available for expenditure shall be deposited in such banks or depositories as may from time to time be designated by the Executive Committee.

7. Any trust company entrusted with the custody of securities by the Finance Committee may, by resolution of the Board of Trustees, be made Fiscal Agent of the Institution, upon an agreed compensation, for the transaction of the business coming within the authority of the Finance Committee.

ARTICLE VII

AMENDMENT OF BY-LAWS

1. These by-laws may be amended at any annual or special meeting of the Board of Trustees by a two-thirds vote of the members present, provided written notice of the proposed amendment shall have been served personally upon, or mailed to the usual address of, each member of the Board twenty days prior to the meeting.

ABSTRACT OF MINUTES OF THE FIFTY-FIRST MEETING OF THE BOARD OF TRUSTEES

The annual meeting of the Board of Trustees was held in Washington, D. C, in the Board Room of the Administration Building, on Friday, December 9, 1949. It was called to order at 10:35 A.M. by the Chairman, Mr. Gifford.

Upon roll call, the following Trustees responded: James F. Bell, Robert Woods Bliss, Lindsay Bradford, W. Cameron Forbes, Walter S. Gifford, Ernest O. Lawrence, Alfred L. Loomis, Robert A. Lovett, Roswell Miller, Henry S. Morgan, Seeley G. Mudd, Henning W. Prentis, Jr., Elihu Root, Jr., Henry R. Shepley, Charles P. Taft, Juan T. Trippe, and James W. Wadsworth. The President of the Institution, Vannevar Bush, was also present.

The minutes of the fiftieth meeting were approved as printed and submitted to the members of the Board.

Reports of the President, the Executive Committee, the Finance Committee, the Auditor, the Auditing Committee, and the Nominating Committee, and of the heads of Divisions and Departments and Research Associates of the Institution were presented and considered.

The sum of \$1,630,478 was appropriated for expenditure by the Institution under the general charge and control of the Executive Committee.

Vacancies in the membership of the Board of Trustees caused by the death of James Forrestal and by the resignations of Frederic A. Delano and Herbert Hoover were filled by the election of Omar N. Bradley, Caryl P. Haskins, and Barklie Henry.

Henning W. Prentis, Jr., was elected a member of the Executive Committee for the term ending in 1950 to succeed the late Frank B. Jewett. Roswell Miller was elected Chairman of the Auditing Committee to succeed Mr. Delano for the term ending in 1951. Lindsay Bradford was elected a member of the Nominating Committee for a period of three years, succeeding Henry S. Morgan.

The meeting adjourned at 12:08 P.M.

REPORT OF THE EXECUTIVE COMMITTEE

FOR THE YEAR ENDED JUNE 30, 1949

To the Trustees of the Carnegie Institution of Washington:

GENTLEMEN: In accordance with the provisions of the By-Laws, the Executive Committee submits this report to the annual meeting of the Board of Trustees.

The detailed record of the activities of the Institution is presented in the reports from the Departments and Divisions, which are contained in the Year Book, a review of some of the highlights being given in the report of the President. The estimate of expenditures for the calendar year 1950 contained in the report of the President has been considered and approved by the Executive Committee, and the Committee has also provisionally approved and recommends to the Board the proposed budget based thereon.

The Board of Trustees at its meeting of December 10, 1948, appointed the firm of Haskins & Sells to audit the accounts of the Institution for the fiscal year ending June 30, 1949. The report of the Auditor, including a balance sheet showing assets and liabilities of the Institution on June 30, 1949, is submitted as a part of the report of the Executive Committee.

In addition to the report of the Auditor there is also submitted a financial statement for the fiscal year ended June 30, 1949, showing funds available for expenditure and amounts allotted by the Executive Committee, and the customary statement of aggregate receipts and disbursements, together with a schedule of real estate and equipment. These statements together with the tables in the Auditor's report comprise the statement of the finances of the Institution.

Three vacancies exist in the membership of the Board of Trustees, resulting from the death in May 1949 of James Forrestal and the resignations of Herbert Hoover in September 1949 and Frederic A. Delano in October 1949.

The term of Mr. Morgan as a member of the Nominating Committee will end at the annual meeting, and a vacancy has resulted in the membership of the Auditing Committee because of the resignation of Mr. Delano.

WALTER S. GIFFORD, *Chairman*
ROBERT WOODS BLISS
VANNEVAR BUSH
FRANK B. JEWETT
HENRY S. MORGAN
ELIHU ROOT, JR.
HENRY R. SHEPLEY
LEWIS H. WEED

October 20, 1949

FINANCIAL STATEMENT FOR THE YEAR ENDED JUNE 30, 1949

	Balances available June 30, 1948	Trustees' appropriations	Net allotments and transfers	Other credits	Total available	Expenditures	Balances available June 30, 1949 for	
							Expenditure	Allotment
Administration	\$6,129.85	\$126,750.00	\$10,670.19	\$55.98	\$143,606.02	\$140,407.51	\$3,198.51
Carnegie Corporation Emergency Fund.....	115,900.41	115,900.41	\$115,900.41
General Contingent Fund....	351,460.18	101,600.00	-78,104.63	374,955.55	374,955.55
General Operations.....	9,032.00	70,790.00	-59,684.29	715.00	20,852.71	20,852.71
Harriet H. Mayor Relief Fund	9,750.00	9,750.00	650.00	9,100.00
Pension Fund.....	187,974.09	95,000.00	7,100.23	290,074.32	110,763.85	179,310.47
General Publications.....	47,478.88	30,000.00	9,265.64	86,744.52	31,387.68	45,402.87	9,953.97
Office of Publications.....	5,711.68	35,550.00	832.71	42,094.39	36,221.86	5,872.53
Research Projects, Fellowships, Grants, etc.....	166,892.08	95,024.48	42,200.00	304,116.56	99,053.95	186,090.94	18,971.67
Special Reconversion Fund. . .	12,630.81	-12,630.81
Departmental Research Operations;								
Plant Biology.....	16,644.62	86,100.00	-4,885.93	97,858.69	87,663.68	10,195.01
Genetics.....	29,015.95	132,995.00	704.75	32,881.18	195,596.88	175,478.08	20,118.80
Geophysical Laboratory....	18,703.15	201,785.00	2,199.52	150.74	222,838.41	212,179.09	10,659.32
Historical Research.....	4,774.15	124,213.00	-242.10	128,745.05	118,251.22	10,493.83
Mount Wilson Observatory.	22,317.48	248,400.00	-15,838.24	980.86	255,860.10	238,692.63	17,167.47
Terrestrial Magnetism	28,563.45	272,750.00	25,723.80	12.44	327,049.69	298,639.57	28,410.12
Embryology.....	9,094.27	100,853.00	-514.59	11,425.00	120,857.68	118,153.73	2,703.95
	<u>\$1,042,073.05</u>	<u>\$1,596,786.00</u>	<u>\$355.09</u>	<u>\$97,686.84</u>	<u>\$2,736,900.98</u>	<u>\$1,667,542.85</u>	<u>\$528,723.82</u>	<u>\$540,634.31</u>

AGGREGATE CASH RECEIPTS AND DISBURSEMENTS FROM ORGANIZATION, JANUARY 28, 1902, TO JUNE 30, 1949

RECEIPTS		DISBURSEMENTS	
<i>Securities Sold or Redeemed</i>	\$141,088,600.74	<i>Securities Purchased</i>	\$156,964,217.81
<i>Income from Securities and Bank Balances</i> ,	59,250,958.94	<i>Accrued Interest on Securities Purchased</i>	795,206.16
<i>Proceeds from Sale of Stock Dividends</i>	124,545.40	<i>Pension Fund</i>	2,052,688.22
<i>Sales of Publications</i>	401,513.16	<i>Bickel Fund</i>	90.38
<i>Bickel Fund (Bequest)</i>	300.00	<i>General Reserve Fund</i>	30,477.43
<i>Colour n Fund (Bequest)</i>	52,015.74	<i>Insurance Fund</i>	140,532.24
<i>Hah Relief Fund (Gift)</i>	2,382.28	<i>Harriman Fund</i>	346.44
<i>Harkavy Fund (Gift)</i>	3,050.00	<i>Harriet H. Mayor Relief Fund</i>	900.00
<i>Harriman Fund (Sale of Land)</i>	4,043.70	<i>Harkavy Fund</i>	221.20
<i>Teeple Fund (Bequest)</i>	10,888.42	<i>Special Emergency Reserve Fund</i>	63,819.41
<i>Van Gelder Fund (Bequest)</i>	1,278.58	<i>National Defense Revolving Fund</i>	3,062,974.97
<i>Carnegie Corporation of New York (Endowment Increase and for Specific Purposes)</i>	13,759,381.24	<i>General Contingent Fund</i>	342,289.26
<i>From Other Organizations and Individuals for Specific Purposes</i>	545,885.98	<i>Carnegie Corporation of New York Emergency Fund. Administration Building and Addition:</i>	
<i>Pension Fund (Refunds)</i>	101,482.47	<i>Construction and Site (Old Building)</i>	309,915.69
<i>General Reserve Fund</i>	79,966.54	<i>Construction (Addition to Administration Bldg.)</i>	416,206.07
<i>Insurance Fund (Refunds)</i>	13,076.02	<i>Site (Addition to Administration Building)</i>	68,570.96
<i>National Defense Revolving Fund (Refunds and Advances)</i>	3,095,347.20	<i>Miscellaneous Expenditures*</i>	40,825.37
<i>Administration Building Addition Account, Rentals and Refunds</i>	18,021.09	<i>Departmental Research Operations:</i>	
<i>Employees' Salary Deductions for the Purchase of U. S. Bonds</i>	99,353.65	<i>Departments of Research, Buildings and Equipment</i>	3,286,525.81
<i>Miscellaneous Refunds and Receipts</i>	1,205,222.32	<i>Departmental Operations</i>	38,703,980.72
	<u>\$219,857,313.47</u>	<i>Research Projects, Fellowships, Grants, etc.</i>	5,731,433.48
		<i>Publication</i>	3,201,947.29
		<i>Administration</i>	3,457,360.70
		<i>Employees' U. S. Bond Purchases</i>	99,224.40
		<i>National Research Council</i>	150,000.00
		<i>Miscellaneous</i>	28,108.36
			<u>\$219,049,307.14</u>
		<i>June 30, 1949, Cash in Banks</i>	808,006.33
			<u>\$219,857,313.47</u>

*Includes Equipment \$7,206.41, Repairs and Alterations to Old Building \$18,599.29.

REAL ESTATE AND EQUIPMENT, ORIGINAL COST

JUNE 30, 1949

<i>Administration</i>		
<i>1530 P Street, N.W., Washington 5, D. C.</i>		
Building and site.....	\$797,633.96	
Equipment.....	19,920.73	\$817,554.69
	<hr/>	
<i>Division of Plant Biology</i>		
<i>Stanford, California</i>		
Buildings and grounds.....	\$73,229.67	
Laboratory apparatus.....	40,913.65	
Library.....	23,340.81	
Operating equipment.....	18,991.99	156,476.12
	<hr/>	
<i>Department of Embryology</i>		
<i>Wolfe and Madison Streets, Baltimore 5, Maryland</i>		
Laboratory apparatus.....	\$25,358.38	
Library.....	7,976.34	
Operating equipment.....	5,311.21	38,645.93
	<hr/>	
<i>Department of Genetics</i>		
<i>Cold Spring Harbor, Long Island, New York</i>		
Buildings and grounds.....	\$275,734.60	
Laboratory apparatus.....	49,448.17	
Library.....	65,256.59	
Operating equipment.....	26,474.74	416,914.10
	<hr/>	
<i>Geophysical Laboratory</i>		
<i>2801 Upton Street, N.W., Washington 8, D. C.</i>		
Buildings and grounds.....	\$170,383.79	
Laboratory apparatus.....	140,232.39	
Library.....	36,510.70	
Operating equipment.....	39,439.34	386,566.22
	<hr/>	
<i>Division of Historical Research</i>		
<i>10 Frisbie Place, Cambridge 38, Massachusetts</i>		
Library.....	\$10,408.05	
Operating equipment.....	18,891.38	29,299.43
	<hr/>	
<i>Mount Wilson Observatory</i>		
<i>813 Santa Barbara Street, Pasadena 4, California</i>		
Buildings and grounds.....	\$268,629.81	
Instruments.....	539,722.36	
Library.....	69,726.52	
Operating equipment.....	62,489.81	
Hooker 100-inch reflector.....	641,070.49	1,581,638.99
	<hr/>	
<i>Department of Terrestrial Magnetism</i>		
<i>5241 Broad Branch Road, N.W., Washington 15, D. C.</i>		
Buildings and grounds.....	\$400,311.53	
Laboratory apparatus.....	176,696.93	
Library.....	35,651.64	
Operating equipment.....	64,324.92	676,985.02
	<hr/>	
		<hr/>
		\$4,104,080.50
		<hr/>

ACCOUNTANTS' CERTIFICATE

To the Board of Trustees of Carnegie Institution of Washington:

We have examined the balance sheet (and supporting schedule of securities owned) of Carnegie Institution of Washington as of June 30, 1949 and the related statements of income and expenditures and current funds surplus for the year then ended. Our examination was made in accordance with generally accepted auditing standards, and accordingly included such tests of the accounting records and such other auditing procedures as we considered necessary in the circumstances, except that we did not examine the records in support of expenditures made (approximately \$193,000) by five of the seven branch offices of the Institution, but we have reviewed internal audit reports of the Bursar's office covering examinations of all branch records during the year.

Effective as of July 1, 1948 the Institution adopted the policy of recording proceeds from sales of non-cash dividends, and in any case where the non-cash dividend is retained as an investment its market value on date of receipt, as income. However, generally accepted accounting principles do not recognize ordinary stock dividends as income. Therefore, proceeds of \$109,071.83 from sales of non-cash dividends during the year under review should not be taken into account as dividend income; the other non-cash distributions sold, amounting to \$15,473.57, are properly considered as dividend income.

In our opinion, subject to the exceptions stated above with respect to the limitation of the scope of our examination and the inclusion of the proceeds from the sales of ordinary stock dividends as income, the accompanying balance sheet and statements of income and expenditures and current funds surplus present fairly the financial position of the Institution at June 30, 1949 and the results of its operations for the year then ended, in conformity with generally accepted accounting principles applied on a basis consistent with that of the preceding period.

HASKINS & SELLS

October 24, 1949

BALANCE SHEET JUNE 30, 1949

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		ASSETS	
<i>Current Funds:</i>			
General:			
Cash in banks and on hand...	\$475,476.01		
Advances—Departmental Research Operations.....	12,424.09		
Accounts receivable—other....	868.22		
Inventory—books.....	146,366.30		
Deferred charges.....	15,356.88		
Due from Endowment and Other Special Funds.....	377,963.93	\$1,028,455.43	
<hr/>			
Restricted:			
Cash in banks.....	84,533.00	\$1,112,988.43	
<hr/>			
<i>Endowment and Other Special Funds:</i>			
Cash in banks.....	\$263,188.93		
Securities (approximate market value \$41,916,000);			
Bonds:			
U. S. Government.....	\$9,027,873.15		
Foreign and International Bank.....	853,288.88		
Public utility.....	2,300,223.90		
Communication.....	1,168,405.65		
Railroad.....	274,484.45		
Railroad equipment trust....	877,386.21		
Industrial and miscellaneous.	5,225,723.79		
Stocks:			
Preferred.....	3,980,855.10		
Common.....	16,930,579.91	40,638,821.04	40,902,009.97
<hr/>			
<i>Plant Funds:</i>			
Invested in real estate and equipment:			
Office of Administration.....	\$817,554.69		
Departments of Research.....	3,286,525.81	4,104,080.50	
<hr/>			
TOTAL.....		\$46,119,078.90	

		LIABILITIES	
<i>Current Funds:</i>			
General:			
Accounts payable.....	\$273.25		
Reserve for valuation of books and accounts receivable.....	147,234.52		
Current Funds surplus:			
Reserves for unexpended appropriations:			
Administration.....	\$3,198.51		
Departmental Research Operations.....	99,748.50		
General Operations.....	20,852.71		
Publication.....	61,229.37		
Research Projects, Fellowships, Grants, etc.....	205,062.61		
<hr/>			
Total.....	\$390,091.70		
Other reserves:			
Carnegie Corporation Emergency Fund.....	115,900.41		
General Contingent Fund.....	374,955.55	880,947.66	
<hr/>			
Restricted:			
Bickel Fund.....	\$209.62		
Harkavy Fund—income account	195.31		
Harriman Fund—income account	84,128.07	84,533.00	\$1,112,988.43
<hr/>			
<i>Endowment and Other Special Funds:</i>			
Due to Current Funds.....	\$377,963.93		
Capital Funds*:			
Endowment Fund.....	\$32,000,000.00		
Capital Reserve Fund.....	5,645,235.43		
Colburn Fund.....	103,310.80		
Harkavy Fund.....	2,828.80		
Harriman Fund.....	304,043.70		
Teeple Fund.....	10,888.42		
Van Gelder Fund.....	1,278.58		
Special Funds:			
General Reserve Fund.....	2,140,012.86		
George E. Hale Relief Fund..	3,491.58		
Harriet H. Mayor Relief Fund.	9,100.00		
Pension Fund.....	179,310.47		
Special Income Reserve.....	124,545.40	40,524,046.04	40,902,009.97
<hr/>			
<i>Plant Funds:</i>			
Income invested in plant*.....	\$3,929,591.43		
Harriman property (gift).....	134,418.07		
Harkavy property (gift).....	2,070.00		
Solar Laboratory (Hale gift) (equipment valued at \$20,803.39 included above*).....	38,000.00		
Bickel property (bequest) (nominal value).....	.100	4,104,080.50	
<hr/>			
TOTAL.....		\$46,119,078.90	

*Represents proceeds from sale of non-cash dividends.

STATEMENT OF INCOME AND EXPENDITURES FOR THE YEAR ENDED JUNE 30, 1949

INCOME:

Investment income:			
Interest and dividends on securities	\$1,694,577.46		
Less—Amortization of bond premiums	<u>11,858.35</u>		
	\$1,682,719.11		
Proceeds from sale of stock dividends	<u>124,545.40</u>	\$1,807,264.51	
Less—Income allocated to:			
Special Income Reserve	\$124,545.40		
General Reserve Fund	85,449.25		
Pension Fund	350.23		
George E. Hale Relief Fund	17.00		
Harkavy Fund	<u>116.63</u>		
			<u>210,478.51</u>

NET INVESTMENT INCOME APPROPRIATED FOR CURRENT

PURPOSES			\$1,596,786.00
Other income:			
Sales of publications	\$9,050.87		
American Cancer Society—grants	23,062.00		
Carnegie Corporation of New York—grants	42,000.00		
Dormitory and mess hall	9,651.68		
Bickel property	\$300.00		
Z*«—Maintenance expenses	<u>90.38</u>	209.62	
Estate of John E. Teepte (deceased)	48.66		
George E. Hale Relief Fund	3,474.58		
Life Insurance Medical Research Fund	1,000.00		
U. S. Public Health Service—grants	9,125.00		
Miscellaneous	<u>9,204.39</u>		
Total	\$106,826.80		
Less—Amounts credited to:			
Restricted funds	\$209.62		
General Reserve Fund	6,402.24		
Special funds	<u>3,523.24</u>	10,135.10	96,691.70
TOTAL INCOME*			<u>\$1,693,477.70</u>

EXPENDITURES:

Pension Fund—annuity and insurance	\$110,763.85		
Harriet H. Mayor Relief Fund	.650.00		
Departmental Research Operations:			
Salaries	\$929,770.45		
Operating expenses	308,353.17		
Dormitory and mess hall—salaries	3,386.00		
Dormitory and mess hall—operating expenses	<u>7,548.35</u>	1,249,058.00	
Research Projects, Fellowships, Grants, etc.:			
Salaries	\$13,536.90		
Grants and miscellaneous	32,125.70		
Fellowship program	<u>53,391.35</u>	99,053.95	
Office of Publications:			
Printing and publishing expenses	\$31,387.68		
Office expenses:			
Salaries	29,376.77		
Stationery, postage, etc.	<u>6,845.09</u>	67,609.54	
Administration		<u>140,407.51</u>	
TOTAL EXPENDITURES			<u>1,667,542.85</u>

Excess of income over expenditures \$25,934.85

*Does not include net gain from sales and redemptions of securities shown in summary of security transactions, page xxxvi.

STATEMENT OF CURRENT FUNDS SURPLUS FOR THE YEAR ENDED JUNE 30, 1949

Balance, July 1, 1948		\$844,348.96
Additions:		
Transfers from:		
Harriman Fund	\$1,000.00	
Pension Fund	9,013.85	
Harriet H. Mayor Relief Fund	650.00	
Excess of income over expenditures	<u>25,934.85</u>	36,598.70
Balance, June 30, 1949		<u>\$880,947.66</u>

SCHEDULE OF SECURITIES OWNED JUNE 30, 1949

Principal amount	Description	Maturity	Cost, amortized cost, or value at date acquired	Approximate market value	*T AVP income
UNITED STATES GOVERNMENT BONDS					
1500,000	U. S. of America Treasury Certf. of Ind. 1 #s	1-1-50	\$500,146.98	\$500,300	—\$34.25
304,000	U. S. of America Treasury 2s	1951-49	304,000.00	304,475	6,080.00
312,000	U. S. of America Treasury 2s	1951-49	312,000.00	313,463	6,240.00
200,000	U. S. of America Treasury 2s	1952-50	200,000.00	201,188	4,000.00
4,500,000	U. S. of America Treasury 2s	1954-52	4,500,000.00	4,599,844	90,000.00
800,000	U. S. of America Treasury 2Ks	1955-52	800,000.00	821,000	18,000.00
400,000	U. S. of America Treasury 2 1/4s	1959-56	420,677.50*	417,750	9,000.00
1,239,000	U. S. of America Treasury 2 Ks	1954-52	1,241,048.67*	1,278,106	30,975.00
50,000	U. S. of America Savings Series "G" 2Ks	1953	50,000.00	48,350	1,250.00
50,000	U. S. of America Savings Series "G" 2Ks	1954	50,000.00	48,200	1,250.00
50,000	U. S. of America Savings Series "G" 2#s	1954	50,000.00	48,050	1,250.00
100,000	U. S. of America Savings Series "G" 2Ks	1955	100,000.00	95,800	2,500.00
100,000	U. S. of America Savings Series "G" 2Ks	1956	100,000.00	95,200	2,500.00
100,000	U. S. of America Savings Series "G" 2Ks	1957	100,000.00	94,700	2,500.00
100,000	U. S. of America Savings Series "G" 2 1/4s	1958	100,000.00	94,800	2,500.00
100,000	U. S. of America Savings Series "G" 2Ks	1959	100,000.00	95,600	2,500.00
100,000	U» S. of America Savings Series "G" 2Ks	1960	100,000.00	98,800	1,250.00
	Income from bonds redeemed				4,245.33
<u>\$9,005,000</u>	Total U. S. Government		<u>\$9,027,873.15</u>	<u>\$9,155,626</u>	<u>\$186,006.08</u>
FOREIGN AND INTERNATIONAL BANK BONDS					
1100,000	Australia, Commonwealth of, S. F. 3 Ks	1956	\$100,000.00	\$94,000	\$3,250.00
50,000	Australia, Commonwealth of, S. F. 3 H%	1957	48,750.00	47,000	1,625.00
90,000	Canadian National Ry. Co., 4Ks Guar	1951	90,091.47*	94,500	4,050.00
100,000	Canadian National Ry. Co., 4Ks Guar	1957	112,000.00	114,000	4,500.00
57,000	Canadian National Ry. Co., 5s Guar	1969	61,008.30*	59,850	2,850.00
35,000	Canadian National Ry. Co., 5s Guar	1970	37,354.52*	37,100	1,750.00
100,000	International Bank for Reconstruction and Development, 2 1/4s	1957	100,000.00	100,750	2,250.00
200,000	Shawinigan Water & Power Co., 1st Mtg. & Coll. Tr. S. F. 3s	1971	207,920.00*	196,000	6,000.00
100,000	City of Toronto Cons. Loan Deb. 5s	1949	96,164.59	101,000	5,000.00
<u>\$832,000</u>	Total Foreign and International Bank		<u>\$853,288.88</u>	<u>\$844,200</u>	<u>\$31,275.00</u>

*After deduction for amortization of premiums on bonds purchased subsequent to January 1, 1940.

SCHEDULE OF SECURITIES OWNED JUNE 30, 1949—Continued

Principal amount	Description	Maturity	Cost, amortized cost, or value at date acquired	Approximate Fair Value	Net Income
PUBLIC UTILITY BONDS					
\$241,000	Columbus & Southern Ohio Electric Co., 1st Mtg. 3Ks.	1970	\$254,705.08*	\$257,870	\$7,832.50
300,000	Consolidated Natural Gas Co., Deb. 2MB.	1968	300,832.60*	306,000	8,250.00
100,000	Detroit Edison Co., Conv. Deb. 3s.	1958	105,242.99*	107,000	1,250.00
37,000	Greyhound Corporation, S. F. Deb. 3s.	1959	37,313.83*	37,370	1,110.00
200,000	Minnesota Power & Light Co., 1st Mtg. 3/8s.	1975	204,338.69*	204,000	6,250.00
100,000	Ohio Power Co., 1st Mtg. 3/8s.	1968	101,500.00	106,000	3,250.00
97,000	Oklahoma Natural Gas Co., 1st Mtg. 2 1/2s.	1961	98,056.57*	97,970	2,788.75
100,000	Panhandle Eastern Pipe Line Co., S. F. Deb. 3 Ks.	1973	101,743.82*	104,000	271.17
50,000	Philadelphia Electric Co., 1st & Ref. Mtg. 2Ms.	1978	49,687.50	51,000	1,437.50
207,000	Philadelphia Electric Power Co., 1st Mtg. 2#s.	1975	211,562.32*	200,790	5,433.75
200,000	Public Service Co. of Indiana, Inc., 1st Mtg. 3/4s.	1975	204,358.12*	206,000	6,250.00
125,000	Puget Sound Power & Light Co., 1st Mtg. 4 1/2s.	1972	129,128.38*	131,250	5,312.50
216,000	Tennessee Gas & Transmission Co., 1st Mtg. Pipe Line 2 1/2s.	1966	218,754.00*	211,680	5,940.00
283,000	United Gas Corp., 1st Mtg. & Coll. Tr. 2 Ks.	1967	283,000.00	283,000	7,782.50
	Income from bonds redeemed				280.00
<u>\$2,256,000</u>	Total Public Utility		<u>\$2,300,223.90</u>	<u>\$2,303,930</u>	<u>\$63,438.67</u>
COMMUNICATION BONDS					
\$150,000	American Telephone & Telegraph Co., Conv. Deb. 2KB.	1961	\$155,378.55*	\$153,000	\$4,125.00
150,000	American Telephone & Telegraph Co., Deb. 2KB.	1975	152,418.75*	147,000	4,125.00
45,000	American Telephone & Telegraph Co., Conv. Deb. 3/4s.	1959	45,000.00	49,050	6,250.00
200,000	Mountain States Telephone & Telegraph Co., Deb. 3 1/2e.	1978	201,680.00*	208,000	3,125.00
100,000	New York Telephone Co., Ref. Mtg. 3 1/2e.	1978	101,380.69*	105,000	3,125.00
200,000	Pacific Telephone & Telegraph Co., Deb. 3 Js.	1978	205,297.66*	210,000	6,500.00
300,000	Southwestern Bell Telephone Co., Deb. 3Ms.	1983	307,250.00*	318,000	9,375.00
	Income from bonds called				2,600.00
<u>\$1,145,000</u>	Total Communication		<u>\$1,168,405.65</u>	<u>\$1,190,050</u>	<u>\$36,100.00</u>
RAILROAD BONDS					
1100,000	Chesapeake & Ohio Ry. Co., Gen. Mtg. 4 1/2s.	1992	\$99,464.29	\$124,000	\$4,500.00
75,000	Chicago & W. Indiana R. R. Co., Cons. 4s.	1952	70,357.66	77,250	3,000.00
<u>100,000</u>	Pennsylvania R. R. Co., Cons. Mtg. 4#s.	1960	<u>104,662.50</u>	<u>114,000</u>	<u>4,500.00</u>
	Income from bonds sold				6.39
<u>^\$275,000</u>	Total Railroad		<u>\$274,484.45</u>	<u>\$315,250</u>	<u>\$12,006.39</u>

* After deduction for amortization of premiums on bonds purchased subsequent to January 1, 1940.

SCHEDULE OF SECURITIES OWNED JUNE 30, 1949—Continued

Principal amount	Description	Maturity	Cost, amortized cost, or value at date acquired	Approximate market value	Net income
RAILROAD EQUIPMENT TRUST BONDS					
\$150,000	Chesapeake & Ohio Ry. Co., Eq. Tr. 2s.	1956-58	\$146,340.34	\$146,500	\$3,000.00
300,000	Chicago Burlington & Quincy R. R. Co., Eq. Tr. 2 [^] s.	1958-63	292,507.12	293,500	6,750.00
150,000	Pennsylvania R. R. Co., Eq. Tr. 2 ^H s Guar.	1958-62	146,358.96	145,500	3,562.50
150,000	Southern Pacific Co., Eq. Tr. 2 [^] s.	1956-58	146,251.10	146,000	3,187.50
150,000	Southern Railway Co., Eq. Tr. 2 Ms.	1956-58	145,928.69	145,500	3,187.50
<u>\$900,000</u>	Total Railroad Equipment Trust.		<u>\$877,386.21</u>	<u>\$877,000</u>	<u>\$19,687.50</u>
INDUSTRIAL AND MISCELLANEOUS BONDS					
#200,000	American Tobacco Co., Deb. 3s.	1969	\$203,051.32*	\$208,000	\$6,000.00
240,000	Bristol Myers Co., Deb. 3s.	1968	241,146.90*	247,200	7,200.00
64,000	Devco & Reynolds Co., Inc., S. F. Deb. 2 ^{pis} .	1965	65,212.16*	64,640	1,840.00
192,000	Eastern Gas & Fuel Associates, 1st Mtg. & Coll. Tr. 3#s.	1965	195,428.99*	188,160	6,720.00
153,000	Food Machinery Corp., S. F. Deb. 2Ks.	1962	152,308.98	151,470	3,825.00
300,000	Goodrich (B. F.) Company, 1st Mtg. 2Ks.	1965	301,184.24*	303,000	8,250.00
291,000	P. Lorillard Co., Deb. 3s.	1963	300,041.08*	299,730	8,730.00
300,000	National Dairy Products Corp., Deb. 2Ks.	1970	304,403.52*	300,000	8,250.00
400,000	Phillips Petroleum Co., S. F. Deb. 2 ^H s.	1964	403,333.13*	407,960	11,000.00
23,000	Pittsburgh Consolidation Coal Co., Deb. 3 [^] s.	1965	23,156.43*	23,460	805.00
150,000	Quaker Oats Co., Deb. 2M ^s .	1964	148,922.50	151,500	3,937.50
300,000	Seagram (Joseph E.) & Sons, Inc., Deb. 2#s.	1966	298,500.00	282,000	7,500.00
400,000	Shell Union Oil Corp., Deb. 2 ¹ / ₄ s.	1971	405,236.36*	388,000	10,000.00
400,000	Socony-Vacuum Oil Co., Deb. 2Ks.	1976	390,278.75	384,000	10,000.00
400,000	Standard Oil Co. of California, Deb. 2 [^] s.	1966	410,219.39*	408,000	11,000.00
300,000	Swift & Co., Deb. 2 ^H s.	1972	301,651.89*	294,000	7,875.00
400,000	Texas Corporation, Deb. 3s.	1965	420,341.89*	420,000	12,000.00
250,000	Union Oil Company of California, Deb. 2K [^] s.	1970	258,919.88*	252,500	6,875.00
100,000	United States Rubber Co., Deb. 2 ^{ffe} .	1976	100,322.09*	94,000	2,625.00
300,000	Westinghouse Electric Corporation, Deb. 2#s.	1971	302,064.29*	297,000	7,875.00
	Income from bonds called.				157.50
<u>\$5,163,000</u>	Total Industrial and Miscellaneous.		<u>\$5,225,723.79</u>	<u>\$5,164,620</u>	<u>\$142,465.00</u>
<u>\$19,576,000</u>	BONDS—Funds Invested.		<u>\$19,727,386.03</u>	<u>\$19,850,676</u>	<u>\$490,978.64</u>

* After deduction for amortization of premiums on bonds purchased subsequent to January 1, 1940.

SCHEDULE OF SECURITIES OWNED JUNE 30, 1949—Continued

Number of shares	Description	Cost, amortized cost, or value at date acquired	Approximate market value	Net income
PREFERRED STOCKS				
1,000	Atuioi Hocking Glass Corp., 54.00 Cum. Pref.	1112,750.00	\$103,000	\$4,000.00
1,500	Appalachian Kleetik Power Co., 4 2/2% Cum. Pref.	159,000.00	163,500	6,750.00
2,000	Anntiong Cork Co., \$3.75 Cum. Prof.	205,500.00	190,000	7,500.00
1,500	Bethlehem Steel Corp., 7 1/4 Cum. Pref.	183,637.50	196,500	10,500.00
1,477	Bristol Myers Co., 5 1/2 Cum. Pref.	156,300.45	149,177	5,538.76
2,000	Buffalo, Niagara Kleetric Corp., 5M/6 Cum. Pref.	207,990.00	178,000	7,200.00
500	Case (J. I.) Co., 7/8 Cum. Pref.	62,225.00	68,500	3,467.19
600	Cleveland Electric Illuminating Co., 58450 Cum. Pref.	68,112.25	66,600	2,700.00
1,900	Consolidated Kdisinn Co. of N. V., \$5.00 Cum. Pref.	202,815.50	203,300	9,500.00
1,125	Continental Can Co., Inc., \$3.75 Cum. Pref.	115,312.50	108,000	4,218.76
145	Corn Products Refining Co., 7 3/4 Cum. Pref.	27,183.25	25,955	1,015.00
1,125	duPont (K. I.) de Nemours & Co., \$4.50 Cum. Pref.	116,125.00	137,250	5,062.51
1,000	KI Paso Natural Gas Co., 4.10 Cum. Pref.	111,442.21	95,000	4,100.00
2,000	General Foods Corp., \$3.50 Cum. Pref.	201,000.00	194,000	7,000.00
1,500	General Motors Corp., \$5.00 Cum. Pref.	187,937.50	186,000	7,500.00
1,000	General Shoe Corporation, \$3.50 Cum. Pref.	102,250.00	83,000	3,500.00
1,000	Grant (W. T.) Co., 3H/6 Cum. Pref.	100,447.91	95,000	3,750.00
1,500	McKehson & Robbins, Inc., \$4.00 Cum. Pref.	144,000.00	139,500	6,000.00
400	Northern Stated Power Co., \$3.60 Cum. Pref.	41,280.00	35,600	1,440.00
695	Ohio Power Co., 4 1/4 Cum. Pref.	76,552.00	75,060	3,127.52
1,500	Pacific Telephone and Telegraph Co., 6% Cum. Pref.	235,220.75	216,000	9,000.00
1,000	Panhandle Eastern Pipe Line Co., 4% Cum. Pref.	104,166.68	98,000	4,000.00
1(KM)	Pillsbury Mills, Inc., \$4.00 Cum. Pref.	107,722.00	103,000	4,000.00
2,0(K)	Reynold-(R. J) Tobacco Co., 3.60% Cum. Pref.	199,683.75	182,000	7,200.00
1,024	Sherwin-Williams Co., 4% Cum. Pref.	112,862.09	110,592	4,096.00
1,400	Standard Oil Co. of Ohio, 3H/6 Cum. Pref. "A"	150,743.69	137,200	5,250.00
250	United States Gypsum Co., 7% Cum. Pref.	45,187.50	44,250	1,750.00
3,100	U. S. Steel Corp., 1% Cum. Pref.	443,407.57	406,100	21,700.00
	Income from stocks called or sold			22,920.13
35,241	Total Preferred Stocks	\$3,980,855.10	\$3,790,084	\$183,785.87
COMMON STOCKS				
7,100	Abbott Laboratories	\$267,476.10	\$276,900	\$10,360.00
500	Allied Chemical & Dye Corp.	96,175.97	84,000	4,500.00
4,000	American Can Company	352,201.17	360,000	15,850.00
4,700	American Gas and Electric Company	202,075.88	202,100	4,525.00
2,700	American Telephone & Telegraph Co.	399,025.39	375,300	24,300.00
5,100	Armstrong Cork Company	251,220.36	209,100	14,700.00
4,500	Boston Edison Company	203,214.54	180,000	11,700.00
1,000	Bristol Myers Co.	39,430.33	27,000	1,600.00
3,000	C. I. T. Financial Corporation	151,369.19	141,000	7,000.00
2,600	Chase National Bank of the City of New York	92,769.35	85,800	4,360.00
8,200	Chrysler Corporation	371,763.95	385,400	36,900.00
5,400	Cleveland Electric Illuminating Company	227,773.54	210,600	7,480.00

(Continued on following page)

SCHEDULE OF SECURITIES OWNED JUNE 30, 1949—Continued

Number of shares	Description	Cost, amortized cost, or value at date acquired	Approximate market value	Net income
COMMON STOCKS—Continued				
1,600	Coca-Cola Company	\$233,577.80	\$208,000	\$7,300.00
2,000	Commercial National Bank and Trust Co. of N. Y.	86,522.22	84,000	4,000.00
6,356	Commonwealth Edison Company	196,326.62	158,900	9,375.10
2,000	Consolidated Edison Company of N. Y.	67,530.37	46,000	3,200.00
1,300	Consolidated Gas Electric Light and Power Company of Baltimore	108,423.09	79,300	4,500.00
1,200	Continental Illinois National Bank & Trust Co. of Chicago	105,810.00	86,400	4,800.00
5,490	Continental Insurance Co.	260,277.60	318,420	13,850.00
9,900	Continental Oil Co. of Delaware	388,856.82	534,600	31,900.00
5,100	Delaware Power & Light Company	105,714.47	96,900	5,610.00
12,400	duPont (E. I.) de Nemours & Co.	506,088.07	570,400	33,325.00
13,100	Eastman Kodak Co.	458,548.61	524,000	21,615.00
1,710	Fireman's Fund Insurance Co.	121,050.00	131,670	4,047.00
5,188	Food Machinery and Chemical Corporation	177,575.97	124,512	8,280.50
13,700	General Electric Co.	540,183.86	479,500	25,830.00
7,600	General Foods Corporation	317,875.40	319,200	16,650.00
8,400	General Motors Corporation	447,819.07	470,400	45,800.00
400	Guaranty Trust Co. of N. Y.	98,003.91	104,800	4,800.00
8,700	Gulf Oil Corp.	391,540.45	513,300	25,500.00
2,000	Hartford Fire Insurance Co.	179,826.06	238,000	5,000.00
8,700	Humble Oil & Refining Co.	290,411.57	600,300	38,250.00
3,1475	Insurance Company of North America	255,748.31	347,500	11,043.75
2,950	International Business Machines Corp.	219,994.84	445,450	11,800.00
4,000	Johns-Manville Corp.	148,016.12	140,000	7,350.00
7,200	Kennecott Copper Corporation	362,303.69	324,000	26,500.00
7,300	Kresge (S. S.) Company	230,158.25	284,700	13,600.00
2,100	Liggett & Myers Tobacco Co.	189,977.30	170,100	10,500.00
320	Mellon National Bank and Trust Company	67,193.07	86,400	2,880.00
6,750	Middle South Utilities, Inc.	98,683.97	101,250
3,200	Minneapolis-Honeywell Regulator Co.	147,869.94	156,800	6,850.00
9,700	Monsanto Chemical Co.	394,552.93	485,000	19,200.00
3,000	Montgomery Ward & Co.	161,894.89	144,000	9,000.00
7,700	National Cash Register Co.	267,801.00	246,400	18,250.00
1,900	National City Bank of New York	76,087.50	74,100	2,720.00
1,800	National Union Fire Insurance Co.	62,056.90	55,800	2,520.00
10,800	Newberry (J. J.) Co.	143,047.69	334,800	21,600.00
4,100	New Jersey Zinc Co.	265,862.52	221,400	15,725.00
3,800	Owens-Illinois Glass Co.	263,802.50	201,400	11,400.00
1,750	Pacific Gai & Electric Company	121,073.09	85,250	5,500.00
11,200	Penney (J. C.) Co.	397,461.99	526,400	27,500.00
1,000	Peoples Gas Light and Coke Company	106,350.00	100,000	5,875.00
3,700	Pfizer (Chat.) & Co., Inc.	215,210.63	170,200	9,625.00
3,500	Philadelphia Electric Company	100,048.39	77,000	4,200.00
7,600	Phillips Petroleum Co.	406,186.99	402,800	22,800.00
9,700	Pittsburgh Plate Glass Co.	312,977.21	300,700	16,275.00
2,200	Procter & Gamble Co.	123,793.05	134,200	6,300.00
1,800	Reynolds (R. J.) Tobacco Co. "B"	72,455.18	64,800	3,600.00
1,400	St. Paul Fire and Marine Insurance Co.	106,987.02	112,000	2,950.00
2,300	Scott Paper Co.	103,055.95	126,500	5,335.00
16,400	Seam, Roebuck & Co.	374,427.57	623,200	45,100.00
6,600	Sherwin-Williams Co.	399,644.64	363,000	18,975.00
5,000	Southern California Edison Company, Ltd.	175,996.33	153,000	3,750.00
11,300	Standard Oil Co. of Indiana	409,733.33	418,100	21,850.00

(Continued on following page)

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SCHEDULE OF SECURITIES OWNED JUNE 30, 1949—Continued

Number of shares	Description	Cost, amortized cost, or value at date acquired	Approximate market value	Net income
COMMON STOCKS—Continued				
7,600	Standard Oil Co. of New Jersey.....	\$452,480.98	1471,200	\$17,219.50
4,600	Texas Company.....	242,815.60	230,000	12,982.50
13,700	Union Carbide & Carbon Corp.....	429,474.16	493,200	25,800.00
5,000	United Fruit Company.....	141,876.60	240,000	20,000.00
9,750	United Gas Corp.....	155,016.03	156,000
4,300	United States Gypsum Co.....	377,236.97	404,200	25,200.00
12,200	Westinghouse Electric Corp.....	333,518.44	280,600	15,050.00
6,300	Woolworth (F. W.) Co.....	281,250.61	296,100	11,800.00
	Income from stocks sold.....			44,479.60
<u>395,639</u>	Total Common Stocks.....	<u>\$16,930,579.91</u>	<u>\$18,275,352</u>	<u>\$1,019,812.95</u>
<u>430,880</u>	COMMON AND PREFERRED STOCKS—Funds Invested.....	<u>\$20,911,435.01</u>	<u>\$22,065,436</u>	<u>\$1,203,598.82</u>
	AGGREGATE INVESTMENTS (BONDS AND STOCKS).....	<u>\$40,638,821.04</u>	<u>\$41,916,112</u>	<u>\$1,694,577.46*</u>

^Represents total interest and dividend income before deduction of amortization of bond premiums.

SUMMARY OF SECURITY TRANSACTIONS JULY 1, 1948 TO JUNE 30, 1949

XXXX

July 1, 1948—Cash awaiting investment..... \$191,355.60

SALES AND REDEMPTIONS

	Gain	Loss	Book value	
Bonds.....	\$122.28		\$4,669,678.84
Preferred stocks.....		\$53,397.59	659,933.76
Common stocks.....	61,802.73		1,353,670.01
Sale of stock rights.....	18,813.77		
Mortgages.....	476.92		
	<u>\$81,215.70</u>	<u>\$53,397.59</u>	<u>\$6,683,282.61</u>
Net gain.....		27,818.11	27,818.11	6,711,100.72
	<u>\$81,215.70</u>	<u>\$81,215.70</u>	
Income applied to amortization of bond premiums.....				11,858.35
Proceeds from sale of stock dividends.....				124,545.40
Total.....				<u>\$7,038,860.07</u>

PURCHASES

Bonds.....	\$4,348,712.31	*1
Common stocks.....	2,426,958.83	6,775,671.14
June 30, 1949—Cash awaiting investment.....		* \$263,188.93

REPORT OF THE PRESIDENT
OF THE
CARNEGIE INSTITUTION OF WASHINGTON
FOR THE YEAR ENDING JUNE 30, 1949

REPORT OF THE PRESIDENT
OF THE
CARNEGIE INSTITUTION OF WASHINGTON

As this annual report is presented to the Trustees in accordance with the By-Laws, the Institution continues to pursue its program of fundamental research. The past year has brought the accomplishment summarized in the annual reports of the Departments, a few of the highlights of which are mentioned below.

In recent reports the way in which scientific programs become formulated by the staffs of the Departments of the Institution has been discussed. There has also been some consideration of the relationship of the Board of Trustees to the programs of an institution of this sort. It would perhaps be well, therefore, to write briefly this year of the position of an individual investigator within the Institution, and the relationship of his program to the whole.

We do not have a rigid hierarchy in our staff. There are senior investigators, junior investigators, aids, and technical supporters of many sorts, but even the titles vary from one Department to another, and there is little of rigidity in the whole structure. This is a desirable situation, for the extraordinary informality of our internal relationships, characteristically American, is one of our great assets. Certainly we have under one title or another senior staff members who, by reason of their attainments, have earned the right to go their own way on their researches, and to explore those puzzles of our environment which most intrigue them. Indeed, the entire organization revolves about the work of these men; the Institution exists in order that they may have the rare opportunity which they

enjoy. Junior investigators in general work in collaboration with these individuals, but with a great deal of freedom of their own and certainly with the opportunity to pursue their own problems to a very considerable extent and thus demonstrate their own effectiveness. The team is completed by younger staff members who have not as yet arrived at the point of independence, together with the technical staff which is most decidedly an integral part of the structure. There are no age requirements, nor are there any seniority rules or scheduled promotions in the whole organization.

The question then naturally arises how the performance of the senior staff is measured. In the large, the answer to this question is that their performance is measured in the same manner that scientific accomplishment generally is measured under the best conditions in any scientific community, namely by the consensus of opinion of colleagues, and of investigators in the same general field who are located elsewhere. But there are certain negative aspects of this subject which are well worth mentioning. Only too often in research organizations the product is weighed, sometimes almost literally, and the staff are under pressure to produce results at intervals, which are not stated explicitly but well understood. When a man is considered for a scientific position or for election to a learned body, only too frequently the number of his published papers is counted, and it is carefully noted whether he has published regularly. The sort of artificial pressure which this tendency creates is absent from

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the Institution. The staff all recognize, of course, that the man who shirks the sometimes disagreeable job of getting his results promptly into print is neglecting an essential part of his undertaking. But the staff also recognize that one good solid article that really constitutes a milestone is worth any number of dribbles, that the article that really pins a subject down completely is far better than the one that is rushed into print while still wobbly in its reasoning. Moreover, the staff recognize that there is such a thing as luck in research, and that the best of investigators may at times miss an opportunity, and struggle long and deviously before he emerges from a temporary obscurity. In fact, the absence of pressure for periodic public performance is one of the strongest reasons why the staff of the Institution are enthusiastic scientists. We take pride in the fact that the stature of our scientists is an affair of a lifetime, and no matter of flashy performance. Yet there is no doubt that the staff regard very seriously the obligation and responsibility that are theirs, because of the rare opportunity they have to advance the sum of human knowledge without arbitrary or artificial pressures or distractions.

The world of science has a place for almost every sort of individual. At the one extreme there are those gregarious cooperative types who can operate more effectively in concert and who become important individuals in a group attack upon a particularly knotty subject. At the other extreme are those detached lonely workers who simply must pursue their own affairs by themselves, without collaboration, and sometimes in the extreme even without assistance. The Institution does not have much of the type of organized group attack which is necessary and sometimes very effective in applied research,

particularly in great commercial laboratories. Neither, on the other hand, do we seem to produce the type of genius who can operate only in isolation. Without definite planning in that regard, our staff, for all its variety, lies somewhere between these two extremes. We expect, and by this we mean that staff opinion expects, that the individual who is pursuing his own line of research will so select his problems and so conduct them that his pursuit of them will reflect favorably upon the progress of the work being conducted by his colleagues in adjacent areas. In addition we expect him to be a helpful individual, who will go out of his way and forget his own pursuits at times to help a colleague out of a difficult situation, and all of this occurs. But if a senior investigator believes he has a lead which will take him to far-reaching results along the lines of our general interests, there is nothing in our rules or in our practice to prevent him from pursuing his individual way with full intensity and with the full support of his colleagues in so doing. This is the sort of fundamental pursuit of knowledge which yields momentous results in the long run. It is only necessary to be sure that those who have the extraordinary privilege are worthy of it, and for this we must rely on their reputations among their own peers.

The Institution differs from a university in many ways. For one thing, the staff are not distracted by the manifold duties that are inevitably a part of the operation of a great university. For another, the business organization of the Institution is designed to lift from the shoulders of investigators as far as is physically possible the burdens that sometimes inevitably intrude in an organization having less singleness of purpose.

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But there is a third way in which we differ, and this is not so favorable. A professor in a university comes in contact necessarily with young minds. An investigator in an independent research organization can shield himself from such contact quite completely if he wishes to do so, or even unconsciously if he naturally shrinks from the give and take, sometimes roughly pursued, which is inherent in contact with young active minds. There is a distinct danger here, for we depend upon young and vigorous minds to keep us from getting stale, and the scientist who removes himself from direct and almost daily criticism is only too likely to get into a groove, or to cherish some personally constructed and bizarre fashion of thought. There are, however, ways in which we offset our otherwise serious isolation from teaching and from youth generally. Several of our Departments are located adjacent to great research centers in universities. Those in the city of Washington participate closely in the scientific affairs of the Federal Government. We have

summer conferences, our staff members occasionally give courses of lectures on invitation, and there are always the important scientific meetings, national and international, attendance at which is sometimes salutary. But we now have in addition a fellowship program which brings young scientists into our midst on a temporary basis, and also a plan for inviting scientists of standing to be our guests. Neither of these plans is rigid in outline. There are, for example, in connection with our fellowships, no rules which limit selections in accordance with academic status or nationality. In inviting guests we have no formalism to consider, but only the question whether a visit will be genuinely beneficial to the guest and to ourselves and thus to the advancement of science. The fellowship program in particular seems to be working well. We have had an excellent group of younger people and they have performed admirably. The program is still in its formative stages and warrants close attention and reasonable expansion.

RESEARCH ACTIVITIES

In the development of modern science it often happens that the creation of a new tool for research is as important a step in attaining the ultimate goal of new knowledge as the actual use of the tool in a research program. This is especially true in astronomy, since most astronomical investigations deal with extremely distant and faint objects. Without telescopes of great light-gathering power to collect an adequate amount of light, the astronomers would be nearly helpless.

Furthermore, the design and construction of such a tool may require as much ingenuity, effort, and expense as its later use for research. Thus the attainment of the great light-gathering power of a

modern telescope means the design and construction of very large optical parts and of huge moving structural members to support them. Moreover, combined with this size must be the accuracy of form and precision of movement of the finest small laboratory instrument. Because of the great rapidity with which flexure, temperature distortions, and errors of manufacture increase with the size of a structure, this requirement poses problems of the greatest difficulty to the designer.

Once successfully constructed, however, such a telescope provides a basic instrument for all kinds of astronomical research during the decades to follow. The instrument may be used by itself to obtain

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direct photographs, or it may be combined with spectrographs or photometers for the study of spectra or the measurement of magnitudes. As new auxiliary instruments are developed during the ensuing years, they may be attached to the telescope to utilize the light it collects, often in ways not originally contemplated when the telescope was built. Thus many of the past "largest" telescopes, constructed thirty, fifty, and even seventy-five years ago, are still active and effective instruments of research today.

The initiation of scientific observations by two notable new instruments, each of outstanding size, was without question the most important research event of the year at the Mount Wilson and Palomar Observatories. The larger of these instruments, the great Hale 200-inch telescope, was first used for taking photographs on a limited observing program during the winter of 1949. Though at the end of the report year some adjustments and minor modifications of this instrument were still necessary before a regular observing program could be scheduled, the earlier trial program provided enough tests to give assurance of the great power of this instrument in attacking many of the outstanding problems of astronomy. Last winter, the Institution, with its friends and collaborators of the California Institute of Technology, had in the Hale telescope a masterful instrument, perfect for average seeing conditions. Powerful as it then was, it was not enough, and the delicate task of further polishing, further refinement, was undertaken. Now, the great instrument is ready, not merely for average seeing conditions, but for those rare occasions when seeing conditions are extraordinary.

The smaller of the new instruments, the 48-inch schmidt camera, was given its

test exposures in the fall of 1948 and was placed in regular service in the following winter. Like the Hale telescope, this instrument is the largest of its type that has thus far been constructed. The schmidt camera is a new development of the past two decades. Because of its ability to give almost perfect definition over a very large plate (14 by 14 inches in the present instrument), it is largely displacing lens-type cameras for survey purposes. The present instrument has fully come up to expectations from both the optical and the mechanical standpoints. These two instruments will make a most powerful asset for astronomical research, the smaller one to find, the larger one to study in detail new objects in the universe about us.

The rare stars with peculiar properties are an object of search by astronomers, since study of them may give important clues to understanding of stellar atmospheres. A thirty-year hunt for several types of these rare objects, including emission-type B stars, planetary nebulae, and T Tauri stars, all of them characterized by bright hydrogen lines, was completed during the year, Dr. Bowen reports. It was a highly successful search. Some 1400 emission line stars, mostly of class B—a many-fold increase in the number of known objects of this type—have been detected in the photographic plates resulting from the study. About 200 new planetary nebulae, more than doubling the observed number of these objects, have been found. In this search, characteristic of astronomy in its patient duration, a 10-inch wide-angle camera with an objective prism and red-corrected lens made it possible to work with the red part of the spectrum, where the strongest of the hydrogen lines is located. This survey of the northern sky having been completed, the search will now turn to the southern sky. The camera

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and its mounting have been loaned to the University of Michigan for a similar survey from the University's station in South Africa.

The investigations at the Geophysical Laboratory are now focused sharply on the formation and properties of rock-forming minerals with and without the presence of mineralizers, such as water, for the general purpose of understanding the complex processes by which the earth was formed. Of the many thousands of minerals that are known to the geologist, only a dozen or so are of any great consequence in the genesis of rocks; and in this limited class two, quartz and feldspar, occupy a pre-eminent position. The Laboratory, which in its earliest days logically gave primary attention to these particular minerals, has now with vastly improved apparatus and techniques turned again to the study of some of their characteristics. Quartz has the interesting property of changing at 573° C. from one crystalline form to another. Recent measurements have shown that the transformation temperature is not a fixed point as it was formerly believed to be, but may vary by nearly 2° , which even at moderately high temperatures is an interval that is readily measurable with modern precision thermometric devices. It appears that the variation in the behavior of different quartz specimens is related to the conditions under which the quartz originally crystallized; and, therefore, that a further correlation of inversion temperature with geologic environment will furnish a valuable clue to the temperature of formation of any given sample of quartz.

Related information has been provided by the measurements on feldspars, which consist mainly of three different types depending on the predominance of lime, soda, or potash. In the work on hydro-

thermal synthesis, it has been discovered that the sodic form, albite, may exist in either of two different modifications depending on the temperature of formation. Natural albite is usually of the low-temperature form, whereas that produced synthetically hitherto has always been of the high-temperature variety. Other peculiarities in feldspars are understandable through the discovery that the solid solutions existing at elevated temperatures sometimes unmix at low temperatures, the extent of unmixing depending on the temperature. An X-ray technique permits ready determination of the composition of many feldspars. Further work is needed, yet already the new method of study appears to make it possible to determine the temperature of formation of feldspar when any two feldspars are found together in a single place. Thus there is now a strong likelihood that both quartz and feldspar will provide reliable geologic thermometers by which the temperature of past earth processes may be inferred.

The Department of Terrestrial Magnetism has brought to successful conclusion a series of experiments focused on one of the few direct and clear-cut puzzles in geophysics, that of the maintenance of the earth's electric charge. Since the voyages of the *Carnegie* during World War I, it has been known that the earth always carries a rather large negative electric charge, shown by the electric field near the surface in fair-weather areas over land and sea. This charge is maintained despite a measured total air-earth current, summed up for all fair-weather areas, of 1500 amperes, flowing in the direction which tends to dissipate the charge. The suggestion was made in England more than twenty years ago that thunderstorms might supply the necessary reverse current, but it has never been possible to prove or

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reject this idea because of the tremendous variability of the air-earth currents observed beneath thunderstorms.

At the end of 1946 the Department requested the U. S. Air Forces to collaborate in an effort to resolve this problem by making appropriate measurements in the clear air above a thunderstorm. The experiments were undertaken and during the past two years have necessitated many hours of flight, often at record heights. The resulting measurements have shown that the electric current between the upper atmosphere and the ground, in localized areas above active thunderheads, is reversed in sign to the fair-weather current and greatly increased in intensity. The activity observed over a typical storm area, resulting in a net current from the solid earth upward to the ionosphere, multiplied by the average number of storms in progress over the whole earth at any one time, is just enough to counterbalance the 1500-ampere current to the earth which was found for the total of all fair-weather areas.

When the staff of the Department of Terrestrial Magnetism resurveyed the status of knowledge of "the electric and magnetic condition of the earth and its atmosphere" in 1946, three conspicuous puzzles were selected as basic problems of primary significance, disregarding the point that each of these seemed at the time largely inaccessible to direct investigation: (a) What are the direct and indirect causes, inside the earth, of the main part of the earth's magnetic field? (b) What mechanism supports and maintains the earth's net electric charge? (c) What and where are the origins of cosmic-ray particles? One of these underlying problems, that of the earth's electric charge, is now resolved with reasonable clarity, and important information on a second

one, the origin of cosmic rays, was unexpectedly provided by the Department's observations, reported two years ago, of sudden cosmic-ray increases accompanying solar flares and radio fade-outs. Since, in general, direct contributions toward the solution of fundamental puzzles, even when the basic questions are recognized and conspicuous, are the exception and not the rule in scientific work, these unexpected successes further encourage a policy which deliberately focuses attention on the formulation and attacking of basic problems even when they seem inaccessible.

For years discussion has been aroused by the theory that the continents of the earth as we know them may have drifted from some earlier configuration to their present pattern, and, indeed, that they may still be imperceptibly moving. Studies of the direction of magnetization of rocks laid down in early epochs now show some promise of yielding evidence bearing on this question. There are rocks in the Blue Ridge Mountains near Washington which are magnetized as though they had originally been laid down in South Africa. Possible instability of magnetic north and possible large-scale local magnetic disturbances from electric current systems inside the molten earth will have to be reckoned with in any effort to explain why this is so. That effort certainly will also have to consider the hypothesis that the crust has moved with respect to geographic north since the ancient epoch when these rocks were laid down*

Studies by the Department on prehistoric changes in the direction of the earth's magnetic field—the compass direction—hitherto made at specific locations in New England and under the Pacific Ocean, by determinations of the residual magnetization of samples of clay and unconsolidated sediments, were extended this year back

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into geological periods earlier than two hundred million years ago. This was done by making observations on a long series of sedimentary rocks. The unfolding of this story of ancient magnetism in recent years at the Department has been dramatic as it progressed, first in painstaking fashion back through twenty thousand years of the last glacial period, then leaping back one million years by measurements on ocean cores. This encouraged an expedition to the west, carrying the record back through eighty million years by measurements on rock samples collected in the region extending from South Dakota through many parts of the Rocky Mountains to the Cascades. This year the story rolls time back more than two hundred million years to a period prior to the folding of the Appalachian Mountains, by studies of sandstones found in Maryland and Virginia which were laid down more than three hundred and fifty million years ago. Studies of the direction of residual magnetization of rock samples taken from different portions of a fold show that the magnetic vectors in the different samples would be parallel if the folded rock were returned to its original flat condition. These "reconstructed compass directions" are found parallel to each other for locations 50 miles apart amid the folded rocks, although the vector found differs violently from the present compass direction. The complex magnetic pattern found in the folded rocks could not be produced by any possible ancient system of localized earth currents, but the parallel vectors in the original flat rocks might well be a record of the compass direction when the rocks were laid down in that ancient Silurian epoch, just as the varved clays of New England show the compass direction of twelve thousand years ago, and clay suspensions redeposited

in the laboratory show the 1949 compass direction in Washington.

Any conclusions on the problems suggested by the findings thus far made in this study will be unjustified until they are supported by long and comprehensive studies of similar and related rocks from widely distributed sites. In support of the initial series of exploratory observations required for this purpose, the Carnegie Corporation has made a special grant to the Institution, and field studies have been started at a series of locations from Alabama to Maine.

It was hardly anticipated that studies of the prehistory of the earth's magnetic field, in search of causes internal to the earth which could produce that field, would lead to these startling questions relating to motions in the earth during early Paleozoic time. It is reasonable to expect that a highly complex but interesting picture eventually will be found in the magnetic pattern of these ancient rocks. It is reasonable also to expect this magnetic pattern in part to be related to "secular variation foci" which we believe are caused today by electric currents deep inside the earth. These special effects may have been much greater in those ancient periods when the earth was younger and presumably more fluid. In any event it will be interesting to follow the further development of these studies of such a delicate and intangible phenomenon as the compass direction and its variations, through one age after another during the long sweep of geologic time.

Steadily increasing pressure of population the world over confronts us with the need for new sources of food. Great changes are surely to be foreseen in the ways by which mankind is fed; some of them will involve new syntheses; others will be simply the improvement and elabo-

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ration of customary methods. One promising possibility is that lower organisms such as yeasts and algae can become sources of the protein and the fats that we must have. Studies of the green alga *Chlorella* which have been proceeding for some years past at the Division of Plant Biology give us a gauge of possibilities. Under controlled conditions, with generous supply of carbon dioxide, *Chlorella* cultures constitute a plant growth that doubles itself every day and that can be made to yield more heavily of protein or of fat by suitable changes in growing conditions. The proteins produced by these cultures contain all the amino acids commonly associated with nutrition. The program has shown the desirability of investigation of the practicability of producing *Chlorella* on a large scale for the possible production of foodstuffs. Much remains to be learned, of course, and the problem becomes primarily one of engineering. The Institution hence has entered into arrangements with Research Corporation providing for support to the Stanford Research Institute in an intensive investigation of the process and products on a pre-pilot plant scale. The Institution will co-operate in the undertaking.

The process of photosynthesis, by which green plants transform water and carbon dioxide into the foodstuffs on which we depend, is a primary focus of the Division's program. The first step in photosynthesis is the splitting of water molecules by the action of light absorbed by the chloroplast pigments in the plant. This has been investigated during the year in colloidal solutions of chloroplast material. In such preparations obtained by ejection of chloroplast suspensions through a needle valve under high pressure, Dr. French reports, the activity has been found to be greatly increased by the aggregation of

the colloidal particles on addition of salt solutions in the presence of dilute methyl alcohol.

From measurements of the effectiveness of different wave lengths of light on corn and bean seedlings which have been grown in darkness, it was found that only the light which is absorbed by protochlorophyll, and not that absorbed by any other pigments present, can lead to chlorophyll formation.

To further the development of improved range grasses with the ultimate aim of better pasturage for food animals, cooperative experiments on forty-six strains of range-grass hybrids have been arranged by the Division of Plant Biology with a number of widely scattered laboratories. This has been done to test the climatic adaptability of *Poa* hybrids over a wide range of climatic conditions such as are found in the state of Washington, in southern California, North Carolina, Scotland, Wales, Holland, Denmark, Norway, Sweden, and Transjordan. Fortunately, many of these improved strains breed true from seed, and thus are easy to distribute. Some of these grass hybrids of particular interest have been selected for use in a quantitative experiment on their growth response in controlled laboratory environments.

At the Department of Embryology the collection, preparation, and study of human embryos proceeded successfully during the year. A human egg in the 2-cell stage and a blastocyst of about 4¹/₄ days were added to the collection by Dr. Arthur T. Hertig, Research Associate, and Dr. John Rock, collaborator. These are stages of normal development never previously seen. At about the time of implantation the human embryo differs from those of other mammals in certain peculiar characteristics. It is therefore of interest that

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these two specimens, earlier than the implantation stage, closely resemble the embryos of other mammals of comparable age. About a dozen valuable human embryos of later stages were serially sectioned by Dr. Heuser for addition to the Carnegie Collection. The collection of specimens bearing on the problem of defective development was augmented by five embryos and fetuses that had been aborted by physicians because the mothers had German measles in early pregnancy. Such defects as these specimens may prove to have will no doubt throw light on the embryological nature of the damage done in such circumstances, which so often leads to blindness, deafness, and mental retardation of the infant.

Dr. J. R. Schlegel, guest of the laboratory on a Rockefeller Fellowship, developed a promising method for determining the time of passage of solutes from the blood vessels to the lymphatic collecting vessels. The method depends upon the use of a dyestuff which is highly specific for the endothelial lining cells of vessels. Being fluorescent, it is readily detectable in ultraviolet light.

Among the important publications of the year was a monograph in the Contributions to Embryology of the Carnegie Institution on the blood vessels of the uterus and particularly of its lining membrane in pregnancy and of the placenta, representing many years of work by Dr. Elizabeth M. Ramsey. Dr. S. R. M. Reynolds and a group of collaborators brought out a series of papers dealing with the physical form of the uterus as it changes during pregnancy to accommodate the fetus, with the adaptation of the uterine blood vessels to pregnancy, and with the physiology of the uterus during labor.

Dr. Louis B. Flexner and his associates,

including Dr. Dean B. Cowie of the Department of Terrestrial Magnetism, made an important contribution to the question of the permeability of blood capillaries, showing that the whole of the capillary wall, not merely the intercellular cement substance, as some have thought, is available as the path for the diffusion of electrolytes from the blood into the tissues outside the capillaries. A striking finding from these studies is that the rate of passage of water through the capillary walls is very high. An amount of water equal to a man's entire weight passes out of his blood capillaries, and is replaced by an approximately equal amount, every 20 minutes.

At the Department of Genetics, additional experimental evidence has been obtained that the unit of heredity, a gene locus, may control several reactions, some of which may have several biological effects. Working with maize, Dr. McClintock by a special technique is able to bring an unstable gene into proximity with a stable gene locus and is thus enabled to analyze more fully the composition and action of the normal locus. One of the normal loci studied is shown by this analysis to be compound, in that it accounts for at least two reactions associated with the appearance of a single end product.

Dr. Demerec's studies of the resistance of bacteria to antibiotics such as streptomycin give another kind of evidence of the great complexity of the gene locus. What appears to be a single gene locus, it is indicated, controls a series of reactions. One change in this locus gives a mutant which is resistant to streptomycin; another change in the same locus produces a mutant which must have streptomycin in order to survive; still other changes produce still other mutants. The im-

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portance of this locus may be great indeed. If "nature strikes back" with bacteria which withstand the antibiotics developed by man as a defense against disease, nature apparently strikes back in one of its reactions to our interference by subtle changes in this minute spot. Different changes here control reactions giving biologically different end results. A study of reverse changes in this locus indicates that they are probably not brought about through a reversal of the original chemical reaction, but through some other change in the same gene locus.

Cancer, on which the massed attack of many sciences is leveled throughout the nation, is an elusive foe. We know little about its basic origins. It takes many forms. One of these is leukemia, the condition due to disarrangement of the blood-making organs which results in the formation of an excess of white cells in the blood. Leukemia in mice has been under study for years by Dr. MacDowell at the Department of Genetics, and he continues progress toward elucidation of the problem. During the report year, for example, his investigations have indicated that among mice the resistance to leukemia previously noted in the offspring of older mothers as compared with the offspring of younger mothers is closely paralleled when the offspring of young mothers are fed by older nurses. It thus appears that in mice the age of the nurse has as certain an influence after birth upon the incidence of spontaneous leukemia and upon length of life as has the age of the mother before birth.

The rodlike bodies called chromosomes in the nucleus of the cell are of extreme importance because of the fact that they are the carriers of genes, the units of heredity. Nearly infinitesimal—typically about seven millionths of an inch in diam-

eter—the chromosome threads are truly the network of vital inheritance. Naturally they are a center of scientific interest. Cytochemical studies at the Department of Genetics by Drs. Kaufmann and McDonald continuing during the year past have utilized purified enzymes in the effort to explain the organization of the chromosome. Successive treatments with nucleases and proteases are used by the investigators to dissect the chromosome. The investigation indicates that structurally the chromosome is an integrated fabric whose proteins constitute an interrelated system and whose nucleic acids are closely linked with the proteins and perhaps with one another. No single protein or nucleic acid, it appears, may be considered the basic structural component of the chromosome.

Studies of the prehistory of Middle America have recently been developing the fact that the early pre-Classic cultures of those regions were much more advanced than once had been thought. This knowledge is important in providing a proper understanding of the amazing burst of civilization that occurred in the succeeding Classic Period, a cultural advance that at one time had seemed almost autogenic in its suddenness and its apparent lack of capable ancestry. The Division of Historical Research has taken a leading part in the study of these early cultural horizons. The work of Mr. Shook has shown that the pre-Classic, sometimes called Archaic, cultures which existed in the Guatemala highlands about the beginning of the Christian era present a considerably more complex picture than had been suspected. Rather than a single cultural phase, it is clearly demonstrated, there were at least three sequent phases. Social organization already was developing the ceremonial and hierarchic, probably theocratic, pattern that was to become so char-

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acteristic of the Classic Period. Technology no longer was primitive. These people had a generous background of accomplishment. The primitive beginnings, the proto-pottery cultures, continue to elude discovery; and so long as that condition exists, one cannot disregard the possibility of distant and very early inter-American, or even trans-Pacific, influences.

Other progress of note was made in the solution of problems concerning the lowland Maya area. Dr. G. W. Brainerd's work in eastern Campeche gives promise of solving, or in part solving, a question of chronology that long has been a bone of contention. Although materials collected are still in process of analysis, it is

believed that relative chronology can be established for the previously undated Chenes-Rio Bee remains of Campeche and Puuc remains of Yucatan. Preliminary results suggest that the Campeche ruins are of Late Classic period, and that the Puuc remains are slightly later but in part Late Classic. This cross-dating by means of pottery will place the ruins of two important archaeological areas within the general framework of the native Maya calendar and should add to the understanding of other areas and later periods in Yucatan. In addition to its bearing on chronology, Dr. Brainerd's work has interesting possibilities relative to cultural contacts and influences between areas.

STAFF

It is a pleasure to report that distinguished recognition has come during the past year from various sources to members of the Institution's staff for their contributions to the advancement of knowledge, and to refer specifically to several among the many instances. The Commander's Cross of the Royal Order of Saint Olav was conferred by the King of Norway on September 7, 1948, on Dr. John A. Fleming, retired Director of the Department of Terrestrial Magnetism and now adviser to the Institution on international scientific relations. To Dr. George W. Morey, physical chemist of the Geophysical Laboratory, on November 12, 1948, was awarded the first Arthur L. Day Medal of the Geological Society of America. The medal, named in honor of Dr. Day, retired Director of the Geophysical Laboratory, came to Dr. Morey in recognition of his achievements in the

application of physics and chemistry to the solution of geological problems.

Dr. Ira S. Bowen, Director of the Mount Wilson and Palomar Observatories, is the recipient for 1949 of the Rumford Premium of the American Academy of Arts and Sciences. In the words of the citation, Dr. Bowen is thus honored "for his many contributions in physics and astrophysics, . . . including . . . the development of numerous devices for testing the 200-inch Palomar reflector, which is under his personal charge in these critical days of installation." The Cyrus B. Comstock Prize of the National Academy of Sciences for 1948 was awarded April 26, 1949, to Dr. Merle A. Tuve, Director of the Department of Terrestrial Magnetism, for his accomplishments ranging from pioneering work in the study of the upper atmosphere and in fields of nuclear physics to the development of the proximity fuze.

FINANCES

June 30, 1949 marked the close of the first complete fiscal year since the Institu-

tion's fiscal closing was changed from October 31 to June 30 by amendment of

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the By-Laws on December 12, 1947. During the fiscal year just ended, income available for operating purposes was substantially greater, approximately \$200,000, than for comparable periods ending in 1947 and 1948. This increase in income came about partly from increased investments in common stocks and partly from higher dividend declarations. The increase was anticipated to a certain extent and was taken into consideration in the preparation of the budget for the calendar year 1949, with the result that it was possible to increase the amount budgeted for 1949 substantially over the preceding year. This action has permitted allocation of

funds for projects which had been held under restraint because of lack of funds, and also made it possible to make some adjustments in staff salaries to bring the salary scale to a level comparable with those of leading educational and research agencies.

At the close of the fiscal period a year ago, it was necessary to transfer a moderate sum from reserves to supplement income collections for that period; it is now possible to report that at the close of the past fiscal year we were able to credit reserves with an amount in excess of the sum transferred a year ago.

FREDERIC C. WALCOTT

The death, on April 27, 1949, of Frederic C. Walcott, former Senator from Connecticut, took from the Institution a counselor of wide experience in affairs, long and thorough knowledge of the Institution's activities, and generous interest in its well-being. A member of the Board of Trustees since 1931, Senator Walcott served as a member of the Executive Committee of the Trustees from 1932 until his resignation in 1948. Manufacturer, banker, conservationist, and humanitarian, Sena-

tor Walcott did distinguished work in association with Herbert Hoover in relief efforts and food administration during the First World War. In the Senate of the United States he served as a member of the Committee on Banking, and was the principal author of the bill creating the Reconstruction Finance Corporation. He drew unsparingly on this wealth of experience in his association with the Institution, and we miss him greatly as a valued counselor and a loyal friend.

JAMES FORRESTAL

One of the most tragic casualties of the Second World War was the death, on May 22, 1949, of James Forrestal, first Secretary of Defense of the nation which he had served with devotion. Of his

selflessness, his dedication to duty, no additional word is needed. It was a matter of profound gratitude to the Institution that he had accepted election to the Board of Trustees in 1948.

REPORTS OF DEPARTMENTAL ACTIVITIES
AND CO-OPERATIVE STUDIES

ASTRONOMY

Mount Wilson and Palomar Observatories

TERRESTRIAL SCIENCES

*Geophysical Laboratory
Department of Terrestrial Magnetism
Special Projects*

BIOLOGICAL SCIENCES

*Division of Plant Biology
Department of Embryology
Department of Genetics*

HISTORICAL RESEARCH

*Division of Historical Research
Special Projects*

MOUNT WILSON AND PALOMAR OBSERVATORIES

OPERATED BY THE CARNEGIE INSTITUTION OF WASHINGTON
AND THE CALIFORNIA INSTITUTE OF TECHNOLOGY

Pasadena, California

IRA S. BOWEN, *Director*

OBSERVATORY COMMITTEE

IRA S. BOWEN, *Chairman*
WALTER S. ADAMS
ROBERT BACHER

EDWIN P. HUBBLE
MAX MASON
H. P. ROBERTSON

The current year was marked by the first actual use for scientific observations of both the 200-inch Hale telescope and the 48-inch schmidt camera. During much of the year extensive tests and adjustments of both instruments have been in progress. Though most of the features of these telescopes have performed remarkably well, some modifications, particularly of the 200-inch instrument, have been found necessary to attain the perfection of operation and performance that has been the continual aim in the design and construction.

Since the inception of the 200-inch project, it has been realized that the key to the success of the telescope lies in maintaining the exact optical shape of a mirror of this size in all orientations and often with rapidly changing temperatures. Thus, a simple dimensional analysis shows that the flexure of such a mirror under its own weight is proportional to the fourth power of the diameter of the mirror and inversely proportional to the square of its thickness. Obviously, if the standard astronomical practice of using a fixed ratio of mirror diameter to thickness (usually 8:1) is followed, the flexure under gravitational forces increases as the square of the aperture of the telescope. Because of the difficulties of carrying even the weight of such an 8 to 1 mirror on a moving telescope, it is impracticable to decrease this ratio of diameter to thickness appreciably. In order

to obtain the greatest possible stiffness within the weight limitations, a ribbed structure was adopted for the 200-inch mirror rather than the conventional solid disk. Nevertheless, actual measurements show that with a simple three-point support similar to that which is customary for very small mirrors the flexure would be 500 to 1000 times as great as the permissible value of a very few millionths of an inch.

Obviously, if distortion is to be eliminated as the telescope moves into various orientations, the support system for the mirror must be much more elaborate and precise than any hitherto devised. For this reason the mirror is supported at thirty-six points by units designed to balance the force of gravity on the section of the mirror assigned to each support by applying the correct components of force in all directions. As the orientation of the mirror changes, each of these force components must vary in such a way as to compensate the force of gravity with an accuracy of at least 0.1 to 0.2 per cent.

Similarly, distortions of a mirror caused by temperature changes incidental to actual operation increase rapidly with the dimensions of the mirror. Every effort was made to reduce these distortions by having the mirror cast of Pyrex glass, which has a coefficient of expansion only one-third as large as that of the glass used in most of the earlier astronomical mirrors. Further-

more, the ribbed structure of the 200-inch mirror has a marked advantage over a solid disk in this regard, since the rate at which thermal equilibrium is reached varies inversely as the square of the thickness of the glass. The maximum thickness of the ribs of the 200-inch is 4 inches, whereas a solid disk would have been 20 or more inches thick. Even with these advantages, thermal distortion is a serious problem for a mirror of this size, and every precaution must be taken to insure an equal flow of heat to all parts of the mirror.

Every effort was made to solve these problems by designing the mechanical parts properly; nevertheless, it has been realized that extensive tests and experiments would be required with the instrument itself before all factors could be adjusted to give optimum performance.

Last year's report outlined the results of the early tests of the mirror support system. These indicated that the friction in the original support system was so great that it would be practically impossible for the system to exert the correct supporting forces with the high precision necessary. During the summer of 1948 the lower part of each support unit was replaced with a new part especially designed to reduce the friction. Further tests of the mirror in October and November 1948 indicated a satisfactory reduction in the vertical friction from 1.3 per cent to 0.12 per cent.

During this same period studies were made of the behavior of the outer edge of the mirror as the zenith distance of the telescope was varied. This outer edge of the mirror overhangs the last row of supports from 10 to 20 inches, and concern had been felt as to the amount that this edge might droop when the mirror axis was changed from the horizontal position, in which all laboratory tests in Pasadena had been made, to the vertical. Contrary

to expectations in the early tests, this outer edge turned up more when the telescope was pointed near the zenith than when pointed near the horizon. Further studies, however, indicated that this change was accompanied by a shortening of the focus of the 200-inch mirror as the zenith distance was increased. By a careful analysis it was possible to show that the decrease in the amount of upturn of the edge with increasing zenith distance was a secondary effect on the ribbed structure of changing focal length. Furthermore, when the change of focal length was eliminated by an appropriate adjustment of the support system, the edge drooped as the telescope approached the zenith, but by an amount which did not cause a sensible deterioration of the image formed by the mirror.

After these problems had been solved, extensive tests were carried out to determine the effect on the mirror of sudden changes in temperature similar to changes that are occasionally encountered in actual observing. From these tests it was at once evident that the outer edge of the mirror was changing in response to a new temperature more rapidly than the central part of the back of the mirror. Thus when a sudden drop in temperature occurred, the edge contracted more rapidly than the back central zone of the mirror, with the result that the outer zones of the mirror were pulled back from their proper position by as much as 20 to 40 millionths of an inch. With rising temperatures the opposite effect occurred and the edge was raised. To speed up the changes in the back central zone, twelve fans were installed in the cell underneath the mirror in February 1949. These have partially corrected the situation, and it is anticipated that still further correction can be attained by properly insulating the outer edge of the mirror.

All tests of the 200-inch mirror, including those made in Pasadena prior to its transportation to the mountain, indicated that its outer edge was too high by amounts ranging up to 20 millionths of an inch. Because of the uncertainty as to how much this edge might droop when the telescope was placed in a vertical position, it was considered inadvisable to correct this outer zone until the mirror could be thoroughly studied in all orientations. This was particularly true since a turned-down edge, which would result if the correction were carried too far, would be much more difficult to rectify than a turned-up edge.

With the completion of the studies and modifications outlined above, it became possible to proceed with confidence with the removal of the proper amount of glass to correct this outer zone. The mirror was taken from the telescope in May 1949 and its original coat of aluminum removed. Polishing off the high zones was started in June with simple equipment designed for local figuring. The polishing is carried out on the observing floor of the dome with the mirror resting on the base of the aluminizing tank. After the removal of as much glass as seems advisable, the mirror is replaced in the telescope and tests of the figure are made using a star as a source. As might be expected, the survey of the whole surface of the mirror with the necessary accuracy of a very few millionths of an inch after each period of polishing is a much more time-consuming operation than the polishing itself.

Two types of tests are made. The first of these is the standard knife-edge test, which provides a qualitative survey of the deviations from a true paraboloid at all points of the surface. To provide a permanent record of the shape of the mirror at each stage of this final figuring process, a large number of photographs are taken of the knife-edge patterns. In general, eight

different angles of approach of the knife edge are studied, and at least four photographs representing different positions of the knife edge are taken for each direction of approach. The second type of test consists in quantitative surveys along twenty diameters across the mirror, made with a full-sized Hartmann screen having twenty holes per diameter.

Because of the closing of the California Institute Optical Shop, Dr. Anderson and Mr. Brown, who had so ably carried out the original grinding and figuring of the mirror, were not available for these final corrections. Mr. Hendrix, of the Mount Wilson Optical Shop, has therefore been in charge of this figuring.

Early in 1949 it seemed desirable to obtain checks on the results of the Hartmann and other tests of the mirror from a study of the performance of the telescope when in actual operation on a limited astronomical program. Consequently, during gaps in the testing program the telescope was used for a moderately extensive series of test photographs with apertures of 140 to 200 inches. Although confirming the fact that the outer 15 or 20 inches of the mirror were only partially effective, these photographs were on the whole remarkably successful. Many objects were clearly recorded that are well beyond the range of any other telescope. In fact, the theoretical gain over previous instruments was so nearly reached that there is every reason to believe that after the correction of the outer zone the telescope will fully come up to all expectations.

The optical parts of the 48-inch schmidt camera, including the very difficult non-spherical correcting plate, were completed by Mr. Hendrix, of the Mount Wilson Optical Shop, in the summer of 1948 and were installed in September of that year. After the usual adjustments and minor

modifications were made, the instrument was placed on a regular schedule of observations in January 1949.

The performance of this instrument has been remarkably fine and has in every way come up to the specifications for it. This camera normally uses plates 14 by 14 inches and therefore photographs about 40 square degrees of the sky at one exposure. The definition of the optical system is so superb that at all parts of this large plate the definition is limited by the resolving power of the photographic emulsion rather than by the optical system. Early tests have shown that a limiting magnitude of 20.3 is regularly reached. The mechanical and electrical parts of the telescope have likewise performed very satisfactorily.

In June 1949 announcement was made of an agreement providing for joint support by the National Geographic Society and the California Institute of Technology of a project for mapping, with the 48-inch schmidt camera, the whole sky visible from the Palomar Observatory. Each section of the sky will be photographed once in red light and once in blue light. Since approximately 1000 plates in each color will be required to cover the sky, it is anticipated that a period of four years will be necessary to complete the survey. This survey makes it possible for the first time to map the whole sky to a depth and in detail comparable with that reached by the large reflectors. In several decades of operation, these large reflectors with their limited fields have covered only one or two per cent of the sky; hence it is anticipated that complete coverage with the new schmidt camera will bring to light a great wealth of objects. These objects will provide material for detailed study with the 200-inch and 100-inch telescopes and with the large instruments of other observatories. In addition, the survey plates will

themselves provide important material for statistical studies of the distribution of many types of faint objects.

During the year rapid progress has been made in the design and construction of auxiliary equipment for the 200-inch Hale telescope. The base frame and many of the mountings for mirrors, plateholders, and gratings of the large coude spectrograph have been installed. This spectrograph will use a four-unit composite grating, designed to accommodate a beam from the collimator 12 inches in diameter. Schmidt cameras having focal lengths of 18, 36, 72, and 144 inches and giving dispersions of 18, 9, 4.6, and 2.3 angstroms per millimeter are to be provided.

As the construction period of the Palomar project has drawn to a close, extensive reorganization of the shops built for this project has been carried out. Since in the future only a small fraction of the capacity of the machine shop on the campus of the California Institute will be required for Observatory operations, it has been reorganized as a central machine shop for the use of all departments of the Institute. Though much of the Observatory construction will continue to be done at this shop, the reorganization will substantially decrease the fraction of the shop overhead to be carried by the Observatories. The optical shop ceased operations on May 1, 1949, with the completion of the last of the major optical parts for the Hale telescope. All future optical construction of the Mount Wilson and Palomar Observatories will be carried on at the Santa Barbara Street shop under the supervision of Mr. Don Hendrix. Mr. Melvin Johnson, of the California Institute shop, has been transferred to Santa Barbara Street to augment the original staff.

During the present year the location of the 200-inch dome has been determined

by the United States Geological Survey as follows:

Latitude: 33 degrees, 21 minutes, 22.41
seconds north
Longitude: 116 degrees, 51 minutes, 50.38
seconds west
or * 7 hours, 47 minutes, 27.359
seconds

The elevation of the observing floor of the 200-inch dome is 5598.5 feet above sea level.

For the past several years reports have been given of the progress of a search for planetary nebulae and early-type stars with emission lines, with a camera having a 10-inch red-corrected lens of the triplet type. This survey has now been completed for all parts of the Milky Way visible from Mount Wilson. During the present year this camera with its mounting and drive was loaned to the University of Michigan, which has transferred it to the Lamont-Hussey station at Bloemfontein, South Africa. At this station Mr. Karl Henize, of the University of Michigan, will use the instrument to complete the survey of planetary nebulae and emission-line stars in the southern part of the Milky Way that is invisible from northern latitudes.

Current investigations at the Mount Wilson and Palomar Observatories are continuing along the lines of the two broad programs that have characterized much of the work of the Mount Wilson Observatory for the past quarter century.

The first of these programs includes a series of investigations planned to extend our knowledge of the large-scale structures of the universe. Studies are first made of the distances, spatial arrangements, and motions of the stars in our own Milky Way system. Already it has been shown that this system is a huge disklike structure some 100,000 light-years in diameter* containing billions of stars and rotating

slowly about a central axis perpendicular to its plane. Similar investigations are then made of the structures, dimensions, distances, and internal motions of the nearer of the extragalactic nebulae, which are now known to be other stellar systems of the same general size and type as our own Milky Way system.

Finally, in another series of studies the spatial distribution and the motions of the more distant of these extragalactic nebulae are investigated. These latter studies are extended out to the greatest distance at which these huge systems can be photographed, and are concerned with such problems as whether the nebulae are uniformly distributed throughout observable space or whether a boundary is finally reached beyond which no further objects are found, thereby indicating a limit to occupied space. Earlier studies of the motions of these nebulae produced the result that all these objects appear to be receding with velocities proportional to their distances. This led to the concept of an expanding universe. The true significance of this phenomenon, however, awaits further velocity measurements on still more distant nebulae.

For the Hale telescope it is estimated that the limiting distance to which these investigations can be carried is one billion light-years, or twice as great as that reached by any previous instrument. In other words, this instrument will extend the boundaries of the observable universe to include a volume of space eight times as large as could previously be reached. Within this volume it is estimated that there are some hundreds of millions of these stellar systems.

Obviously the answers to the most fundamental problems of cosmology, including the structure and extent of the universe, the significance of the expanding universe, the possible curvature of space, and many

other similar problems, depend on further observations in this field. High hopes are held for the effectiveness of the Hale telescope in obtaining the solutions to many of these problems which depend on pushing out to the boundaries of the observable universe.

Much of the information about the structure of the Milky Way and the structure and spatial distribution of the extragalactic nebulae is obtained from a study of direct photographs of these objects taken with the telescopes used as cameras of long focal length and great light-gathering power. With the exception of a few of the closest stars, the distances of the objects are determined from a comparison of the absolute brightness or candle power of the object with its apparent brightness as estimated from a photograph or measured with a photoelectric cell. The facts necessary to assign a star to a given class of known absolute magnitude are found from a study of its spectrum or, if it is a variable star, from a determination of the period and form of its light-variation. Motions of near-by objects across the line of sight are measured by observing the change in position over a period of years; velocities toward or from the observer are readily fixed by a study of the displacement of the lines in the spectrum of the object.

The second program is concerned with the physical and chemical properties of individual stars and other single objects. Since, with the exception of our own sun, all stars appear as points even in the most powerful telescopes, such investigations are limited to a detailed analysis of the star's light by means of spectrographs. In the past century the spectra of all the standard stellar types, as well as those of most of the more unusual types such as variables and novae, have been studied and described with increasing detail as telescopes and spectrographs have become more

powerful. Thus the great light-gathering power of the 100-inch and 200-inch telescopes combined with recent advances in spectrograph design now make it possible to study the spectra of all naked-eye stars in as much detail as was possible for the sun a half century ago. Such spectroscopic studies continue to constitute a substantial part of the observational program of the Observatories.

With the development of theories of atomic structure in the past quarter century, it is rapidly becoming possible to utilize these spectroscopic observations to obtain a more quantitative understanding of stellar characteristics. Thus from measurements of both the positions and the intensities of the lines of a stellar spectrum one may obtain a quantitative chemical analysis of the stellar atmosphere. Similar measurements make it possible to fix the temperature, the pressure, and the strength of the magnetic field in the observable surface layers of the star's atmosphere. Study of the spectrum also throws light on the structure of the star's atmosphere and the circulation of gases in it. With the aid of theory it is possible to extrapolate the temperatures, pressures, and densities to the center of the star and to understand the flow of heat out from the central regions where it is generated.

Recent nuclear experiments and theories point definitely to the hypothesis that the energy which a star radiates in such tremendous quantities comes from nuclear transformations of one chemical element into another. These reactions are of the same general type that gives the atomic bomb its enormous power. Information concerning the central temperature and density of typical stars combined with a knowledge of chemical composition is necessary to determine which of the possible nuclear reactions are most effective in each stellar type. Quantitative determina-

tions of chemical composition also provide information as to the extent to which these nuclear fires have progressed in burning up the available supply of fuel. Eventually such studies may throw light on the origin of the chemical elements.

As new theoretical approaches begin to find solutions to these problems, they almost invariably raise additional problems that must be solved by further observations before the final answers are reached. In particular, recent studies of the types mentioned above are requiring quantitative measurements of the intensities of spectrum lines in addition to the measures of their positions which were provided by earlier observations. The Observatories have recently completed a new direct-intensity microphotometer which will very greatly speed up these quantitative intensity measurements.

Naturally all these spectroscopic studies of stellar atmospheres are checked where possible with detailed observations of surface features of the one star where they can be directly observed, namely, our own sun.

As in all scientific work, these broad programs are built up out of a large number of detailed investigations. It is only as the results of these highly specialized studies, carried out at many institutions, are brought together that the general broad pictures of our universe begin to emerge.

The following sections of this report outline the detailed programs that have been in progress during the current year. As-

tronomy is almost entirely an observational rather than an experimental science. For this reason, any observing program must take into account many factors over which the astronomer has no control. Thus, observations of any given object can be made only during the time of year at which the object is in the sky for a substantial part of the night. Particularly if long exposures are necessary, observations are therefore limited to a period of from two to four months each year. Several years may thus be required to accumulate the necessary observations on a particular object, and parallel studies of other objects must be planned to utilize the remainder of the observing periods during each year. Furthermore, many programs are concerned with changes in the spectra, in the brightness, or in the positions of objects. Many of these changes occur slowly and often may require years to complete a cycle. Again, occasional observations spread over long periods are necessary for these studies.

Because of these conditions it is customary and indeed necessary for effective operation that any given observer have a substantial number of programs in progress at any one time, many of which will require several years for completion. As a result, any record of the projects to which the members of the staff of the Observatories have devoted time in a given year is a long one, much longer than would be normal for a staff of the same size working on experimental problems.

OBSERVING CONDITIONS

The total precipitation for the season of 1948-1949 was 21.63 inches, which is the third lowest on record on Mount Wilson. This dry season following the record low rainfall of the preceding year has had a serious effect on the water supply of the mountain. By the application of moderate

restrictions on the use of water it has been possible, however, to maintain an adequate reserve in the reservoirs, and no serious difficulty is anticipated unless there are additional unusually dry years. The snowfall was 84 inches.

Solar observations were made on 312

days between July 1, 1948 and June 30, 1949. During this period the 60-inch telescope was used on 282 nights, the 100-inch on 263 nights.

SOLAR RESEARCH

SOLAR PHOTOGRAPHY

Solar observations were made by Hickox, Hoge, Nicholson, and Richardson. The numbers of photographs of various kinds were:

Direct photographs	612
<i>Ha</i> spectroheliograms of spot groups, 60-foot focus	972
<i>Ha</i> spectroheliograms, 18-foot focus	1,040
K2 spectroheliograms, 7-foot focus	41,680
K2 spectroheliograms, 18-foot focus	879
K prominences, 18-foot focus	1,098

SUNSPOT ACTIVITY

The magnetic classification and study of sunspots and related phenomena have been continued by Nicholson and Miss Whitney. Co-operative programs have been carried out with the United States Naval Observatory, the Observatory of Kodaikanal, the Department of Terrestrial Magnetism of the Carnegie Institution, and the Central Radio Propagation Laboratory of the National Bureau of Standards.

During the calendar year 1948, observations were made on 334 days, none of which was without spots. The total number of sunspot groups observed was 582, compared with 663 in 1947. The number of groups in the northern hemisphere decreased from 309 in 1947 to 271 in 1948; in the southern hemisphere, from 354 to 311.

The monthly means of the number of groups observed daily during the past two and one-half years are given in the accompanying table.

The number of sunspot groups observed daily has varied in characteristic short-

Month	Daily number		
	1947	1948	1949
January	9.9	10.6	8.7
February	10.5	8.1	13.6
March	11.8	8.0	14.7
April	11.5	14.4	12.1
May	16.8	12.7	10.2
June	13.4	12.2	9.6
July	12.9	11.4
August	14.1	12.4
September	13.7	10.8
October	12.4	10.7
November	10.8	9.2
December	10.1	10.2
Yearly average..	12.3	10.9

period fluctuations. Three marked maxima have occurred at intervals of 11 months, in May 1947, April 1948, and March 1949, with monthly means, respectively, of 16.8, 14.4, and 14.7 groups per day. The great activity of May 1947 and of the other months of that year marks the middle of 1947 as the maximum of the cycle.

SUNSPOT POLARITIES

Magnetic polarities in each spot group have, so far as possible, been observed at least once. The classification of groups observed between July 1, 1948 and June 30, 1949 is indicated in the table on the next page. "Regular" groups in the northern hemisphere are those in which the preceding spot has S (south-seeking) polarity and the following spot N polarity; in the southern hemisphere the polarities are reversed.

Hemisphere	Regular	Irregular	Unclassified
North	226	6	79
South	214	4	71
Whole sun—	440	10	150

FLARES

As a result of the discovery of apparent relations between solar flares and such phenomena as radio transmission, terrestrial magnetism, and cosmic rays, there has been a steadily increasing interest in data on flares and the spot groups associated with them. The automatic patrol program was amplified in March 1949 to take five times as many spectroheliograms as before. The photographs are now taken at the rate of one per minute in series of five exposures, each exposure with a different diaphragm over the collimator. These graded exposures serve as an intensity calibration, furnish a much finer time scale, and make the detection of faint flares more certain.

PROMINENCES

The eruptive prominence of June 3, 1949 was photographed by Hickox with the 13-foot spectroheliograph, and variations of its velocity with time were studied by Pettit. The prominence rose to a height of 870,000 km in 12 hours. The time-distance diagram gave successive velocities of 1.5, 7, 33, 85, 137, and 192 km/sec. Of these, the 15 and 33 km/sec velocities were very definite. The motion was somewhat erratic during the periods having average velocities of 7 and 192 km/sec and appeared to accelerate slowly during the periods having average velocities of 85 and 137 km/sec.

SOLAR GRANULATIONS

Spectra of the solar granulations have been taken at the center of the solar disk by Martin Schwarzschild, of Princeton University Observatory, and Robert S. Richardson, using the 425-mm image of the sun at the 150-foot tower. Differences in radial velocity are readily apparent from inspection on plates taken with exposures of 10 seconds during periods of fine seeing. Preliminary measures show the hotter material to be rising with respect to the cooler background with a relative velocity of about 0.2 km/sec.

THE H AND K LINES AND MAGNETIC STORMS

Richardson has remeasured the plates taken during the great magnetic storms of September 18, 1941 and March 1, 1942 to obtain improved data for a test of the suggestion by Chapman that a cloud of charged particles moving earthward during a magnetic storm might be detected by a faint absorption line shortward of the solar lines.

The intensities of 48 selected points in the continuous spectrum from A3888 to A4007 were compared on plates taken during magnetic calm and magnetic storm. For the great storm of September 18, 1941, three plates taken a few minutes apart 20 hours 30 minutes after the storm were measured. For the great storm of March 1, 1942, eight plates were measured taken from 15 hours before to 16 hours after the storm. Flares of intensity 3+ were observed about 20 hours before the beginnings of both storms.

Results were obtained in fair agreement with those published in 1944, which were based upon fewer plates and a different method of reduction. For the storm of September 18, 1941, apparent absorption bands dropping 0.06 below the normal

spectrum were observed to the violet of both H and K. For the storm of March i, 1942, apparent absorption bands dropping 0.03 below normal were observed. The bands indicate a range in velocity of inter-solar ionized calcium of from -600 to -1400 km/sec, although the uncertainties in these velocities are large.

If the absorption bands are real, the effect is barely above the limit of observation by photographic photometry. Even these improved measurements fail to furnish definite observational evidence for the assertion that magnetic storms are due to corpuscular emission from the sun.

GENERAL MAGNETIC FIELD OF THE SUN

Fifteen sets of visual measures of the sun's general magnetic field were made between October 1, 1948 and April i, 1949 by Nicholson and Hickox using the *Fe* line at 6173 Å. The individual sets gave values ranging from +43 to -28 gauss, reduced to the north pole of the sun, with a mean value of -2.3 ± 3.3 gauss. The

probable errors indicate that the range in the measures was accidental and not due to a varying field.

RELATION BETWEEN SOLAR AND TERRESTRIAL PHENOMENA

Nicholson and Dr. Oliver Wulf, of the U. S. Weather Bureau, have extended their studies of the relation of bright solar flocculi to geomagnetic activity, previously reported for 1943 and 1944, to include the years 1941 and 1942. They have compared geomagnetic activity with the declination of the moon over 294 lunar cycles (1925-1946) and have found a tendency for activity to be highest near and following the northerly and southerly maxima of declination, when the lunar air tide is asymmetric with respect to the earth's axis of rotation and to the average air circulation. These and other observations give further evidence that the circulation of the upper atmosphere plays a role in the production of recurrent geomagnetic activity.

STELLAR INVESTIGATIONS

LIGHT-CURVES OF NOVAE

The visual brightness of Nova Puppis 1942 as measured by Pettit with the wedge photometer has now fallen to mag. 11.7. A plot of all the measures since November 10, 1942 on a logarithmic time scale shows a general drop from maximum light with some long-period fluctuations of a few tenths of a magnitude. From Sanford's measures of the distance to the nova and the expansion velocity of the shell it can be estimated that the present diameter of the expanding shell is about $1V2$. Since the minimum disk detectable under ordinary circumstances is probably about 2" in diameter, the shell cannot be expected to be visible before 1952.

The visual brightness of T Coronae Borealis fell gradually from the secondary maximum of mag. 8.0 in the summer of 1946 with some small fluctuations to mag. 9.8 during the year July 1947 to July 1948. It then brightened and is now about mag. 9.6. Slow oscillations of this kind were observed in 1867 at the same interval, 1238 days after primary maximum, and in the same magnitude range. It appears that the light-curve has behaved like the 1866 curve in considerable detail.

SPECTRAL ENERGY-CURVES OF STARS

Pettit has investigated the adaptability of a panchromatic photomultiplier tube to the measurement of spectral energy-curves

of stars. For this purpose the tube in the regular nebular photometer mounting was attached to a Bausch and Lomb quartz monochromator at the Newtonian focus of the 60-inch telescope. Deflections at hundred-angstrom intervals of the spectrum were taken between A7000 and X3000 on a Leonis and ν Virginis. When properly reduced, these measures yielded spectral energy-curves outside the earth's atmosphere.

The energy-curve of ν Virginis, mag. 4.2, type Mi, shows a general drop in the visible spectrum and a rapid drop from A4500 to A3900, after which it is about constant at about one-tenth the intensity at A5500. The curve for a Leonis, mag. 1.34, type B8, rises from about one-eighth maximum intensity at A6000 to a maximum at A4800, drops rapidly to about half this value at A3700, then rises throughout the ultraviolet.

STELLAR SPECTROSCOPY

RADIAL VELOCITIES

Completion of the older Observatory radial-velocity programs was delayed, mainly by unfavorable weather conditions during the winter months, when most of the remaining observations must be made. About 65 plates of 53 stars are still needed.

During the report year, 411 plates were taken with the 60-inch telescope on these and the supplementary radial-velocity programs, 319 by R. E. Wilson. In addition, 676 plates were obtained with the 60-inch in connection with special investigations by various observers. Measures of the plates taken on the regular programs are up to date.

Radial velocities have been completed for about 2000 stars. The results together with revised spectral classifications are being prepared for publication by R. E. Wilson and A. H. Joy.

The card file for a general catalogue of radial velocities has been kept up to date; a study has been made of systematic differences between the various observatories engaged in radial-velocity determinations; and the format of the catalogue has been discussed and adopted.

E. R. Dyer, Jr., Carnegie Fellow, who is on a two-year leave from the University of Virginia, is measuring the radial velocities of dwarf M stars selected from objective-

prism plates by Vyssotsky and others at the Leander McCormick Observatory by means of spectrophotometric criteria. It is expected that the inclusion of these stars will give a solution for space velocity less affected by selection than previous material. These stars have visual magnitudes from 8.9 to 11.5 and are distributed over the sky north of -20° declination. From October 1948 to June 1949 slit spectrograms of no A/mm dispersion have been obtained for 148 stars, mostly with the 60-inch reflector, and about half of these have been measured. Eight new stars with Balmer emission have been found, and more than 40 stars with bright H and K lines of *Can*.

The spectral types and radial velocities of 90 dwarf stars of late type which have been under observation for parallax at the McCormick Observatory were published by Joy and Mitchell.

VARIABLE STARS

Spectrograms of 34 of the high-luminosity variable stars of globular clusters were obtained by Joy. By use of the spectral characteristics and the periods it is possible to determine the type of variation of the star. A tabulation indicates that with a few exceptions the cluster variables belong to the RR Lyrae, W Virginis, RV Tauri,

and semiregular (periods 60 to no days) classes. The radial velocities yielded by the spectrograms serve to identify the stars with the clusters as well as to help determine the velocities of the clusters.

Forty-four coudé spectrograms of cepheid variables and spectroscopic binary stars have been obtained by Sanford for the determination of radial velocities. The spectrum of W Virginis, a cepheid of stellar population II, was found to have double absorption lines for the first couple of days after light-maximum.

Further studies have been made by Sanford of the radial velocities of RR Lyrae from high-dispersion spectrograms. The absorption lines of hydrogen and of H and K were found to become double in each cycle for a short time near median magnitude on the rising branch of the light-curve.

Joy has obtained additional spectrograms of RR Lyrae stars, bringing the number of unpublished stars to 60.

Sanford has continued his investigation of the recurrent nova T Coronae Borealis. Spectrograms obtained in January and February 1949 are essentially similar to the one obtained in May 1948. The study of earlier plates has shown a velocity variation of the lines of the class M spectrum. This variation follows a sine-curve with a semi-amplitude of 21 km/sec and a period of 230.5 days. No velocity variation is shown by the source of the emission lines; hence the assumption that T Coronae Borealis is a binary is beset with difficulty.

Spectrograms of Nova Cygni 1948 were obtained by Sanford, Mowbray, and Joy in June 1948. Emission and absorption lines characteristic of the phases shortly following maximum were present. Velocities of the expanding shells were 560 and 1270 km/sec. A spectrogram obtained on July 1, 1949 showed an advanced nebular stage with weak continuous spectrum and

strong emission lines of H, *Hen*, Nni, [Ne m], and [Oin].

STARS WITH EMISSION LINES

The Mount Wilson survey of the northern Milky Way for the detection of stars whose spectra have bright hydrogen lines was completed during the past year. This survey, begun by Merrill in 1919, combined the advantage of (1) objective-prism photography with the wide-angle 10-inch telescope with (2) the use of the strong red *Ha* line. Recent photographs have been made with a lens especially designed for red and yellow light; they are far superior to the early plates. Many observers have taken part in the program, the principal ones having been P. W. Merrill, M. L. Humason, W. C. Miller, and A. G. Mowbray. A very large part of the assessment of plates and preparation of catalogues has been done by Miss Cora G. Burwell. The third section of the general catalogue, now ready for printing, brings the number of Be stars to 1088. Of these, about 800 have been detected at Mount Wilson. Awaiting future listing are about 600 additional bright-line objects discovered at Mount Wilson whose spectral types have not been determined; but it is reasonably certain that most of them are of class B.

The spectra of most of the brighter objects discovered in the survey have been photographed again, in more detail, with slit spectrographs attached to the 100-inch or the 60-inch telescope. Several stars have become the subjects of extensive investigation.

Shell stars offer special inducements to detailed study because many of them have extremely active outer atmospheres. About 35 are now known (not counting P Cygni stars or those with combination spectra), of which only a few have been adequately observed. In several stars (HD45910,

187399, 218393) studied during the year by Merrill, the hydrogen lines undergo striking changes in structure and displacement from plate to plate. Of special interest in certain stars is the progression in radial velocity from line to line along the Balmer series. Studies have been continued of the long-period damped oscillations in 48 Librae and in HD33232. In DM—27⁰ 11944 an outflow of gas at the rate of 300 km/sec has continued since 1921. It is hoped eventually to obtain reliable interpretations of the remarkable velocities and accelerations which occur in the extended atmospheres of these objects.

O. C. Wilson has nearly completed the observational part of the spectrographic survey of the northern Wolf-Rayet stars. Three stars with variable displacements presumably due to orbital motion are HD177230 and 197406 and BD + 40°4220. Another star, HD50896, shows large variations both in displacement and in shape of some of the emission bands, but it is not yet known whether these effects can be attributed to orbital motion.

An orbit for the Wolf-Rayet binary HD190918 has been completed and published. The velocity variation is relatively small, and if this fact is attributed to small inclination of the orbit, the results show that the observed red shift of A4686 He 11 in such stars is probably independent of the direction from which they are viewed.

A complete list of the stars whose spectra are known to have H and K (*Can*) in emission was prepared by Joy and R. E. Wilson. The intensities of the emission lines were estimated from Mount Wilson spectrograms. Of a total of 445 stars, there are 37 supergiants, 93 giants, 15 subgiants, and 277 dwarfs. Calcium emission evidently occurs frequently among stars of various kinds, but is largely limited to the cooler atmospheres of stars of spectral types later than G5. In the giants and super-

giants the emission lines are divided by an absorption reversal.

ABUNDANCES OF CHEMICAL ELEMENTS

Greenstein is continuing his investigation of abundance, pressure, temperature, and turbulence in stellar atmospheres. Additional studies have been made of F stars, and a detailed comparison of the spectra of giant, subgiant, and dwarf G stars has been started with the highest resolution available. These F and G stars are sufficiently like the sun to provide fairly direct comparisons when detailed studies are made with high dispersion. A preliminary investigation of the subdwarf Groombridge 1830 reveals large differences from a normal dwarf, due to the higher pressure and the higher opacity in the subdwarf.

Two stars with apparent peculiar abundances of certain elements are also under investigation. The carbon-rich, hydrogen-poor star R Coronae Borealis normally shows a metallic-line spectrum remarkably close to that of a late F-type supergiant, the C₂ bands, and a few as yet unidentified features. A detailed program of measurement and identification of the lines in the helium-rich, hydrogen-poor star υ Sagittarii is in progress; high-dispersion spectra are available from A3200 to A6600. In the red, weak emission of *Nai*, *Feu* is observed, besides the strong absorption of *Ne* 1, *N*1, and the ionized metals. The variation of line intensities around the radial-velocity orbit has been studied by Dr. Adams and found to be small.

GENERAL MAGNETIC FIELD OF EARLY-TYPE STARS

The search for and analysis of stars showing evidence of strong magnetic fields has been continued by H. W. Babcock. Construction and use of a new optical

phase-shifter have permitted effective extension of the observing program to stars north of the equator. This phase-shifter, or compensator, largely overcomes the limitation imposed by the oblique reflection from the coudé flat of the 100-inch telescope. One result of this extension is the discovery that the well known bright spectrum variable α^2 Canum Venaticorum has a strong magnetic field, and that the field not only varies, but reverses its apparent polarity in a manner rather similar to that previously found for HD125248. The magnetic variations are very probably synchronous with the 5.5-day period of line-intensity variations. This observation is significant for the interpretation of the spectrum of α^2 Canum Venaticorum, which has been almost intractable heretofore.

Two other stars, HD10783 and HD 153882, have been found to be spectrum variables of type A, and to possess strong magnetic fields that reverse their apparent polarity. Periods have not yet been determined. Considerable evidence on magnetic fields in other stars has been accumulated.

A number of additional spectrograms of HD125248 have been obtained; together with the plates of last year they make a valuable collection, well distributed over the 9-day period of spectral and magnetic variation. Measurement of these plates, with the assistance of Miss Burd, is now well advanced. Tentative results show that elements of the three groups characterized by the rare earths, iron, and chromium are generally consistent among themselves, but that at certain phases systematic differences appear among the three groups in radial velocity and in indicated polar magnetic field strength. Additional data are being assembled.

The elementary theory of magnetic intensification of stellar absorption lines in

the presence of a general magnetic field has been developed during the year. It is shown that the maximum intensification factor for a saturated line in the presence of a strong field is n/i , where n is the number of components in the Zeeman pattern. Conditions favoring the effect include high atomic weight, moderate temperature, and, of course, a Zeeman pattern of many widespread components. Many lines of *Eu* 11 and of *Cr* 1 are particularly sensitive to magnetic intensification. Evidence for the presence of the effect in α^2 Canum Venaticorum is given in the form of a comparison of theoretical intensification factors with variations in line intensity observed by Struve and Swings. It is apparent that many phenomena observed in spectrum variables still require explanation.

Microphotometer tracings of the spectrum of the white dwarf 40 Eridani B obtained with the analyzer show no evidence of polarization. It is concluded that the great widths of the hydrogen lines in the spectrum cannot reasonably be accounted for by Paschen-Back broadening, as suggested by Blackett.

MISCELLANEOUS

The near-by dwarf star L 726-8 was observed by Joy and Humason at the request of W. J. Luyten. At the time of the first spectroscopic observation with the 100-inch telescope, August 25, 1948, the star was found to be a visual binary. The spectra of both components were estimated as $dM5.5e$. Measurements of a direct photograph showed a separation of $1''.5$ in position angle 117° . At the time of a second spectroscopic observation, September 25, 1948, one of the components, which could not be identified because of poor seeing, increased its brightness noticeably. The spectrogram showed that the outburst pro-

duced a strong continuous spectrum which nearly blotted out the normal M-type spectrum, the emission lines of hydrogen being strengthened. The line of ionized helium at $\lambda 4686$ appeared in emission. This type of spectral activity had previously been found by Joy in the T Tauri variables and in the bright-line stars involved in the Taurus clouds.

Among the dwarf stars of lowest luminosity, seven were observed whose spectra were dhfy or later but without emission lines. No subdwarf characteristics were seen.

The intensity distribution of the continuous spectrum of the well known subdwarf Wolf 489 was estimated to be about that of a G₀ star. No lines could be distinguished in the spectrum.

Sanford has shown that the radial velocities derived from the interstellar Ca II lines in the stars in open clusters, as well as the stellar velocities, manifest differential galac-

tic rotation. Furthermore, there is evidence that both equivalent widths and velocities from the interstellar lines have merit as criteria of distance.

Additional spectrograms of stars of standard spectral types have been obtained. One set, dispersion at $H\gamma$ 35 Å/mm, was obtained largely by A. H. Joy.

Microphotometer tracings of the widened lines in the spectrum of the G-type star HD 17555 indicate a rotational speed at the equator of at least 75 km/sec. This observation by Merrill would indicate that hydrogen atoms thrown out from the equatorial zone may be responsible for the bright $H\alpha$ line.

Zwicky, Johnson, and A. G. Wilson have completed the coverage of the Milky Way both with direct photographs and with objective prism on the 18-inch schmidt telescope. This provides the base comparison plates for an extensive search for novae in the Milky Way.

GALACTIC NEBULAE AND STAR CLOUDS

PLANETARY NEBULAE

The search for planetary nebulae by Minkowski has led to the discovery of 34 new planetaries during the past year. The total number of planetaries discovered on the 10-inch objective-prism plates is 194. Objective-prism exposures with the 18-inch schmidt have to date led to the identification of 20 new planetaries; a considerable number of objects found with the 18-inch schmidt still remain to be investigated. Of the total of 370 planetaries now known, 114, or almost one-third, appear within 10° of the galactic center. This demonstrates the high concentration of planetaries toward the center of the Galaxy. It is hoped to obtain information on the kinematics of the central region of the Galaxy from an investigation of the radial velocities of planetaries; such an investi-

gation has been started, but no results are as yet available.

The survey of internal motions in the brighter planetary nebulae with the coude spectrograph has now been completed by O. C. Wilson. In general, relatively large velocities of expansion of the nebular shell are found. Except in the case of hydrogen, larger velocities are more normally associated with high-excitation particles than with low-excitation particles. Moreover, slitless spectrograms show that $[N\text{v}]$, which always gives a smaller than average velocity of expansion, also always gives an image slightly smaller than hydrogen, whereas lines of $[O\text{ II}]$ and $[N\text{ III}]$ tend to give slightly larger slitless images. These facts suggest that radiation pressure, arising from radiation produced within the bright rings, is responsible for the observed

effects. Such considerations have led to a somewhat different picture of the structure and dynamics of planetary nebulae from that now current.

NOVA HERCULIS

Plates taken by Baade in the summer of 1948 at the Newtonian focus of the 100-inch telescope show no marked changes in the structure of the ejected shell within the past four years. Images of A3727 [On] and A4959, 5007 [O in] are elliptical rings with weak condensations at the ends of the major axis. The image of *Ha* A6548, 6584 shows the same remarkable structure noted in 1942.

VARIABLES IN THE NUCLEAR REGION OF THE GALACTIC SYSTEM

The observations of the field around the globular cluster NGC 6522 ($A=328^\circ$, ($3=-4^0$) were concluded by Baade with extended series of plates taken from July to September 1948. Continued search for variables has brought their number to 284 for the field investigated, or to 750 variables per square degree. Magnitudes for the variables so far found have been estimated by Dr. Sergei Gaposchkin, of Harvard College Observatory. The derivation of the light-curves, the joint work of Dr. Gaposchkin and Dr. Cecilia Payne-Gaposchkin, is completed for more than half the material. The results confirm the earlier surmise of Baade that the stars of the nuclear region of our galaxy belong to population II. Roughly 50 per cent of the variables are cluster-type variables of large amplitude with very asymmetrical light-curves and periods of less than 0.4 days. The semiregular variables come next in frequency.

During the present year observations of

a new field of the nuclear region centered on CD-28°14334 ($X=332^\circ$, $\xi=-6.5^\circ$) were begun. In contrast with the field centered on NGC 6522, which shows some irregularities caused by absorption, the new field is exceedingly uniform.

STAR CLUSTER WITHIN THE ORION NEBULA

The large star cluster in the Orion nebula recently discovered by Baade, using plates sensitive to the A6900-7500 region, has been searched for variables. A comparison of two pairs of plates led to the discovery of 50 new objects of this type.

GALACTIC STRUCTURE

With the 48-inch schmidt camera the central region of our galaxy was covered by Baade with red exposures, the exposure times ranging from 1 hour to 3 hours. A search on these plates led to the discovery of a few new globular clusters. All are rather close to the galactic equator (the line of greatest obscuration) and are exceedingly faint.

Baade is making an attempt to single out the near-by spiral arms of our own galaxy, using the highly luminous B and O stars and the emission nebulosities which from studies of the Andromeda nebula are known to occur only in these arms. Because of heavy obscuration, it appears simplest to search for the emission nebulae in *Ha* light. This search will be followed by the identification of exciting stars, and the determination of their spectral types, absolute magnitudes, apparent magnitudes, and color excesses from which distances can be calculated. As a first step in this program, the Milky Way was covered along the galactic equator from $A=310^\circ$ to $A=90^\circ$ with red exposures of 1 hour's duration with the 48-inch schmidt camera.

EXTRAGALACTIC NEBULAE

NEBULAR PHOTOMETRY

The program of photometry of the extragalactic nebulae for which Humason has derived velocities has been continued by Pettit with the 1P21 photomultiplier cell, principally with the 60-inch telescope. Magnitudes on the photographic scale and color indices have been obtained for each object. Of over 500 systems, approximately 70 remain to be measured. Many of the remaining systems are large and require measurement of numerous galactic stars. Some systems will have to be measured with the 20-inch telescope.

Of the 175 nebulae measured by Stebbins and Whitford, 22 have been remeasured, one-half of which are indicated by them as being of doubtful accuracy. The average departure regardless of sign is 0.1 mag.

RADIAL VELOCITIES

Final revisions of measured red shifts and spectral types for over 500 nebulae have been completed and are now being prepared for publication by Humason.

Two spectrograms of NGC 4151 have been obtained with the coudé spectrograph (10.3 A/mm) by O. C. Wilson. The emission bands have been measured for wave length on one plate, with the result that AX/A appears to be constant to within about 1 per cent from A3400 to A6600. The second spectrogram is somewhat stronger and shows unmistakable structure in some of the emission bands; no analysis of the structure has yet been attempted.

SURVEY OF THE ANDROMEDA NEBULA

The 100-inch survey of the Andromeda nebula with ultraviolet, blue, red, and near infrared exposures, covering an area 1°5 by 5°0, was practically completed by Baade during the present season. Among the

final results will be a complete list of all emission nebulosities brighter than -3^M (some 300) and a similar number of star clusters to about the same limit.

MEMBERS OF THE LOCAL GROUP

Intercomparisons with Selected Area 68 were made by Baade for both NGC 6822 and the Andromeda nebula, since there exists some uncertainty regarding the magnitude of the brightest stars in these systems. Long exposures of the Andromeda nebula with the 48-inch Schmidt in the visual and red regions revealed what appears to be a tidal effect in its elliptical companion, NGC 205. Besides its two axes due to projection, this nebula shows a third axis which is directed toward the center of the Andromeda nebula. This third axis or bar extends in either direction beyond the projected disk of NGC 205.

DISTRIBUTION OF NEBULAE

A substantial part of the first photographs taken by Hubble with the 200-inch Hale telescope and by Hubble, Baade, and Zwicky with the 48-inch schmidt camera were devoted to exploratory investigation of the distribution of extragalactic nebulae in space. Hubble, with the assistance of A. R. Sandage, is studying on some of these plates the distribution of faint nebulae in standard apparently normal regions of the sky.

Almost a dozen distant clusters of nebulae were found in an area of 300 square degrees in Virgo by Baade, and several additional clusters were picked up by Zwicky, especially in the Corona Borealis region. A number of clusters discovered on plates taken with the 48-inch schmidt telescope were checked on plates taken by Baade with the 100-inch. It is obvious from these tests that the few

clusters of nebulae previously known represented a very meager sample indeed, and that the rich material to be expected from the 48-inch schmidt survey will greatly facilitate all investigations in which one must fall back on clusters of nebulae (velocity-distance relations, etc.).

Zwicky has used the 48-inch schmidt plates to study the luminosity function of nebulae, particularly at the low-luminosity end of the distribution-curve. An additional dwarf system was found which presumably is within the perimeter of the local group, and many dwarf systems were picked up which belong to other groups of nebulae. As a limit of dwarf systems, the suspected existence of intergalactic matter was confirmed through some preliminary investigations of the central regions of the Coma cluster, where luminous intergalactic matter seems to be recorded on the 48-inch schmidt plates over a region about one-half million light-years in diameter.

The work on the constitution of clusters of nebulae with the 18-inch schmidt was continued by Zwicky and was extended to the 48-inch schmidt for the Cancer cluster and the Coma cluster. The studies made originally with the 18-inch were corroborated; in particular, it was found that bright and faint nebulae segregate in the sense of heavy and light masses in a Boltzmann assembly.

An area 18° by 18° covering the Virgo cluster was photographed by Baade. The plate material will permit an accurate classification of all members of this cluster, since the scale of the 48-inch schmidt (1 mm = 67 seconds of arc) is large enough for study of the detailed structure of the nebulae.

Plates taken by Baade in connection with a study of the group of nebulae associated with M 81 and 101 revealed a third dwarf member associated with the physical pair NGC 5236 and 5253.

THEORETICAL STUDIES

The so-called galactic radio noise has been studied theoretically by Greenstein. Since it may originate in thermal agitation in interstellar clouds of ionized hydrogen, an attempt has been made to determine the effective optical thickness of the ionized regions in our galaxy. This appears to be small except along the spiral arms, and galactic radio noise, if produced in interstellar space, should come only from the spiral arms.

With Professor Leverett Davis, Greenstein has developed a theoretical explanation of the recently observed polarization of light transmitted through interstellar clouds of absorbing dust particles. If the particles are not spherical but elongated, as most crystalline materials are, they absorb light preferentially of a plane of

polarization perpendicular to their long axes. To produce observable polarization, the long axes should remain nearly parallel over long regions in space. This orderliness is disrupted by the bombardment of the particles by the interstellar hydrogen. At $10,000^\circ$ K, an average collision with hydrogen produces an angular velocity of the particle of 10^6 radians per second. Thus only at very low temperatures (10° K) will there be orderliness unless a decelerating mechanism is found. If the particles have some ferromagnetism, their rotation in a galactic magnetic field will dissipate energy and slow them down, by hysteresis. A field of 10^{16} gauss is required. Recent theoretical progress suggests that perhaps the particles may continue to spin and nutate about the magnetic fields, with

a statistical preference for the long axis to lie perpendicular to the field. A much smaller field, with smaller energy loss, is then required. The existence of a magnetic

field, either general or associated with the interstellar clouds, is of significance in recent theories of the production and trapping of cosmic rays.

INSTRUMENTATION

The program for ruling improved gratings for spectrographs of the 200-inch Hale telescope and other instruments has been actively pushed by Prall under the supervision of H. D. Babcock until February 1, 1949, and of H. W. Babcock since that date. Between April and December 1948, five large gratings (6 by $7\frac{1}{2}$ inches) of excellent resolving power were made, and during this period the advantages of the graphite-on-diamond end-thrust bearing for the screw were proved. In 1949, three additional 6 by $7\frac{1}{4}$ -inch gratings have been ruled, as well as one slightly smaller. There has been a progressive rise in the quality of the gratings as small residual sources of error have been reduced or eliminated. Periodic error has been reduced to such a level that Rowland ghosts have intensities of less than 0.5 per cent in the sixth order, with normal slit widths. Ghosts are not seen in useful orders of the latest gratings. The periodic error has

been practically eliminated by the use of a cam which introduces a compensating periodic displacement of the grooves having an amplitude of 3×10^{-7} inch. One remaining problem is connected with proper lubrication of the ways of the diamond carriage when long grooves are ruled; this seems to represent the one serious obstacle to the production of gratings of high quality.

The integrating exposure meter for the coude spectrograph has been rebuilt by H. W. Babcock on a new design. It is superior to the old one and has proved useful in routine work for several months.

A low-dispersion grating spectrograph for use in nebular studies at the Newtonian focus of the 100-inch telescope was designed by Nichols and Minkowski and constructed in the Observatory shop. It uses one of the gratings recently ruled by Prall and Babcock.

GUEST INVESTIGATORS

In addition to the permanent staff, a substantial number of guest investigators from other institutions have made use of the facilities of the Observatories to obtain material for their studies. Plates already in our files have been used, and additional observations have been obtained with the large telescopes.

Dr. Lyman Spitzer, Jr., of the Princeton University Observatory, continued his investigations of interstellar absorption lines during the fall of 1948. About one hundred of Adams* high-dispersion plates showing

interstellar K and H were microphotometered to obtain equivalent widths of these lines and their components. New plates of some 20 stars were taken in the yellow, in a search for components of the interstellar D lines. About half of these showed complex structure, with components at the same radial velocities found by Adams in K and H. In addition, a search was made for interstellar *U* and *Be* lines, with negative results. If the abundance of *Be* relative to *Na* is the same in interstellar matter as in the earth and in meteorites, this cl-

ment should be about at the threshold of measurement with the 32-inch camera used.

In the summer of 1948 Dr. A. E. Whitford, of Washburn Observatory, University of Wisconsin, extended the previous study of colors of remote extragalactic nebulae to include spirals in the Corona Borealis cluster. In the small sample available, late-type spirals do not show the excess reddening found in elliptical nebulae in the same cluster. This observation supports Schwarzschild's suggestion that near-by elliptical nebulae have become bluer in the past hundred million years, owing to the burning out of the red supergiants. The photoelectric calibration of magnitudes and colors of stars in Selected Areas 57 and 61, begun by Dr. Joel Stebbins in 1947, was continued, and work started on Selected Area 68. The study of interstellar reddening out to 2 microns, begun in 1947, was extended to several additional highly reddened B stars, using an improved lead sulfide photometer with refrigeration.

Dr. John C. Duncan, of Whittin Observatory, Wellesley College, continued his photographic studies of nebulae. Negatives were obtained with the 18-inch schmidt camera and red-sensitive films which showed much more extensive nebulosities in the Cygnus region than had previously been observed.

During the summer of 1948 Dr. George Herbig, on leave from the Lick Observatory as a National Research Fellow, made a study, on coude plates, of the occurrence of the titanium isotopes in M-type stars in which the titanium oxide bands appeared in adequate strength. A precise determination of the abundance ratios was not feasible owing to the photometric and other difficulties inherent in quantitative work with such heavily lined spectra, and to the present impossibility of identifying all the

TiO lines which were confused with the weak isotope features. The results indicate that, in the stars examined, and within the rather large uncertainty of the observations, there are no large deviations from the isotopic constitution of terrestrial titanium. Certainly there is no enhancement of the relative abundance of any of the rarer titanium isotopes in the M-type stars such as is apparently observed for C^{13} in some carbon stars.

A rather strong molecular absorption band with head at A_{3682} was found by Merrill and Joy in the spectra of a number of long-period variables. Herbig identified this band with *ZrO* by comparison with a spectrogram of the zirconium arc in air taken by Dr. R. B. King. The rotational structure of the band on the laboratory plate is that to be expected of an electronic transition with $\Delta A=0$; a ${}^1Z \rightarrow {}^*Z$ transition seems most probable. If this is the case, this band is probably the (0,0) head of a new system of *ZrO*.

The "channeled" appearance of the *H δ* line in Omicron Ceti was shown to be due largely to absorption by rotational structure of the (2,0) and (3,1) bands of the blue-green system of titanium oxide.

In two visits to the Observatories in 1948 and 1949, Dr. Sergei Gaposchkin, of the Harvard College Observatory, carried out an extensive investigation of variables in the vicinity of the galactic nucleus around the globular cluster NGC 6522. Estimates were made of the brightness of several hundred variables, previously discovered by Baade on over 100 plates of this region. The classification of the variables on the basis of these measures was used for a general discussion of the problem of the population of the galactic nucleus. In addition, some colorimetric studies of the globular clusters NGC 6522 and 6528 and a spectroscopic investigation of a few in-

dividual stars such as SX Cassiopeiae and AE Aquarii were carried out.

During the summer of 1948 Dr. Cecilia Payne-Gaposchkin, of the Harvard College Observatory, examined and classified over 2000 spectra of cepheid variables, cluster-type variables (RR Lyrae stars), and RV Tauri stars, from the Observatories' files. For some stars (especially on spectra of higher dispersion) the intensities of selected lines were estimated by eye in lieu of classification. The material obtained from this examination is being used for the formation of mean spectral and velocity curves of these stars as a function of their period, and for an analysis of the atmospheric and physical conditions of the stars.

Dr. Daniel M. Popper and Dr. Everett C.

Yowell, of the University of California at Los Angeles, have carried out spectroscopic observations of a group of eclipsing binaries.

A study of the intensities of the lines of C, AT, O, Ne, Mg, Al, Si, and S has been undertaken for early B stars by Dr. Lawrence H. Aller, of the University of Michigan, using coudé plates from the Mount Wilson Observatory files. The survey includes nearly all objects with sharp lines between O and B5. These intensities are being used for a determination of the abundances of these light elements.

Mr. W. C. Miller and Dr. A. G. Mowbray, both of Pasadena, have made further important contributions to the Be star program by obtaining many slit spectrograms with the large telescopes.

THE LIBRARIES

During the year 1948-1949 the library on Santa Barbara Street has acquired 562 volumes, 276 by gift, 103 by purchase, and 183 by binding, making a total of 16,892 volumes. Volumes from the Hale collection still form a large proportion of the gifts, but a number have come from other sources, including a selection from Dr. Pease's library, presented by the executor of his estate. His copy of Smith's *Optics*, 1738, is an excellent addition to the collection in the Hale Room.

About 500 volumes have been selected from Dr. Hale's library to send to Palomar Mountain, for the book room of the 200-inch Hale telescope. Some of these are duplicates of astronomical books already in both the Santa Barbara Street and Robinson Laboratory libraries; others, of a more

general scientific character, will furnish good reading during inclement weather.

To the 4000 volumes in the library at Robinson Laboratory, 387 volumes have been added during the year: 141 by purchase, 22 by binding, and 224 gifts. Among the latter, 85 volumes came from Mrs. Richard C. Tolman as a memorial to Dr. Tolman, 24 from the Bateman library, and 82 from the Hale, Pease, and van Maanen collections. Although not yet complete, particularly in reference works, this "working library" began in the fall of 1948 to serve the new department of astronomy of the California Institute of Technology and those staff members of the Mount Wilson and Palomar Observatories whose offices are in Robinson Laboratory.

STAFF AND ORGANIZATION

Dr. Alfred H. Joy retired on October 1, 1948 after thirty-three years as a member of the staff of the Observatory. After first

participating in the solar program of the Observatory, Dr. Joy gradually shifted his efforts to stellar spectroscopy. He col-

laborated extensively with Dr. Adams in the study of the effect of absolute magnitude on stellar spectra and in the use of these relationships for the determination of stellar parallaxes. In recent years Dr. Joy has investigated the spectra of a large number of variable stars. These studies have very substantially advanced our knowledge of the classification and characteristics of this difficult group of objects. Radial-velocity measurements of these stars have been applied by Dr. Joy to studies of galactic rotation and similar problems.

From 1920 until his retirement Dr. Joy held the post of Secretary of the Observatory. In this position he very ably handled most of the official correspondence. He was also responsible for the public relations of the Observatory, including arrangements for visitors, press releases, and the preparation of official literature. Mr. Milton Humason was appointed Secretary of the Observatory on Dr. Joy's retirement.

Miss Cora Burwell retired on July 1, 1949 after forty-two years of service on the computing staff of the Observatory. In addition to assisting in many investigations in the field of stellar spectroscopy, she was co-author of the very extensive *Mount Wilson catalogue of B- and A-type stars having emission lines*.

Mr. Edison Hoge transferred to the Hydrodynamics Laboratory of the California Institute of Technology in March 1949. For twelve years Mr. Hoge had been in charge of the photographic laboratory of the Observatory and had assisted in the observations of the solar department. Mr. William Miller was appointed photographer and will devote his whole time to the photographic laboratory. Simultaneously much of the routine photographic finishing work of the Observatory was transferred to the photographic laboratory of the California Institute. With these

changes it is expected that the photographer will be in a position to carry out regular tests of the speed and color sensitivity of each batch of plates used by the Observatories. He will also investigate methods of hypersensitization and other procedures designed to obtain the maximum effectiveness of the plates when used for the long exposures that are necessary in most astronomical investigations.

RESEARCH DIVISION

Solar Physics: Seth B. Nicholson, Edison R. Hoge, Edison Pettit, Robert S. Richardson, Joseph O. Hickox, Irene Whitney.

Stellar Spectroscopy and Motions: Paul W. Merrill, Horace W. Babcock, Ira S. Bowen, Jesse L. Greenstein, Milton L. Humason, Alfred H. Joy, Rudolph Minkowski, Roscoe F. Sanford, Olin C. Wilson, Ralph E. Wilson, Fritz Zwicky, Sylvia Burd, Cora G. Burwell, Mary F. Coffeen, A. Louise Lowen, Barbara Olsen.

Nebular Photography, Photometry, and Spectroscopy: Edwin P. Hubble, Walter Baade, Milton L. Humason, Josef J. Johnson, Rudolph Minkowski, Edison Pettit, Albert G. Wilson, Fritz Zwicky, Alice S. Beach.

Secretary of the Observatory: Milton L. Humason

Editor: Paul W. Merrill

Assistant Editor and Librarian: Elizabeth Connor

Photographer: William C. Miller

INSTRUMENT DESIGN AND CONSTRUCTION

Design: Bruce Rule, project engineer; Edgar C. Nichols, chief designer; Harold S. Kinney, draftsman.

Optical Shop: Don O. Hendrix, superintendent; Floyd Day, Melvin Johnson, opticians.

Instrument Shop: Albert McIntire, superintendent; Elmer Prall, instrument maker; Fred Scherff, Oscar Swanson, Albert Labrow, Donald Yeager, machinists.

MAINTENANCE AND OPERATION

Mount Wilson Observatory and Offices

Office: Anne McConnell, bookkeeper; Wilma Berkebile, secretary; Dorothea Otto, stenographer and telephone operator.

Operation: Ashel N. Beebe, superintendent of construction; Hugh Couch, carpenter; Kenneth de Huff, engineer; Murdoch McKenzie, janitor and relief engineer; Thomas A. Nelson, Ralph Bennewitz, Eugene Hancock, night assistants; Emerson W. Hartong, truck driver and machinist helper; Anthony Wausnock, Margie Wausnock, Alexander Kochanski, stewards; Arnold T. Ratzlaff, Homer N. Joy, janitors.

Palomar Observatory and Robinson Laboratory

Office: Eleanor G. Crawford, secretary and librarian; Dorothea Davis, secretary.

Operation: Byron Hill, superintendent, Palomar Observatory; Fred Feryan, Gladys M. Feryan, Harley C. Marshall, George W. Pettit, Joe Stehlik, Benjamin B. Traxler, Gus Weber, Raymond L. White.¹

¹ The Palomar Observatory is still in a transition stage from construction to operation. In most cases the final positions in the operating organization have not been assigned at the time of this report

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GEOPHYSICAL LABORATORY

Washington, District of Columbia

LEASON H. ADAMS, *Director*

Research may be likened to the exploration of a series of small fields spread in a broad pattern over the landscape and traversed by a network of paths along one of which the investigator chooses to proceed, pausing now and then to observe, to ponder, and to experiment. He seeks to understand all that he perceives; and if after a while he is satisfied with what he has learned in one particular field, he passes on to another, sometimes following along the original path and sometimes along an intersecting one. Steadily he pushes forward from that which is known into that which is unknown and, therefore, mysterious. As he acquires experience and confidence, he is encouraged to enter areas of greater complexity and to attack more formidable problems.

Usually the true scientist finds the greatest satisfaction in extending the limits of knowledge and selecting paths that lead into new fields of inquiry, but it may happen that in undertaking step by step the solution of one of nature's broad-gauge problems, he finds that the path he must follow to success will turn back toward its starting point. Two striking examples of the way in which fruitful research may curve back on itself after many years are furnished by the Geophysical Laboratory's current program in experimental petrology. Although this is a forward-looking program and advances are being made into hitherto unexplored fields, it has seemed desirable also to re-examine two old subjects because of an increasing realization of their place as key factors for the unraveling of some mysteries concerning the formation of the rocks of the earth's crust.

These two items are the minerals feldspar and quartz.

Probably the most important group of rock-forming minerals consists of the feldspars, which are aluminosilicates containing other oxides, principally lime, soda, and potash. The first paper published from the Laboratory (1905), entitled *The isomorphism and thermal properties of the feldspars*, is a monograph now generally considered to be one of the great classics of petrology and to have laid the foundation for the subsequent program of silicate research. Day and Allen gave the first proof that the interrelationships and genesis of minerals may be studied with success by quantitative laboratory methods. Their work still evokes the admiration of the most critical, and it was done the hard way. Improved experimental methods, especially the quenching techniques and the automatic control of temperatures, were developed later, but these investigators had to make use of the method of heating curves in its most difficult application, and they were obliged to regulate temperatures laboriously by hand for long periods of time.

After the quenching method was developed, several years later, it was applied to the feldspars with resulting refinement of the original work. Feldspars are found as mineral phases in many of the silicate "systems" that have been studied at the Geophysical Laboratory, and there has been a strange lack of correlation between the synthetic feldspars and the natural ones. Pure feldspars made in the laboratory show an unbroken series of solid solutions, but careful determination of the

crystal structure of natural feldspars reveals that the two end members of the plagioclase series, albite and anorthite, have certain differences of lattice structure which seem to forbid belief that they could form a joint lattice with continuous range in composition from end to end. Also, in the laboratory the soda feldspar, albite, and the potash feldspar, orthoclase, form complete solid solutions; but in deep-seated rocks, where the concentration of potent volatile fluxes may be greater by reason of greater pressure, and where the temperature of crystallization is lower, albite containing very little potash and potash feldspar with only moderate amounts of soda are found side by side. Moreover, artificial crystalline albite does not have properties corresponding to those ordinarily given for natural albite. The field evidence, then, has seemed to run counter to the laboratory evidence.

The reason for this seeming lack of desired correlation between natural and artificial feldspars is becoming evident from the new work, made possible on the one hand by improved methods of subjecting materials to the simultaneous action of high temperature and high pressure in the presence of water, and on the other hand by the commercial development of new types of X-ray spectrometers. The recent investigations have shown that the difference between natural albite, as usually found, and artificial albite is that the natural albite is a low-temperature form. This dual character is exhibited by other plagioclases as well as by albite, and is especially marked in feldspars close to albite in composition.

Furthermore, the peculiarities in the alkali feldspars are now seen to be a consequence of an unmixing at lower temperature of the continuous series of solid solutions that may form at high temperatures. The extent of this unmixing depends on

the temperature; and, by a special technique the essence of which is to measure very precisely one of the crystal spacings, it is possible to determine rapidly and easily the compositions of the feldspars formed at any temperature. The situation with respect to the plagioclase feldspars has not yet been cleared up completely, but the indications are that by means of the new methods it will be possible to determine the temperature of formation of any pair of feldspars associated in a single rock.

Thus, it may be seen that the return to one facet of an old problem, after improved apparatus and techniques became available and after further field evidence was obtained, has led to a major advance in our knowledge of the minerals of which igneous rocks are composed. Previous discrepancies are being removed and a possible method for determining the temperature of mineral formation has resulted.

A second area of early exploration to which the orderly prosecution of silicate research has impelled us to return is the fundamental nature of the mineral quartz. This and other forms of silica have been subjected to study from the early days of the Geophysical Laboratory. In 1906 Day and Shepherd described a method for making clear and bubble-free silica glass by fusing quartz crystals under pressure—a method that subsequently was adopted by industry. The use of quartz as a geologic thermometer was suggested in 1909; Fennel's classic work on the various forms of silica and their mutual relations appeared in 1913; and the effect of pressure on the high-low inversion of quartz was studied in 1928.

The transformation of quartz at 573° C. from one crystalline form to another is one of the important characteristics of this mineral. Up to the present it has been considered to be a fixed point, like the melting point of pure Ice, independent of

the origin and previous history of any particular sample. Recent work with improved apparatus, details of which are presented in a later section, has cast doubt on the immutable character of this transformation. Indeed, parts of the same crystal may show transformation temperatures that differ by a large fraction of one degree, and individual samples of quartz may vary by nearly 2° . It is not yet possible to give a complete explanation of the

variations found in natural quartz, but enough has already been learned to justify the conclusion that the variations in properties of quartz from different localities will provide a new geological thermometer for indicating the temperature and the general conditions prevailing in rocks with which quartz is associated.

The following is an account of the principal investigation carried forward during the past year.

ANHYDROUS SILICATES

METHOD OF STUDY OF COMPLEX MIXTURES

Knowledge of the melting relations of anhydrous silicate mixtures is of fundamental importance in the application of physical-chemical methods to problems of rock formation. Much of the past work of the Laboratory has been on dry melts, from which has come information of basic interest not only in connection with the Laboratory's problems, but also in connection with those of industry. This work has progressed from the simple to the complex, the complexities arising almost entirely from the difficulty of comprehending and explaining melting relations in systems of several components.

In studying these multicomponent systems, it is our practice to separate the various systems into smaller parts, each of which can be treated as a unit. One object of this is to simplify both the theoretical discussion and the practical work. For example, the ternary system nepheline—diopside—silica, discussed below, is part of the quinary system $\text{CaO—MgO—Na}_2\text{O—Al}_2\text{O}_3\text{—SiO}_2$; but it is a part which can be studied and discussed as a unit. Another reason for the separation of a complex system into simpler parts is that we are thus able to approach our objective by a series of steps each of which may be con-

sidered a research that is complete in itself. Although a full knowledge of all the interrelations of all the rock-forming constituents is a goal to be attained in the far-distant future, we advance toward the goal in such a way that our progress may be described in terms of completed projects.

This method of subdividing a complex system is illustrated by current work on the four-component system $\text{K}_2\text{O—MgO—Al}_2\text{O}_3\text{—SiO}_2$. Here the compositions are represented graphically by means of a tetrahedron, but, to minimize the necessity for trying to think in terms of three-dimensional space, the system is separated into smaller parts by treating each group of three compositions as end members of a triangular "join." This is equivalent to passing a plane through these three points in the tetrahedron. Such a join may be a true ternary system, by which is meant that, when any mixture of the three end members is melted and crystallized, all compounds and compositions remain within the triangle. An example of this situation is the join leucite—forsterite—silica, discussed in last year's report, on which additional measurements have been carried out.

This condition is not found in the join leucite—clinoenstatite—potassium tetrasilicate, also discussed last year, on which im-

portant progress has been made. During crystallization of mixtures in this join, the compounds $2\text{MgO} \cdot \text{SiO}_2$ and $\text{K}_2\text{O} \cdot 5\text{MgO} \cdot 2\text{SiO}_2$ are formed, and, since these are outside the plane under consideration, the resulting residual liquid also must be outside, and on the opposite side, of the join. In spite of such complications, the study and discussion of the quaternary system is always greatly facilitated by separating it into smaller portions. Much progress has been made on the joins mentioned in last year's report and on some additional ones.

THE JOIN JADEITE—ACMITE IN THE
QUATERNARY SYSTEM Na_2O —
 Al_2O_3 — Fe_2O_3 — SiO_2

Substantial progress has been made on the study of other anhydrous systems. Some of this has been motivated, in part at least, by the puzzling problem of the mineral jadeite ($\text{Na}_2\text{O} \cdot \text{Al}_2\text{O}_3 \cdot \text{SiCfe}$). The stability relations of jadeite are not known, and it never has been made in the laboratory. Previous studies have shown that natural jadeite on being heated begins to decompose as low as 800°C . and that at about 850° it melts to a clear glass which later crystallizes to nepheline and albite.

Since some of the natural pyroxenes are solid solutions of jadeite and its ferric iron analogue, acmite ($\text{Na}_2\text{O} \cdot \text{Fe}_2\text{O}_3 \cdot 4\text{SiO}_2$), it was believed that a study of the join jadeite—acmite would be desirable and might clarify the stability relations of jadeite. During the past year nine compositions between jadeite and acmite have been prepared, and these are now being studied. For those compositions between 100 per cent acmite and 20 per cent acmite, the primary phase is hematite or a hematite-corundum solid solution. Between 100 per cent and 80 per cent jadeite, the primary phase is a nepheline-albite solid solution. As a consequence, during crystal-

lization of all melts in the join jadeite—acmite, the composition of the liquid phase leaves the join, and its behavior may be described only by reference to the quaternary system Na_2O — Al_2O_3 — Fe_2O_3 — SiO_2 . For those compositions in the join between 100 per cent acmite and 48 per cent acmite, the primary phase (hematite or a hematite-corundum solid solution) is next joined by acmite (or an acmitic pyroxene) below liquidus temperatures. For those between 52 per cent and 80 per cent jadeite, the second solid phase to appear is a nepheline-albite solid solution; and for those between 80 per cent and 100 per cent jadeite, the second solid phase to appear is a hematite-corundum solid solution. At subliquidus temperatures, and especially where only a small amount of a liquid phase is present, equilibrium between crystals and liquid is attained only slowly, and some necessary runs of long duration are now in progress. The fine-grained crystalline phases present at the completion of a run are being identified by their X-ray diffraction patterns.

THE JOIN JADEITE—DIOPSIDE AND COMPOSITIONS
IN THE JOIN NEPHELINE—
DIOPSIDE—SILICA

Some years ago fifty separate compositions in the triangular join nepheline—diopside—silica were prepared, and from time to time as the opportunity occurred the liquidus relations in this join were determined. This triangular join is not ternary and is a plane in the quinary system CaO — MgO — Na_2O — Al_2O_3 — SiO_2 . Some of the fifty compositions prepared were chosen to lie in the joins albite—diopside and jadeite—diopside. The results (which have not yet been published) show that albite—diopside is not a binary system and that between pure albite and 90.5 per cent albite the primary phase is not pure albite, but an albite-rich plagi-

clase. The join jadeite—diopside cuts the fields of nepheline-albite solid solutions, plagioclase, and diopside. The observation that those compositions in this join which lie in the diopside field do not reach the boundary surface plagioclase-diopside at the same temperature indicates that the diopside is not pure $\text{CaO} \cdot \text{MgO} \cdot 2\text{SiO}_2$, but a more complex pyroxene.

During the past year the subliquidus relations in the join jadeite—diopside have been investigated in greater detail, and the solid phases have been identified by means of X-rays. No jadeite has been encountered at the temperatures investigated. Those compositions in the triangular join nepheline—diopside—silica near the side line nepheline-diopside have been completely crystallized, and a study of the solid phases is now in progress. These studies should throw light on the mutual stability relations between nepheline, forsterite, diopside, melilite, and plagioclase.

THE QUATERNARY SYSTEM MAGNESIA—
FERROUS OXIDE—ALUMINA—SILICA

During the past year some further data were obtained on subliquidus relations in the join FeO-Mg cordierite—Fe cordierite. Primary phases encountered in the join are only three: mullite, spinel, and wüstite. Cordierite is obtained only as a subliquidus phase in the mullite field and in the low-iron part of the spinel field in mixtures containing up to about 40 per cent FeO. At around 50 to 60 per cent FeO in the spinel field, a pyroxene is obtained as a subliquidus phase. Charges containing more than 60 per cent FeO, still in the spinel field, are opaque on quenching because of rapid growth of dendrites of fayalite and possibly wüstite below the liquidus, with the result that it is very difficult to establish the liquidus temperature.

It is planned to continue the laboratory study by completing two additional joins: Mg cordierite—clinoenstatite, and Mg cordierite— SiO_2 -FeO. Both these planes should intersect the cordierite phase volume and assist in outlining the stability relations within the tetrahedron, besides supplying valuable data on the cordierite solid solution series.

Some investigation was made of the factors that influence the attainment of equilibrium between metallic iron (of the crucible) and iron-bearing silicate melts. As a result of previous work it is known that in a neutral atmosphere an equilibrium condition is reached between the metallic iron and the ferrous and ferric iron in a melt. In the course of attaining this equilibrium (minimum of ferric iron), there is a consequent change in the total iron content of charges, the composition changing along a line that on the diagram may be drawn through the FeO corner and the point representing the initial composition. It was found that the amount of change of total iron content of a charge depends primarily upon the total available oxygen in the surrounding gas. Charges heated above the liquidus in an iron crucible sealed in evacuated silica glass tubes for periods up to 14 hours show only slight change (maximum 2 to 3 per cent) in total iron content. On the other hand, charges heated for 2 hours in an atmosphere of purified nitrogen change by an amount which varies from 5 to 15 per cent, or more, dependent upon the partial pressure of oxygen in the nitrogen. The previously used technique for purifying the nitrogen supply was improved by passing the tank nitrogen through cleaned steel wool at 700° to 800° and by reducing the resultant oxide coating on the steel wool with a slow stream of hydrogen fed through overnight between runs.

STUDIES ON THE INVERSION CHARACTERISTICS
OF QUARTZ

Preliminary studies on the inversion temperature of quartz (see last year's report) indicated that the temperature of the high-low inversion of quartz varies by almost 2° in different samples. This variation is apparently related to the geologic environment in which the quartz was found, and there are grounds for believing that with sufficient information on the behavior of a variety of samples of natural and synthetic quartz it will be possible to make a direct correlation between the inversion characteristics and the conditions of formation.

A new method has been developed for studying the inversion and specifically for measuring the magnitude of the heat effect, the temperature at which the inversion begins, and the temperature range through which the transition takes place.

The present apparatus for determining the inversion characteristics involves the use of a silver sample block with four chambers in which are placed crushed and sized samples of the quartz, cryolite (Na_3AlF_6), arkanite (K_2SO_4), and granular alumina. Cryolite and arkanite have inversions at 562° and 584° , respectively, and give reference temperatures for the quartz inversion, which is at 573° . Thermoelements of gold palladium-platinum rhodium are placed in each of the four materials and connected in such a manner that no electromotive force is generated when all four materials are at the same temperature. If, on cooling or heating, any of the materials absorbs or gives off heat, its temperature will be temporarily above or below that of the others, and an emf will be produced, which deflects a sensitive galvanometer. The galvanometer deflec-

tion is recorded on a Brown strip chart recorder by means of a light spot reflected from the galvanometer mirror and falling on a divided photocell connected so as to form a "light-follower" circuit.

Preliminary runs with the apparatus showed that the inversion temperature of a quartz sample can be bracketed between the cryolite and arkanite inversions with an error of repetition of less than 0.05° C. The preliminary and calibration runs also show that some quartz samples differ from others not only in temperature of beginning and duration of inversion, but also in the magnitude of the thermal effect accompanying inversion and in the amount of hysteresis between heating inversion and cooling inversion. All quartz samples studied also exhibit an as yet unexplained difference between the temperature of beginning of inversion in the initial heating run and the temperature of beginning of inversion in subsequent runs.

One particularly interesting quartz specimen from Minas Geraes, Brazil, is a perfectly clear and colorless crystal the basal part of which was formerly used as the reference quartz. Upon exposure to X-rays, sections of the crystal are selectively colored and exhibit three distinctly separate growth zones. The inner and outer zones of this crystal have inversion temperatures which differ by from 0.6° to 0.8° , the greater difference being obtained on cooling. It appears that a further detailed study of even this one crystal may furnish a valuable clue to the relation between inversion temperature and structure and composition, and measurements on quartz samples prepared in the laboratory under controlled conditions may provide quantitative relationships for making quartz a reliable geological thermometer.

SILICATES IN THE PRESENCE OF WATER UNDER PRESSURE

Fundamental though they are to geology, the results of studies of dry systems cannot be applied directly to many geological problems without some knowledge of the effect of volatile components, especially water, in altering the phase-equilibrium relations. Water is an essential constituent of rock magmas, and its presence in magmatic systems raises many problems both theoretical and experimental. The techniques that have been developed will carry us a long way toward the solution of such problems. The positive control of pressure obtained by the use of a pump with a pressure regulator to inject water into the pressure vessel has increased both the ease of manipulation and the certainty of results and has eliminated the doubtful calculation of pressure by means of equations of state known not to be applicable. Two successful types of pressure vessel are in daily use; both offer possibilities for almost unlimited investigation in fields that are just being opened up.

FELDSPARS

Among rock-forming minerals the feldspars are of the highest importance. In the igneous rocks of the accessible crust of the earth they far outweigh all other minerals in abundance; in the metamorphic rocks they are but little less important; in sedimentary rocks they occur not merely as detrital grains derived from other rocks, but also as new, authigenic growths. It is only natural that the feldspars have been much studied and that more is known about them than about any other rock-forming mineral group. As is ever true in scientific investigation, the knowledge gained serves to reveal and to emphasize what there is yet to learn.

Studies are now being carried out that are designed to throw further light on the

conditions under which feldspars develop. The work consists principally of laboratory experiments in which feldspars are grown under a wide range of measured pressures and temperatures, followed by determination of the properties of the feldspars and the manner of the variation of these properties with the controlled conditions of their growth, and this again by careful measurement and comparison of the properties of the synthetic feldspars with those of natural feldspars of widely variant geologic environment. By reason of the ubiquitous character of natural feldspars, it is expected that quantitative knowledge of the conditions of formation of a wide range of natural rocks will thus be greatly extended and refined. Significant progress has been made, some details of which are now presented.

There are three principal feldspars: potash feldspar (KAlSi_3O_8), soda feldspar ($\text{NaAlSi}_3\text{O}_8$), and lime feldspar ($\text{CaAl}_2\text{Si}_2\text{O}_8$). No natural feldspar ever has the composition indicated by any one of these chemical formulas. They are always mixtures of the three, not mechanical mixtures but true solid solutions, or "mixed crystals." In the language of the theory of phase equilibrium, these chemical compounds are the components of the solid solutions; or in the language of crystal-structure theory, K, Na, and Ca (also Si and Al) can proxy for each other in the crystal lattice within certain limitations which are variable and whose variation is controlled by the conditions of formation of the feldspar—and, of course, by the composition of the medium (solution or melt) from which the crystals formed.

The components of the feldspars are also conveniently referred to by mineralogists as end members; and mineral names are given to these end members^ the soda

feldspar being known as albite, the lime feldspar as anorthite, and potash feldspar as orthoclase or microcline depending on its crystal form. Pure albite, anorthite, and orthoclase can be made in the laboratory, but, as has been noted, when formed in nature each of them contains some of the others. Certain occurrences of albite are known in which there is a total of little more than 1 per cent of anorthite and orthoclase. Usually, however, significant or even notable amounts of all three components enter into the composition of the natural crystals, though the potash component is always low when the lime component is high.

We may now state in more specific terms the special problems posed by the feldspars. Igneous and other rocks contain feldspars that appear to show every gradation of composition between albite and anorthite; and these two components have, therefore, been regarded as forming an unbroken series of solid solutions known as the plagioclases. Plagioclase is the principal constituent of the most common lava flows, the basalts. Early studies at this Laboratory of the melting temperatures of pure synthetic mixtures of these components seemed to confirm the view that an unbroken series exists. On the other hand, examination of natural feldspars by the new and powerful methods of X-ray diffraction shows that anorthite and albite have differences in atomic arrangement that seem to preclude the possibility of continuous and complete substitution of one end member for the other. It is possible that at high temperatures the lattices are such that unbroken gradation of composition can occur, but that at the ordinary temperatures at which X-ray studies have been made this situation no longer prevails. The solution of this particular feldspar problem is important because, if the plagioclases represent an unbroken series,

the composition of a plagioclase occurring in any rock will depend only upon the composition of the medium from which it is formed; if, on the other hand, the series has a hiatus at some temperatures, then the composition of a plagioclase will depend as well upon the temperature at which it is formed. It thus might prove possible to determine the temperature of formation of any plagioclase.

Over against the great subgroup of lime-soda feldspars known as the plagioclases stand the other subgroup commonly referred to as the alkali feldspars. These are potash feldspar, occurring in nature in two principal forms, orthoclase and microcline, and the soda feldspar, albite. Albite is thus a member of both subgroups. The sodic end member of the plagioclases is sufficiently like potash feldspar in mode of genesis to warrant grouping them together. Not only that, but these two feldspars also form solid solutions, that is, homogeneous crystals which are intermediate between them in composition. These alkali feldspar solid solutions show much more complicated relations than the plagioclase series have usually been regarded as showing.

Crystals of alkali feldspars, when formed as early crystals in lavas and, therefore, presumably grown at high temperatures, may show a wide range of mutual solid solution of the potash and soda components. On the other hand, when grown in deep-seated rocks, probably in the presence of volatile materials held in by the greater pressure, with consequent lowering of the temperature of crystallization, the soda and potash feldspars in nearly pure form occur side by side as separate crystals. In natural rocks the indications are, therefore, strong that in this series of feldspars the extent of solid solution is a function of temperature.

In order to determine the quantitative aspects of the relation thus qualitatively

stated, studies have been made on the behavior of mixtures of various compositions between $\text{NaAlSi}_3\text{O}_8$ and KAlSi_3O_8 in the presence of water vapor, which is the principal natural volatile flux, at pressures up to 2000 atmospheres and at temperatures up to those at which the mixtures are completely liquid. In the language of phase-equilibrium theory, we have investigated the liquidus, solidus, and subsolidus relations in the system $\text{NaAlSi}_3\text{O}_8$ — KAlSi_3O_8 — H_2O at various pressures. The synthetic mixtures approach closely the composition of the natural volcanic rock trachyte and its deep-seated equivalent syenite, since these rocks consist essentially of alkali feldspars.

A necessary, or at least a most desirable, preliminary to the study of any system having water as one of the components is a study of the system without water, in this case the system $\text{NaAlSi}_3\text{O}_8$ — KAlSi_3O_8 . Fortunately this work had previously been done, and in addition we were fortunate in having many mixtures available for our studies with water, for the mere making of the mixtures is in itself no mean task. Since liquids of these compositions are extremely viscous, attainment of a homogeneous liquid requires long periods of special treatment.

The extreme viscosity of the liquids also introduces great difficulties in the attainment of equilibrium between crystals and liquid, but these have been overcome by increasing the time factor, and a satisfactory equilibrium diagram for the system without water (the dry system, as it is called for convenience) is available. This diagram gives only the liquidus relations, that is, the temperature of completion of melting of various mixtures. The solidus relations, that is, the temperatures of beginning of melting, were not determinable in the dry way. Although not altogether conclusive, the relations found in the dry

way strongly suggest that KAlSi_3O_8 and $\text{NaAlSi}_3\text{O}_8$ form a complete series of solid solutions at high temperatures, of the type with a minimum melting temperature, complicated only by the incongruent melting of compositions rich in the potash component.

With the mixtures and the information available from previous work, a study of the behavior with water was undertaken. For this purpose, the Tuttle apparatus (last year's report) has proved very convenient, and in the detailed study of the crystalline phases the Norelco X-ray spectrometer with Geiger counter attachment has been of inestimable value. As was known from previous work, the recalcitrant behavior of alkali feldspar compositions examined in the dry way largely disappears in the presence of water vapor under pressure.

The homogeneous glasses of intermediate composition crystallized readily under hydrothermal conditions, and it was quickly established that the two components do indeed form an unbroken series of solid solutions or mixed crystals at high temperatures. Examination of the crystalline products in the X-ray spectrometer established this fact, and preliminary studies of optical properties indicate that there is a continuous change in these properties with composition. The X-ray powder spectra of the crystalline end members are very similar, but close examination reveals differences, one notable difference being the position of a rather prominent peak (maximum of intensity of X-ray reflection) on the spectrometer chart. The peak has a position corresponding to a 2θ value of about 22° for the soda end member and of about 21° for the potash end member. By reference to the single-crystal studies of W. H. Taylor this peak is identified as due to the [201] reflection, and its position is, of course, determined by the [201] spacing. Careful determinations of the position of

this peak were made for the end members and for 13 intermediate compositions by measuring the intensity of reflection as determined by the total count on the Geiger counter during a 5-minute period, at successive fixed positions spaced only 0.05° apart, all measurements being referred to a constant standard having a peak close to 23° . It was thus found that, for compositions crystallized at 900° and 300 atmospheres pressure of water vapor, the position of the peak changes continuously from end to end of the series, and that the variation with composition is sensibly linear. Since it was found that measurements on the same material could be duplicated within 0.02° and the total variation is about 1° , this must be regarded as a very satisfactory demonstration of the linear variation of the [201] spacing in an unbroken series of solid solutions.

Conversely, by measuring the position of this peak, the composition of any feldspar of the series can be determined within an error of 2 per cent in proportions of the end members, which is at least as high accuracy as can be obtained in silicate chemical analysis of high grade.

Starting with a series of compositions crystallized as indicated above and proved to be homogeneous mixed crystals, we have proceeded to determine the solidus and liquidus relations at two isobars, 1000 atmospheres and 2000 atmospheres. It was established beyond question that at both isobars the series is of the type showing a minimum-melting composition, and at both pressures this composition is 70 per cent NaAlSi₃O₈. The ability to determine the composition of crystals by the position of the [201] peak on the X-ray spectrometer was here of the greatest value, for in mixtures quenched from any temperature within their melting interval, and thus consisting of crystals and glass, it was still possible to determine the composition of

the crystals. This procedure, of course, locates a point on the solidus curve for that temperature. During progressive melting of the mixture with 70 per cent NaAlSi₃O₈ there was no change in the composition of the crystals, whereas during the progressive melting of a mixture on the potash side of this minimum the crystals moved still farther toward the potash side and in mixtures on the soda side the crystals moved toward the soda side. At the composition of this minimum-melting mixture and at the minimum temperature (840°) the liquid contains 7.7 per cent H₂O at 1000 atmospheres.

It is noteworthy that the minimum temperature at the 1000 atmospheres isobar lies 220° below the minimum temperature in the dry melts, but an additional 1000 atmospheres lowers the minimum only 70° farther, bringing it down to 770° . It may therefore be stated that alkali syenites under sufficient overburden to permit a pressure of 2000 atmospheres, say at a depth of about 5 miles in the earth, and given an adequate supply of water, will crystallize from the molten state at a temperature close to 770° . At greater depths and again with adequate water available, syenitic magma would crystallize at a still lower temperature, but not much lower; for, as has been seen, the effect of increased pressure rapidly diminishes. Not all mixtures have yet been studied, but as the work progresses from the minimum out toward the end members it is increasingly evident that the results will agree very closely with the early measurements at this Laboratory by Goranson on the melting of the end members at corresponding pressures of water vapor.

Studies of subsolidus relations have also been made, and here again the X-ray method of determining the composition of the crystals proved invaluable. It was found that, when crystallized at low tern-

peratures, the intermediate compositions gave two $[\bar{2}01]$ peaks, the one corresponding to a potash-rich feldspar and the other to a soda-rich feldspar, and the composition of each is determined by the position of its peak. There is, then, as had been deduced from the evidence of natural rocks, a failure of complete solid solution of the alkali feldspars at lower temperatures, and two feldspars are formed side by side. Moreover, the compositions of the two feldspars in equilibrium with each other at any temperature can be determined experimentally. The hiatus increases as the temperature falls. Much has already been accomplished in determining the width of the hiatus, but the work is very time-consuming, and determinations for some temperatures in the critical range are yet to be made. When they are completed it should be possible to determine rather closely the temperature of growth of any associated pair of alkali feldspars in natural rocks. It will be necessary only to determine the composition of the two feldspars.

One item of outstanding importance in the results of this work has not yet been mentioned. It is that the crystalline NaAlSi₃O₈ prepared in this work does not have properties corresponding to those ordinarily given for natural albite. The reason for this is not far to seek. The best source of crystals of feldspar approaching the albite end member is in pegmatites, where the feldspar is formed at relatively low temperatures. It is optically positive and has a large optic axial angle. The soda feldspar produced in the laboratory is optically negative with a small angle, and has notably lower refractive indices than the pegmatite albites. There are also quite marked differences in their X-ray patterns. Now there are soda feldspars in natural rocks that are optically negative with a small angle and low refractive indices.

They were studied long ago by F. Fouqué and were obtained by him from lava flows in which they occurred as phenocrysts. They are, accordingly, to be regarded as a high-temperature variety. To be sure, they were described by Fouqué as "anorthose" and some of them have a high potash content, but some approach albite reasonably closely (nearly 90 per cent NaAlSi₃O₈) and render it clear that there are in nature high-temperature albites. Our synthetic albite is the high-temperature variety; and, unfortunately, no matter how low a temperature or what combination of ingredients is used, only high albite has yet been produced, though it is certain that at the lower temperatures the high albite formed metastably. It is not an unusual experience with silicates to find great difficulty in producing low-temperature modifications in the laboratory, but usually some device is hit upon which turns the trick. The secret for albite has not yet been found.

It has been possible, however, to determine approximately the temperature of inversion by finding the lowest temperature at which the low-temperature modification (ordinary pegmatite albite such as that from Amelia County Court House in Virginia) can be transformed into the high-temperature variety. The change is very sluggish at any temperature, but can be brought about simply by heating the dry powdered mineral at 1080° for one week. This temperature is far above the inversion temperature, in spite of the slowness of transformation. With the aid of a flux of Na₂Si₂O₅ and water vapor at 1000 atmospheres, the change could be induced at 725° in a week, but not at 675° in two weeks. It is concluded, therefore, that the inversion temperature is approximately 700°.

The existence of this inversion in the sodic end member of the plagioclase series

necessitates an inversion in adjacent plagioclases at least. It has been confirmed that plagioclase with up to 30 per cent anorthite has high- and low-temperature modifications distinguished by their X-ray diffraction patterns. Beyond that range of composition, plagioclases suffer changes on heating which may or may not be of the same character. This is the work on plagioclases which was mentioned earlier and which is being further prosecuted. Clearly one cannot solve the problem of solid solution in the plagioclases by selecting natural plagioclases haphazard and determining their crystal structure by X-rays, as has been attempted. The crystals studied must be selected with reference to their manner of occurrence and probable temperature of formation, and the results must be correlated with measurements on synthetic feldspars grown at controlled temperatures.

SOLUBILITY OF QUARTZ IN STEAM

The investigations with water at high temperature and pressure may be considered to open up a new field of chemistry. The usual experimental conditions are such that the water is above its critical temperature and pressure and hence will ordinarily be called a vapor or gas, but in some of the work the water is under so high a pressure that its density becomes of the same order as that of liquid water at room temperature. Owing to its high density, the "gaseous" water may have a great solvent power for solids, and the often-discussed gas transport of solid material becomes a demonstrated fact. The current work on the solubility of quartz in steam furnishes an excellent example of this phenomenon.

Water under pressure is pumped into a heated pressure vessel containing crushed quartz. The pressure is held constant by

a regulator, and on entering the heated pressure vessel the water is changed to steam. This steam is slowly passed through the pressure vessel, cooled, collected, and weighed, and the amount of dissolved silica is determined by analysis. The rate of flow is controlled by a needle valve on the exit side of the apparatus, and rates ranging from less than 0.1 to 10 grams of water per minute have been used. Temperatures have ranged from 300° to 500° and pressures from 2000 to 15,000 pounds per square inch. The lower pressures are comparable with those used with modern high-pressure steam turbines, and the temperature range includes that of superheaters. This is of practical interest because the deposition of quartz and amorphous silica on the blades of high-pressure turbines leads to a serious decrease in efficiency and requires frequent shutdown for its removal.

The experimental results are rather striking. Even at the lower pressures and temperatures, the solubility of the solid, quartz, in the gas, steam, can easily be measured. At 400°, and still more at 500°, the solubility increases rapidly with the pressure. At 1000 atmospheres pressure (15,000 psi), the gas contains 0.25 per cent silica. This corresponds to a partial pressure of silica of 2.5 atmospheres—and silica is one of the least volatile of substances. Reaction takes place rapidly. Even when the rate of passage is such that the steam is in contact with quartz for less than 10 minutes, the vapor phase becomes three-quarters saturated, and 0.8 gram of silica is carried over per hour. At this easily attainable pressure, the solubility of silica in steam is ample to account for the formation of the quartz in pegmatite deposits.

THE SYSTEM H₂O—NA₂O—SxQs

The first studies on the silicates of sodium and potassium in the presence of

water were published in 1914, and a detailed study of the system $\text{H}_2\text{O}-\text{K}_2\text{O}-\text{SiO}_2$ in 1917. The methods and apparatus used in that study are not suitable for $\text{H}_2\text{O}-\text{Na}_2\text{O}-\text{SiO}_2$, because of the much greater pressures encountered and the resulting solubility of both Na_2O and SiO_2 in the gas. A successful technique was not developed until 1941, and progress was interrupted by the war.

In the report for 1940-1941 (Year Book No. 40) it was stated that sodium metasilicate and sodium disilicate have strongly retrograde solubilities, which fall practically to zero at the critical point of water, 374° and 212 atmospheres. This is the first intersection of the critical curve and the solubility curve; at the relatively low pressure that obtains here, the solubility in the gas is negligible. The second intersection of the critical curve must take place on the upper part of the solubility curve, a part of the curve that may be regarded as representing the lowering of the melting point by dissolved water. It is important to note that as the temperature is lowered the pressure required to hold the water in solution continuously increases. At 400° , 26° above the critical point of water, pressures on the isothermal saturation curve of sodium disilicate are greater than 1200 atmospheres, and at this pressure the solubility of solid in gas is important. Moreover, the gas does not dissolve Na_2O and SiO_2 in the same proportions that are present in the liquid; hence the gas and liquid must be regarded as ternary. The composition of gas, liquid, and, when present, crystalline phase must be determined.

In studying the system, it is found most convenient to operate at constant temperature and constant pressure. Most of the work has been at 400° , and the results for this isotherm are nearly ready for publication. Several series of isobars have been made. A silicate mixture of known com-

position is placed in a shallow platinum crucible, mounted on a pedestal in a pressure vessel (or bomb) of the metal Inconel. Stainless steel bombs were abandoned because when the Na_2O content of the gas was high it drilled holes through the 1-inch bottom overnight. The bomb with charge is placed in a furnace held at constant temperature, and water is slowly pumped in until the desired pressure is reached, the pressure then being held constant by an automatic regulator.

Usually after an overnight run the bomb is shut off from the pressure system and cooled, first in air, then in water, and opened. The contents are divided into three parts. Almost all the gas condenses to a liquid outside the crucible. This liquid is removed, weighed, and analyzed. In some experiments with high Na_2O content, it is necessary to dilute the condensed gas with water, otherwise it will partially solidify as NaOH before it can be removed from the bomb. The contents of the crucible consist of glass (representing a hardened liquid), or glass plus crystals, or crystals, plus some extra condensed gas which has reacted with the glass or crystals. This material is poured off and analyzed, and from this analysis the composition of the glass or the crystals or both, and the composition of the gas, the amount of this additional condensed gas, and the amount of material it has dissolved can be calculated.

GRAPHICAL REPRESENTATION WITH A VOLATILE COMPONENT

The method of study will be made clearer from a consideration of figure 1, which refers to a constant temperature and a constant pressure. The sides of the composition triangle $\text{H}_2\text{O}-\text{Na}^{\wedge}\text{O}-\text{SiO}_2$ are omitted, except for a part of the side $\text{Na}_2\text{O}-\text{SiO}_2$ on which is located the composition of sodium disilicate (*Di*), Na_2O^*

2SiO_2 . The lines I-I, 2-2, and *Di-Di* go from the H_2O apex to the indicated points on the side $\text{Na}_2\text{O}-\text{SiO}_2$; any mixture of *Di* and H_2O , for example, must lie on the line *Di-Di*. The dot-dash curves *Gd* and *G₂G* represent the compositions of gases in equilibrium with unsaturated liquids along the lines *LLi*, or *L₂L*, shown by solid lines. The dotted curve *GIG₂* shows the gaseous solubility of sodium disilicate. The curve *E₁L₁L₂E₂* is the solubility curve of sodium

bomb, the gross composition of the mixture must be on the line from the H_2O apex to the composition of sodium disilicate, i.e., the line *Di-Di*. If the amount put in is less than that corresponding to the solubility of sodium disilicate in steam at this temperature and pressure, the composition will be on *Di-Di* to the left of point *a*. At 700 atmospheres this is equivalent to about 0.7 gram, in the bomb used. When a little more is added, a clear glass

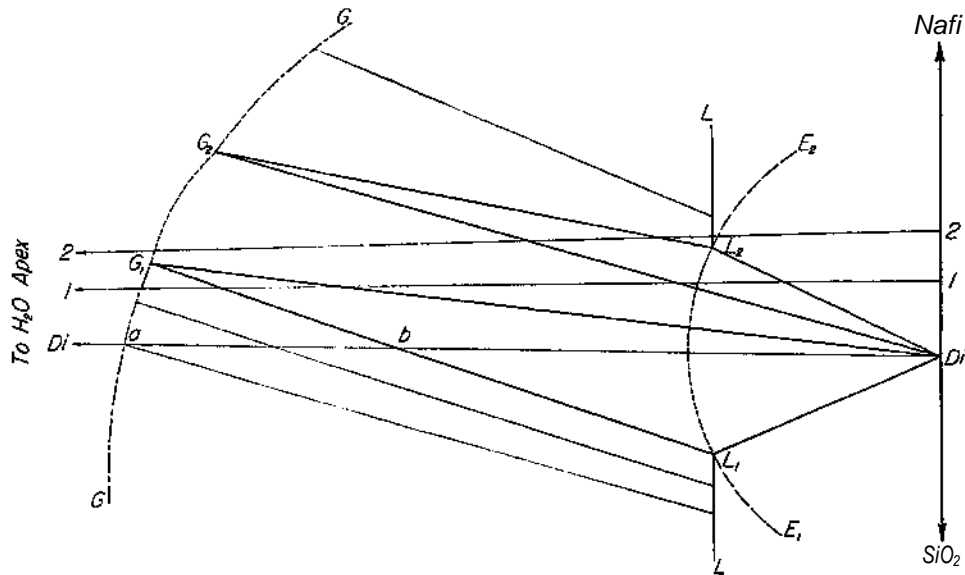


FIG. 1

disilicate in water; but, since at constant pressure and temperature there cannot be a series of solutions in equilibrium with gas and sodium disilicate, the curve is metastable except for the points *h* and L_2 . The parts *E₁L₁L₂* and *L₂E₂* represent solutions having vapor pressures less than that for which the diagram is made, and, therefore, unstable; and the part *L₁L₂*, solutions having vapor pressures greater than that of the diagram. Only the liquids L_1 and L_2 can coexist in equilibrium with gas and solid at this pressure and temperature.

When sodium disilicate is placed in the

is found in the crucible, which is an unsaturated solution on the line *LLi*; the corresponding gas compositions are indicated by tie lines. When the amount of disilicate corresponds to point *b*, crystals of disilicate begin to form. The composition of the gas is G_1 , that of the liquid L_1 , and these remain constant as long as there are present gas, liquid, and crystals. Addition of more disilicate results only in more crystalline disilicate; the composition will remain within the 3-phase triangle *G₁-L₁-Di*, all compositions are fixed, and only the proportions change.

If the original mixture is richer in SiO_2 than Li, disilicate can be formed only at lower pressures; and as the proportion of silica is increased, the pressure gets lower and lower until the boundary of the quartz field is reached, after which quartz becomes solid phase and the pressure increases rapidly. This disilicate-quartz boundary is at a low pressure, 250 atmospheres; the liquid contains 25 per cent H_2O , and the gas 99.7 per cent H_2O .

When a mixture of the composition of sodium disilicate is put into the bomb, the gas and liquid compositions, that is, the points G_i and Li of the 3-phase triangle, depend on the pressure. At 700 atmospheres, G_i corresponds to 95 per cent H_2O , 1.8 per cent Na_2O , and 3.2 per cent SiO_2 ; and Li to 21.5 per cent H_2O , 20.5 per cent Na_2O , and 58 per cent SiO_2 . The compound $\text{Na}_2\text{O} \cdot 2\text{SiO}_2$ contains 66 per cent SiO_2 . The solid dissolved in the gas contains 64 per cent SiO_2 , and the solid dissolved in the liquid 73.9 per cent SiO_2 , so that the disilicate has been divided between a gas phase containing a lesser ratio of SiO_2 and a liquid containing a greater ratio of SiO_2 .

At 1200 atmospheres pressure the corresponding values are: gas, 93.8 per cent H_2O , 2.8 per cent Na_2O , 3.4 per cent SiO_2 , and liquid, 29 per cent H_2O , 22.7 per cent Na_2O , and 48.3 per cent SiO_2 . The solid in the gas now has 55.6 per cent SiO_2 , and the solid in the liquid 68 per cent SiO_2 . Even this higher pressure is not enough to liquefy sodium disilicate completely, although the percentage of silica in the dissolved liquid is now 68, not far from the 66 per cent of disilicate. It is a remarkable fact that at 374° the vapor pressure of the solution is about 212 atmospheres, whereas at only 26° higher it has increased to over 1200 atmospheres. If a solution of sodium disilicate in water is cooled from high

temperature, a pressure of over 1200 atmospheres will be developed as a result of the cooling and consequent crystallization. It may also be mentioned that gas and liquid are still far apart in composition, and that at a critical end point they must be identical.

Another significant fact is brought out by figure 1. The join $Di-Di$ passes from the field of unsaturated gas into the field of gas plus unsaturated liquid, then into the 3-phase region. Starting with a mixture containing more Na_2O , along the line 1-1, the sequence of fields is the same, until it emerges into a field of gas plus crystalline disilicate. The dotted line G_iG_2 represents the gaseous solubility of crystalline disilicate, and mixtures along the line 2-2 pass directly into this region.

Further work at a little lower temperature will throw light on these and other interesting phenomena in a field that as yet has scarcely been investigated either theoretically or experimentally.

FILTER AUTOCLAVE

A solubility determination with the high-pressure-steam filter autoclave comprises four distinct phases: first, heating a reaction mixture to the desired temperature in the presence of steam at a predetermined steam pressure and then stirring it under these conditions of temperature and pressure until equilibrium is reached; second, filtering the reaction mixture with maintenance of the equilibrium temperature and pressure; third, cooling the filtrate to room temperature and atmospheric pressure without a change in its over-all chemical composition; fourth, analyzing the filtrate and identifying the solid phase.

A year ago it was reported that the first complete experiment had just been performed with this apparatus. Besides indicating the need for some mechanical

improvements in the apparatus, that experiment showed that the initial procedure for the third phase of the determination (cooling) was unsatisfactory. The problem of the change in water content of the filtrate during the cooling still remains, though a great many variations in cooling schedule have been tried. Solution of this problem was the major objective of the forty-two solubility determinations and four special experiments performed since the fall of 1948. It has been learned that liquid water enters the lower chamber of the autoclave and floods the crucible holding the filtrate. The source of the pressure difference that pushes the water into the lower chamber has not yet been discovered. Absorption of water by the cooling filtrate from the atmosphere of steam that surrounds it, which at first was supposed to be the only mechanism for the introduction of extraneous water, is so far overshadowed by flooding that it is not yet possible to tell whether it has taken place at all.

The search for the source of the extraneous water may be made by performing simulated solubility determinations in which no charge of silicate is present; and this has been done in some instances. In general, however, it was considered preferable to carry out complete solubility determinations. In this way experience was gained in the manipulation of the apparatus, and the various parts were given endurance tests. The value of this policy is attested by the fact that the number of unsuccessful solubility determinations has decreased steadily, as causes of failure have been discovered and overcome. The most troublesome one, which accounted for nine of the seventeen unsuccessful determinations, was leakage of nitrogen around the flange of the filter crucible during filtration. Although this condition prevented

the obtaining of a filtrate, it did not interfere with the acquisition of information applicable to the problem of the source of the extraneous water.

The leakage of nitrogen past the filter crucible has been prevented by several successive changes in design of the holder for the filter crucible. Other improvements made in the apparatus during the past year include a thrust bearing for the stirring shaft, a centering device for the autoclave (which permits alignment of the stirring shaft with the axis of the gear and thrust bearing), a keyway for mounting the stirrer on the shaft, and a turbo-compressor for forced cooling of both the autoclave and the steam boiler.

Another advantage of making complete solubility determinations before the cause of extraneous water has been eliminated is that some information has been gained about the attainment of equilibrium. Using as a criterion the K_2O content of the glass obtained by dehydration of the filtrate, the same result was obtained when KOH and quartz or glassy $K_2Si_2O_5$ and silica gel were used as starting materials instead of crystalline $K_2Si_2O_5$ and quartz. Several experiments in which the time of stirring under equilibrium conditions was reduced to 1 hour (normally it was 4 hours) indicated that this was not long enough for equilibrium to be reached.

The variation in K_2O content of the glass obtained from determinations performed under normal conditions was so small as to suggest that the limit of accuracy of a solubility determination performed with the filter autoclave (when the extraneous water is absent) will be that imposed by the analysis of the filtrate. Furthermore, the mean value of the K_2O content at 300° and 50 atmospheres pressure agrees well with that obtained by interpolation of previous data.

EXTREME HIGH PRESSURE

An important aspect of work with high pressure is the effect of pressure, in the absence of volatile components, on the melting and transition of minerals. Apparatus has been designed and constructed capable of simultaneously producing and maintaining pressures up to 15,000 atmospheres and temperatures up to 1400°. The immediate experiments planned involve temperatures between 500° and 800° and pressures up to 10,000 atmospheres. Data on the behavior of silicates in the range of conditions that can be produced by the ap-

paratus are essentially nonexistent. For this reason, the primary program involving the apparatus will be the determination of the change of melting point of minerals with pressure. From these data, thermodynamic properties can be calculated and correlated with the results obtained by other methods now being applied at the Laboratory. Having obtained this fundamental information, we shall be in a position to make an intelligent selection of other programs for high-pressure experimentation.

STATISTICAL PETROLOGY

Further work on application of statistical methods to petrology has been carried out, and a preliminary study of the relation between grain size, area of measurement, and sample variance has been completed. It turns out that the critical problem here is an instrumental one, since it will be necessary to establish a quantitative measure of grain size, if variances for rocks of different grain size are to be compared. The problem of measurement is very simple, for by substitution of a finer thread in the mechanical stage of the point counter almost any reasonable distance between points can be obtained, and the distance traversed is given by $(n-1)^{-1}$, where n is the number of points and λ the distance between points. The best method of recording the measurement has not yet been determined. Several schemes are under consideration, and at present it appears that the best results would be obtained by substitution of a recording-tape adding machine for a counting cell.

Relations between ratios are often used in chemical petrology without full realization of the difficulties and ambiguities involved in passing from ratio correlations to inferences about relations between the

absolute values from which the ratios are formed. Usually it is these absolute values that are of primary concern. By application of a series of restrictions to the variables used in the Pearson general formula for index correlation, statements governing product-moment correlation between ratios in the special cases commonest in petrology are derived. Each special case is illustrated by one or more numerical examples drawn from the literature of petrology, and the general conclusion is that where interest centers on the absolute values, as is often the case, careless application of ratios is liable to be misleading and even their careful use is likely to yield ambiguous or uninterpretable results.

End-stage reactions in the crystallization of magma often take the form of pseudomorphous replacements, and the question as to whether these reactions occurred in a still homogeneous paste of solids and liquids, or after the separation of liquid residues from crystallized material, is one of recurring interest. Qualitative approaches to this problem have been largely unsatisfactory, since it is to be expected that fabric and habit will be very similar. In late magmatic and early hydrothermal crystal-

lization. Provided that the reaction is truly pseudomorphous, however, a replacement occurring at about the same time in the entire mass would lead to a positive correlation between the amount of original mineral available to the reaction and the amount of replacement product formed, for the extent of replacement would be largely a function of the amount of original mineral available. If, on the other hand, the replacement occurs only after the magmatic residue has been strained away from some parts of the rock and concentrated in others, there will be no correlation between original mineral and replacement product. By means of the conventional part-whole correlation formula, it may be shown that under such circumstances there would be negative correlation between the amount of replacement product and the amount of original mineral surviving the reaction. On this basis it has been concluded that muscovite pseudomorphously replacing plagioclase in the Barre, Vermont, granite is late-magmatic rather than post-magmatic or hydrothermal. The argument is subject to certain

limitations which do not seriously hinder its practical application.

A detailed test has been made of the homogeneity of the well known granites from Westerly and Bradford, Rhode Island. Hand specimens were taken at each of the larger quarries in each granite; and from each hand specimen three thin sections were cut in such a fashion that variance analyses would provide information on variance attributable to differences between the two granites, between hand specimens of each granite, and within each hand specimen. This latter source of variation could be further analyzed into a location and an orientation factor. The end result is that differences between the two granites, though small, are readily detectable, that orientation and location effects within hand specimens of either granite are negligible, that small differences between hand specimens of the Westerly are detectable, but that the existence of such differences between hand specimens of the Bradford granite may not be inferred from the data.

STUDIES ON VOLCANIC PRODUCTS

In last year's report on the study of the rocks extruded at Santiaguito, the new volcanic cone halfway up the side of Santa María in Guatemala, it was stated that a study of the acid constituents of the emanations from the fumaroles would next be undertaken. The detailed studies have been concluded. In addition, considerable work was done on the incrustations that were found at and near the vents of the fumaroles.

Studies in the field revealed the fact that the fumaroles could be divided into two types which for the purposes of this report may be called A and B. At type A, steam at about 300° issued freely from open vents

and no free sulfur was visible. These fumaroles were on the eastern slope of Santiaguito and were situated about 300 feet above the floor of the crater developed on Santa María in 1902. At type B, about 700 feet above the crater floor, clouds of suffocating steam vapors issued from fine cracks. At a depth of about 12 inches below the surface, a layer of sulfur approximately 3 inches thick was found. The sulfur varied from solid crystalline form near the surface to liquid form increasing in degree of fluidity with depth. This mantle of sulfur, shot through with fine cracks, helped to maintain the rather surprisingly steep slope of nearly 50°.

ACID CONSTITUENTS OF THE EMANATIONS

Samples of condensed steam were collected from type A fumaroles in 1932 and 1939. Samples were collected from types A and B in 1940. Analysis showed that all contained appreciable amounts of the following constituents: hydrochloric acid, sulfuric acid, and sulfur dioxide. The concentrations of the condensate seem to vary from year to year. From 4 to 15 grams per liter of hydrochloric acid were found. In some cases the content of sulfuric acid was about the same as that of hydrochloric, but more frequently the latter dominated. In addition, the condensates were almost saturated with respect to sulfur dioxide. In type B the condensates were led into a solution of cadmium acetate, but, as was expected, no cadmium sulfide was precipitated, a fact which indicated the absence of hydrogen sulfide. The same procedure was followed at the fumarolic area type B, where the great deposits of free sulfur were found. A copious yellowish precipitate was formed, but analysis in the laboratory showed that it was free sulfur mixed with extremely minute amounts of selenium and tellurium. It is possible for H₂S and SO₂ to coexist at temperatures above about 300° (the steam temperature at both areas), but on cooling, they promptly react to form sulfur. This circumstance is considered to provide an explanation of the presence of sulfur at type B fumaroles. At the type A area the conditions are completely oxidizing, owing, we now believe, to aspiration of air into the edifice.

In addition to the main acid constituents mentioned above, hydriodic, hydrobromic, hydrofluoric, and boric acids were found in small but readily determinable amounts in the acid emanations from type A and type B. Even though the amounts of hydriodic and hydrobromic acid were small relative to the main acid constituents,

the amounts, about 3 milligrams of the former and from 2 to 20 milligrams per liter of the latter halogen acid, are not only significant but also surprising. It seemed desirable to ascertain whether or not other fumarolic areas exhibited a similar concentration of these acids. Water samples had been collected from the crater lake of Santa Ana in El Salvador and from the crater lake of Kawah Idjen in Java. The two lakes receive the volatile products from intensely active fumaroles located below and also above the lake level. Iodides and bromides in easily determinable amounts were found in the samples from both lakes. It appears that these halogen acids are probably more abundant in volcanic emanations than has heretofore been supposed.

INCRUSTATIONS

In general the particle size of many mineral constituents in the incrustations is so small that their identification has been exceedingly difficult and in some cases, up to the present, impossible. As many of the mineral species as possible were identified by means of the microscope and X-ray analysis. Chemical analysis yielded considerable knowledge concerning the chemical environment. Additional mineral species were identified by the combination technique of breaking down each incrustation into groups by chemical methods and examining these by microscopic and X-ray methods,

As a result of study of the incrustations from fumaroles of type A by the technique just referred to, it appears that the chemical environment is so definitely an oxidizing one that only salts of the various bases contained in the andesitic rock are found in the stalactitic incrustations. Where these had been subjected to temperatures above 100°, only sulfates and partially decom-

posed rock minerals were found. In products subjected to lower temperature, chlorides of the various bases were abundant. It is interesting to note that where sulfates predominate they belong to the relatively water-insoluble group of complex aluminum sulfates; the more soluble alkaline salts are present only in subordinate amounts.

At fumaroles of type B, the mantle of sulfur, referred to above, had an orange color. Chemical analysis showed that the deep color was due to the presence of arsenic sulfide (As_2S_3), selenium, and tellurium. Microscopic examination showed that the sulfide was amorphous orpiment. Below the sulfur, a grayish-black indurated material was found. The microscope revealed the presence of opal (a hydrated form of SiO_2), sassolite (boric acid), and anhydrite ($CaSO_4$). It seemed obvious that the induration was due to the formation of opal and anhydrite. It is of interest to note that boric acid had previously been found in the condensates of the steam issuing from both fumarolic areas. The evidence seems clear that these products, which are readily volatile in this chemical environment at temperatures above 300° , have been brought up from the hotter lava below. As a matter of fact, they have an appreciable vapor pressure even at 300° , but have been held in place by the mantle of sulfur, the upper surface of which was at a much lower temperature. This gray-

black incrustation was further studied by the combination technique referred to above. It was shown that the dark color was due to about 1 per cent of exceedingly finely divided pyrite (FeS_2). In addition, between 5 and 10 per cent of the low-temperature form cristobolite (SiO_2) and a lesser amount of anatase, one of the crystalline forms of titanium oxide, were found. These minerals were formed by the interaction of the mineral constituents in the lava and the acid aqueous vapors given off by the hot lava below. The presence of the pyrite shows that at fumarole type B the chemical environment is definitely of a reducing nature. In view of the fact that the steam 5 inches below the surface has a temperature of about 300° , it is not likely that the chlorides could form; as a matter of fact, only an insignificant amount of the chloride ion was found in this incrustation.

Examination of a thin section of the indurated gray-black incrustations revealed the fact that the rock minerals had been completely altered, but the "ghosts" of the original minerals were plainly visible.

The information so far obtained from these chemical studies has an important bearing on rock alteration by means of the acid emanations and fluids that must exist on a large scale in many regions exposed to igneous activity. There is reason to believe that the results throw light on the earliest stages of such alteration.

THERMAL PROPERTIES OF MINERAL SUBSTANCES

Investigations of the thermal properties of mineral substances have been part of the recognized program of the Laboratory from the beginning, and upon the resumption of research in petrology at the close of the last war, greater emphasis was placed on this subject. Phase-equilibrium studies

show what crystalline substances are formed from silicate mixtures, the compositions of the liquids which can be in equilibrium with them, and the pertinent temperatures. But for the complete thermodynamic description of any system, it is necessary to know also the changes in

volume, and especially the heats of fusion and solution at all points on the solubility curves. Not until such knowledge is available can our results be applied to petrologic problems with full effectiveness.

In view of the scarcity of reliable thermal data on silicates, the diversity of silicate compounds, and the much greater geological importance of some than of others, a careful selection of the substances for study at this Laboratory has been made. Among the more important series of silicate minerals are the feldspars, pyroxenes, hornblendes, feldspathoids, micas, aluminum silicates both hydrous and anhydrous, garnets, melilites, and olivines, together with a galaxy of hydrous silicates and aluminosilicates. In order not to scatter efforts too widely over the field, it has been decided to concentrate at first on the feldspars and the chemically adjacent substances jadeite, nepheline-carnegieite, leucite, and kaliophilite. At present carefully "purified" natural minerals are being used, but since these are almost never pure chemical compounds, it will be necessary to study synthetic preparations as well.

Work on this project during the past year has consisted of purifying mineral samples, making heat-of-solution measurements, preparing for the measurement of specific heats, and synthesizing artificial preparations.

PREPARATION OF SAMPLES FOR HEATS OF SOLUTION

The Laboratory now has a series of excellent samples of feldspars, of some feldspathoids, and of the aluminosilicates sillimanite, andalusite, and kyanite, which have been purified by mineral separation methods and prepared in a very finely divided form suitable for solution in the hydrofluoric acid calorimeter. Analyses have been obtained for most of the samples.

THE SOLUTION CALORIMETER AND MEASUREMENTS OF THE HEATS OF SOLUTION IN HYDROFLUORIC ACID

Certain modifications have been made in the methods of measurement of the temperature rise in the gold calorimeter described in last year's report. In place of the Mueller bridge and type K potentiometer combination, a White double potentiometer has been installed as the measuring instrument, and the potential-drop method has been replaced by a method using a Wheatstone bridge with three fixed arms. The unbalance of the bridge is read on the potentiometer, the arrangement being in principle like that used in reading the pressure in the compressibility apparatus of this Laboratory. In order to utilize the sensitivity of the galvanometer to the fullest extent, the bridge has equal ratio arms for dividing the current of 2000 ohms each, and the resistance of the third arm and of the thermometer is of the order of 100 ohms. An important consideration in choosing the constants of the bridge is the current carried by the thermometer, which should be as small as possible in order that the heating effect of the thermometer be small. The third arm of the bridge is always kept at a lower resistance than the thermometer, so that the unbalanced emf will always be positive and will increase with the temperature of the calorimeter. When the temperature rise is less than 1° , the unbalanced emf is linear to better than one part in 10,000, with respect to the resistance change of the thermometer. With the setup as ordinarily used, the unbalanced emf is more than 500 microvolts per degree change in temperature, and the emf can be read to better than 0.1 microvolt, so that the error in temperature measurement does not need to be much greater than 0.0001° .

The electrical energy for calibration is

supplied to the calorimeter at a rate somewhat comparable with the rate of generation of heat by the dissolving sample. Heat-exchange correction is made using a computational procedure based on the Regnault-Pfaundler method. The calorimeter assembly is completely immersed in an oil bath kept at a constant temperature near 74.5°. The actual temperature variation is within 0.01° at present; and it is planned to improve this further, if it turns out that the accuracy of the results can be augmented by doing so. For the solution experiments, the calorimeter is filled with 819.1 grams of 20 per cent hydrofluoric acid, prepared by mixing distilled water with 48 per cent acid, of reagent grade.

The apparatus and methods were first tested by dissolving certain salts in water. These experiments gave results which were in good agreement with data found in the literature. A few orienting experiments with selected samples showed that the natural feldspars may be expected to dis-

solve in the acid without complications. Synthetic nepheline, on the other hand, though it dissolved rapidly in the initial decomposition, gave a post-precipitation of chiolite ($\text{Na}_5\text{Al}_3\text{F}_4$), the heating effect of which complicates the attainment of a constant heat-exchange rate after the solution is finished. There are still troublesome items of experimental procedure to be worked out.

Results have already been obtained for the heats of solution of quartz, albite, bytownite, anorthite, and adularia, which gave, respectively, 553, 564, 624, 646, and 527 calories per gram (rounded off to the nearest calorie). The rounded value obtained for quartz (99.95 per cent SiO_2 , 0.05 per cent impurities—mainly iron oxides) uncorrected for impurities, 553 calories per gram in 20 per cent acid, compares well with the final corrected value of Sahama and Torgeson, 549 in 20 per cent acid. Other values obtained in 20 per cent acid are: Mullert (1913) 498, Roth (1928) 517, and Troitsch (1932) 548.

DEEP SEISMIC PROSPECTING

Jointly with the Department of Terrestrial Magnetism, the Laboratory carried forward the investigation of the earth's crust by obtaining accurately timed records of the arrival of vibrations from charges of explosives set off at distances up to several

hundred kilometers. Further information concerning the thickness and nature of the crustal layers has been obtained. Details of the results are to be found in the report from that Department.

The following is a list of the papers published during the report year in technical journals. In addition there are several papers that have been prepared and are awaiting publication. These are: F. Chayes, "On a distinction between late-magmatic and postmagmatic replacement reactions"; G. L. Davis and H. H. Hess, "Radium content of ultramafic igneous rocks. II. Geological and chemical impli-

cations"; G. L. Davis, "Radium content of ultramafic igneous rocks. III. Meteorites"; R. C. Kracek, "Phase transformations in one-component silicate systems"; J. F. Schairer, "Phase transformations in poly-component silicate systems"; A. H. Stone, "On supersonic flow past a slightly yawing cone. II"; and O. F. Tuttle, "The variable inversion temperature of quartz as a possible geologic thermometer."¹

SUMMARY OF PUBLISHED WORK

- (1104) A simple point counter for thin-section analysis. F. Chayes. *Amer. Mineralogist*, vol. 34, pp. i-n (1949).

A manually operated point counter for thin-section analysis is described. The machine is sturdy, inexpensive, and easily operated. Its precision has been tested by analyzing in duplicate 47 thin sections of rocks and computing the analytical error, or standard deviation of a single analysis, from the observed variance of the differences. The error distribution is effectively binomial, and the precision of the instrument is somewhat better than that of the Wentworth-Hunt and Hurlbut integrators. Average operating speed is about four times that of the Wentworth-Hunt and twice that of the Hurlbut machine.

- (1105) A new hydrothermal quenching apparatus. O. F. Tuttle. *Amer. Jour. Sci.*, vol. 246, pp. 628-635 (1948).

A simple apparatus has been developed for the study of equilibrium relations at high temperatures and pressures in mineral systems including volatile components. Investigations have been carried to pressures of 30,000 pounds per square inch (approximately 4.5 miles depth) at temperatures up to 900° C. Results on the system $K_2O-Al_2O_3-SiO_2-H_2O$ show that a water pressure of 15,000 psi lowers the liquidus about 100° in the orthoclase field. In compositions approaching the quartz-orthoclase join, a pressure of 30,000 psi gave relatively insignificant additional lowering.

- (1106) The radium content of varved clay and a possible age of the Hartford, Connecticut, deposits. William D. Urry. *Amer. Jour. Sci.*, vol. 246, pp. 689-700 (1948).

The radium content of the summer and winter portions of the varves in the clay deposits at Hartford, Connecticut, varies rhythmically. When the radium contents of the summer and of the winter portions are plotted against time as measured by the varve count, the curves exhibit slopes of opposite sign. The total radium content of any varve, how-

ever, is practically constant. These phenomena may be due to a disturbance of the radioactive equilibrium, but this hypothesis, though plausible, is far from proved. A greater concentration of uranium relative to ionium in the winter clay than in the summer clay would explain these phenomena. Such a disturbance of the equilibrium provides a means of determining the age of the deposits. On this basis, there is derived a tentative figure for the age of the Hartford clay (varve 3700) of 18,000 years. The hypothesis of a disturbance of radioactive equilibrium is supported by the fact that the analyses of the summer and winter curves, which are completely independent, give very nearly the same age.

- (1107) Radioactivity of ocean sediments. VI. Concentrations of the radioelements in marine sediments of the southern hemisphere. William D. Urry. *Amer. Jour. Sci.*, vol. 247, pp. 257-275 (1949).

It has been reported in previous publications of this series that the mode of variation of the radium concentration below the ocean bottom affords a method of determining time in ocean sediments. Hitherto, these researches were confined to the northern hemisphere. Similar studies in the southern hemisphere, combined with the necessary geological and biological investigations, should provide an answer to the question of the contemporaneity of glaciation in the northern and southern hemispheres. Measurements of the radium content as a function of depth in the sediment are presented here for ocean-bottom cores obtained by the U. S. Navy Antarctic Expedition of 1946-1947.

- (1109) Radioactivity of ocean sediments. VII. Rate of deposition of deep-sea sediments. William D. Urry. *Jour. Marine Research, Sverdrup Sixtieth Anniversary*, vol. 7, no. 3, pp. 618-634 (194B).

A study of the variation of the radium content during the period of re-establishment of radioactive equilibrium in the buried deposits of deep-sea sediments provides a method of

dating the record of past events in the ocean bottom. The results of such studies can be readily applied to determinations of the rate of deposition provided that knowledge of the distortion involved in obtaining core samples of the deep-sea sediments is available.

Application of the method of discerning rates of deposition is not limited, as was the application of earlier methods, to sediments deposited during postglacial time; it is possible to study the variation of the rate of deposition in the past as far back as the method of dating is applicable, i.e., for about half a million years. Rates of deposition as a function of time are reported here for red clay, globigerina ooze, foraminiferal marl, glacial marine deposit, and calcareous blue mud from areas extending from the Antarctic Ocean to the North Atlantic.

Outstanding features of these determinations are as follows: Deposition of practically all the sediments is more rapid at present than during the past half million years. The repeated climatological changes of the ice age did not have a particularly noticeable effect on the rate of deposition in general. Only during the long middle interglacial stage did the rates tend to be somewhat higher than the low rates generally prevailing in the past half million years. The lowest rates of deposition are associated with the last glacial stage and the early period of the middle interglacial stage or possibly the end of the second glacial stage. Locally, there are often interesting short-period changes in the rate of deposition which appear to be caused by climatological changes.

However, the amount of detail in an analysis of the rates of deposition varies greatly: in cores of equal length, far more detail can be obtained *in one* where the sediment was deposited in a hundred thousand years than in one where the sediment required a million years for deposition.

Average rates of deposition, in so far as they are comparable, are in good agreement with previous estimates by the *stratigraphic* and *supply* methods.

(mo) On ratio correlation in petrography. F. Chayes. Jour. Geol., vol. 57, no. 3, pp. 239-254 (1949)-

The same ratio correlation may be generated by many different combinations of relations between absolute measures, but a single set of absolute-measure statistics leads to one, and only one, correlation between any particular set of ratios formed from these absolute measures. The passage from ratio correlation to inference about relations between absolute measures is ambiguous at best and often misleading.

Algebraic statements exhibiting ratio correlation as a function of absolute-measure statistics are offered for types of ratios commonly used in petrography. These statements are all derived from Pearson's general formula for index correlation. They yield good approximations only if the fraction s/\bar{x} for each absolute measure is suitably small.

Several practical examples drawn from petrographic literature are described. In most of these cases the ratios seem to have been used either to order the data or *in* the hope that they would throw some light on relations between absolute measures. The results are shown to be on the whole indecisive and ambiguous and in a few cases decidedly misleading.

The formation of ratios should be confined to those problems in which hypotheses being tested deal with ratios. Absolute measures are always preferable when large numbers of observations must be recorded without benefit of satisfactory hypothesis. Ratios can always be drawn from tables of absolute measures; frequently, absolute measures cannot be reclaimed from tables of ratios.

(mi) The system MgO—SiO₂—H₂O. N. L. Bowen and O. F. Tuttle. Bull. Geol. Soc. Amer., vol. 60, pp. 439-460 (1949).

Equilibrium in the system MgO—SiO₂—H₂O has been determined at temperatures up to 1000° C. and at maximum pressures of water vapor varying from 15,000 pounds per square inch at this maximum temperature to 30,000 psi in the range 900°—600° and 40,000

psi in the range 600°—300°. Thus were fixed the univariant pressure-temperature curves of the following five reactions: I, serpentine + brucite \rightleftharpoons forsterite + vapor; II, serpentine ± 5 forsterite + talc -j- vapor; III, forsterite + talc ± 5 enstatite + vapor; IV, talc \rightleftharpoons enstatite -h quartz + vapor; and V, brucite \pm periclase +• vapor. Pure magnesian serpentine has a maximum temperature of existence at approximately 500°, varying only about 10° in the whole range of pressure, 2000 to 40,000 psi. Forsterite is stable in contact with water vapor down to a temperature of about 430° (at 15,000 psi). Only below that temperature is it transformed into serpentine and brucite. Iron-bearing olivines are stable in contact with water vapor down to still lower temperatures.

No liquid is formed in any composition of the system throughout the range of temperatures and pressures at which experiments were conducted, a condition which remains unchanged when the mixtures have upwards of 7 per cent FeO.

There is consequently no likelihood that any magma can exist that can be called a serpentine magma and certainly no possibility of its existence below 1000°. There seems no escape from the conclusion that ultramafics can be intruded only in the solid state.

(1112) Some examples of the application of thermochemistry to petrology. Th. G. Sahama and D. R. Torgeson. *Jour. Geol.*, vol. 57, no. 3, pp. 255-262 (1949).

A brief summary is presented of measurements of heats of solution of minerals belonging to the forsterite-fayalite and to the enstatite-orthoferrosilite series and of artificial ilmenite and geikielite.

In the olivine and orthopyroxene series, the heat of solution of HF is found to be a linear function of the Mg : Fe ratio, indicating perfect isomorphism between the corresponding end members. The importance and possibilities of applying calorimetry to the study of isomorphism are emphasized. On the basis of the calorimetric data available for the minerals in question, the heat and free energy

are given for the reaction: olivine + quartz \rightarrow 2 pyroxene. The difference in the stabilities of MgSiO₃ and FeSiO₃ is illustrated.

The stability reaction of ilmenite and geikielite in the presence of olivine or orthopyroxene is calculated from the calorimetric data. The influence of the entropy of mixing isomorphous minerals upon the stability relation is emphasized.

(1113) Significance of radioactivity in geophysics—thermal history of the earth. William D. Urry. *Trans. Amer. Geophys. Union*, vol. 30, pp. 171-180 (1949).

It appears that the effects on the earth's thermal history of the exponential decay of the sources of atomic (radioactive) heat within the earth are such that the upper crust was heating in its early history and that subsequent cooling has been more nearly linear than had been supposed. In the deeper parts of the crust and below, the thermal history has been complex, with simultaneous heating at one depth and cooling at another depth. Temperatures in the past beneath a Pacific-type ocean have not varied in the same manner.

(iri4) Melting relations of chalcocite. Einar Jensen. *Norske Videnskaps-Akademi, Oslo, Mat.-Naturv. Klasse, No. 6* (1947).

A study of the melting relations of chalcocite by the method of differential thermal analysis shows a maximum melting temperature of 1129° C. for the composition Cu_{1.99}Ag_{0.01}S, which melts sharply. Mattes containing more or less sulfur than this, including pure Cu₂S, melt lower and over a temperature interval—pure Cu₂S from 1107° to 1x27°. The limit of solubility of liquid copper in liquid chalcocite is found at a total composition of 80.21 per cent copper at 1105°. The solubility of copper in solid chalcocite is too small to be observed at 1105° and 1127°. The limit of solubility of sulfur in chalcocite could not be observed. No transformations in the solid state could be observed between 404° and melting temperatures in the composition range 77 to 82 per cent copper.

(1115) The system silver sulfide—antimony trisulfide. Einar Jensen. *Norske Videnskaps-Akademi, Oslo, Mat.-Naturv. Klasse, No. 2* (1947).

Nine minerals are known with a composition within this binary system. Previous thermal studies of the phase relations by Pelabon, Jaeger and van Klooster, and Konno indicate the existence at the melting point of the intermediate compounds $\text{Ag}_2\text{S} \cdot \text{Sb}_2\text{S}_3$ and $3\text{Ag}_2\text{S} \cdot \text{Sb}_2\text{S}_3$ only, corresponding to the minerals miargyrite and pyrargyrite, respectively. The present study of the phase relations was performed by differential thermal analysis on pure samples of exact composition, heated in sealed Pyrex tubes provided with thermocouple wells. The results indicate that the compound $\text{Ag}_2\text{S} \cdot \text{Sb}_2\text{S}_3$ has a polymorphous transition at some temperature below 380°C . and melts congruently at 518.7° . The compound $3\text{Ag}_2\text{S} \cdot \text{Sb}_2\text{S}_3$ melts at 485.8° . As the proportion of Ag_2S in the preparations increases, the liquidus curve first falls from 554°C , the melting point of Sb_2S_3 , to a eutectic with $\text{Ag}_2\text{S} \cdot \text{Sb}_2\text{S}_3$ at 462° , 22 per cent Ag_2S , then rises to the melting point of this compound, and again falls to a second eutectic, between $\text{Ag}_2\text{S} \cdot \text{Sb}_2\text{S}_3$ and $3\text{Ag}_2\text{S} \cdot \text{Sb}_2\text{S}_3$, at 464° , 59 per cent Ag_2S . The melting curve of $3\text{Ag}_2\text{S} \cdot \text{Sb}_2\text{S}_3$ rises from this eutectic and after passing through its maximum descends to a third eutectic at 470° , 77 per cent Ag_2S . From this point the curve remains almost horizontal to the neighborhood of 80 per cent Ag_2S , from which point it finally rises to the melting point of Ag_2S at 837.0° . The author suggests that the nearly horizontal portion of the melting curve may be the result of intrusion into the binary system of the liquidus surface of some ternary compound in the system $\text{Ag}-\text{Sb}-\text{S}$. The transition in $\text{Ag}_2\text{S} \cdot \text{Sb}_2\text{S}_3$, mentioned above, was found to begin, on heating, at temperatures as low as 344° , and to end at $370-380^\circ$. On cooling there was no reverse transition; on reheating after some weeks at room temperature a negative heat effect shows up near 207° . This leads to the conclusion

that the equilibrium transition temperature lies between 207° and 344° (359° in the pure compound).

(1116) Pressure-volume-temperature relations in solutions. VIII. The behavior of some solutions of electrolytes in water. R. E. Gibson and O. H. Loeffler. *Ann. New York Acad. Sci.*, vol. 51, pp. 727-752 (1949).

This paper presents the results of measurements of specific volumes of solutions of sodium bromide and sodium chloride over a wide range of pressure, temperature, and concentration. From these data various thermodynamic properties of the solutions, namely, the volume change on mixing, the pressure-temperature coefficient, the energy-volume coefficient, and the change in specific heat with volume at constant temperature, are calculated; the data are presented in detail in the form of tables and curves.

The effects of pressure, temperature, and concentration on the various thermodynamic properties of solutions discussed in this paper are all in qualitative agreement with the current theories of the molecular distribution in water. A comparison of the thermodynamic properties of sodium bromide in water and in glycol indicates that the contractions on mixing, the thermal expansions, and the energy-volume coefficients of the aqueous solutions at the lower temperatures are largely determined by the effects of short-range forces, and that the effects of ion-molecule interactions are only secondary. At higher temperatures the effects of short-range forces have a decreasing influence on these volumetric properties. Indeed, there is good reason to believe that above 150°C , the ion-molecule interactions and other long-range forces will play the significant part in determining the volumetric behavior of aqueous solutions and that simple regularities will be revealed that are masked at the temperatures where most of our information is now available.

(1117) Annual report for 1948-1949.

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DEPARTMENT OF TERRESTRIAL MAGNETISM

Washington, District of Columbia

MERLE A. TUVE, *Director*

Three years ago, beginning afresh on "significant research toward philosophical goals" after the interruptions of World War II, the staff of the Department stated three simple problems in geophysics which remained conspicuously unsolved, despite two generations of study on "the magnetic and electric condition of the earth and its atmosphere." These were as follows: The causes, inside the earth, of the earth's magnetic field are unknown; the mechanism which replenishes the earth's measured electric charge is not identified; mechanisms which give rise to particles of cosmic-ray energies are not yet recognized. The staff considered that these three basic puzzles might well serve as landmarks for orienting the geophysics program.

It is surprising to be able to report that the second of these questions, relating to the maintenance of the earth's electric charge, has been resolved by measurements in the clear air high above thunderstorms. One must also recall that unexpected evidence for a close relation of cosmic rays to our sun and to other stars was found in 1946, as reported two years ago, when cosmic-ray increases were discovered to occur at the times when large solar flares were in progress. All three of these problems in 1946 seemed basic in our thinking, yet probably beyond the reach of direct

investigation. It is encouraging indeed to find that formulation of a central problem in the simplest and most direct fashion can, with good fortune, be followed by significant new knowledge. The measurements above thunderstorms, carried out with the help of the U. S. Air Forces, are described below.

It remains to be seen whether direct knowledge can be obtained as to the causes, inside the earth, of the earth's magnetic field. No direct approach to this puzzle has yet presented itself, but new evidence, brought out in the course of a study of prehistoric changes in the compass direction, has instead opened up surprising questions relating to the possibility of great movements of the earth's crust with respect to the core and geographic poles during ancient geological epochs. This unexpected finding has been brought to light through studies of the residual magnetization of rocks.

In the laboratory phase of the Department's work, a steady development of the biophysics program is a strong indication that men trained in the physical sciences find ample challenge in the organized behavior of living matter, can formulate problems, and can obtain answers of interest and significance in this field.

EXPERIMENTAL GEOPHYSICS

THE EARTH'S CRUST

PALEOMAGNETISM

In the last annual report, there was extensive discussion of a program for investigation of the residual magnetism of

the sedimentary rocks in the earth's crust. This program was initiated with the hope that the results might provide a detailed history of the changes of the earth's magnetic field extending back millions of years in geologic time.

Our investigations have demonstrated that when sedimentary rocks are laid down in tranquil water they may acquire a direction of magnetization as the result of the statistical alignment of magnetized grains parallel to the earth's field. Therefore, a record of the past directions of the earth's magnetic field is found preserved in some sedimentary rocks. Recent tests here have demonstrated that sediments may retain a direction of magnetization without change for as long as 200 million years, and it now appears that it should be possible to trace far back in time the history of the changes in direction of the earth's magnetic field.

Rock magnetism reconnaissance expedition of 1948. The Department's studies of the magnetization of crustal materials have heretofore been confined to relatively young unconsolidated sediments from New England glacial deposits and from ocean cores. The results of these studies indicated that for the past million years the earth's magnetic field has had much the same orientation that it has today, and that its strength has remained approximately constant. In an effort to extend our knowledge of the changes of the earth's magnetic field farther back in geologic time, a three-month expedition to the flat-lying sedimentary deposits of the western United States was carried out during the summer of 1948. Samples were taken at eight sites scattered from Colorado north-westward to Washington and eastward into Wyoming and South Dakota. The age of the rocks measured ranged from approximately 10 to 100 million years. Because many rocks are not perceptibly magnetized, and because of difficulties with the techniques of preparing samples, only 96 separate observations of magnetic polarizations could be made. The results are summarized in figures 1 and 2, in which the directions of the horizontal com-

ponent and of the inclination of the rock magnetizations are indicated. The limited number of sites and samplings makes it unwise to draw extensive conclusions from the data, but the pronounced maxima in the two graphs suggest that for the past 100 million years, roughly, the earth's magnetic axis has remained centered, on the average, on the geographic axis. This interesting possibility is consistent with various different theories of the origin of the earth's magnetic field, and therefore cannot be advanced as an argument favoring any one of them.

Stability of magnetization. Workers in the field of rock magnetism have long recognized the necessity for demonstrating that rocks are capable of retaining their initial directions of magnetization from the time of their origin to the present. Definitive results on this question were obtained in 1948 from experiments which satisfactorily take into account long periods of time as a factor in the behavior of magnetization. This factor was treated by utilizing in the magnetism studies changes in geologic structure which took place millions of years ago. It is possible in many localities to locate rock exposures where sedimentary beds were long ago squeezed into contorted arches by mountain-building forces. Observations in these squeezed and folded beds have yielded knowledge of the permanence of the directions of magnetization.

One particular series of observations on Silurian rocks (350 million years old) that are well exposed at Hancock and Pinto, Maryland, is of special interest. In one of the folded beds, shown in plate 1, it was possible to obtain 20 suitably spaced measurements of the directions of magnetization. The directions were found to vary systematically throughout the fold in a manner that has an intimate relation to the attitude, or position in space, of each

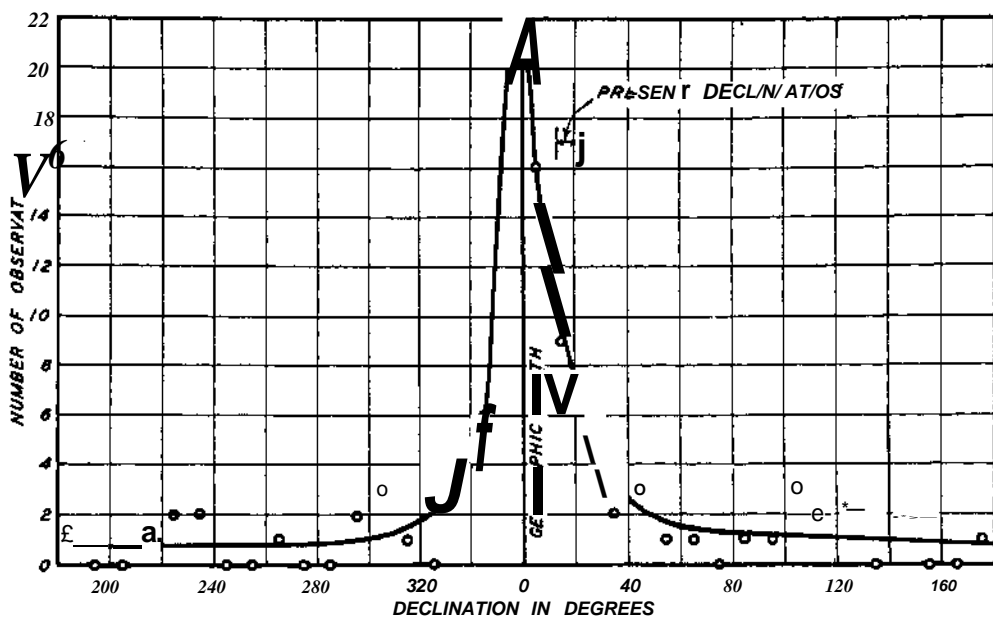


FIG. 1. Frequency distribution of declination measurements on rock samples

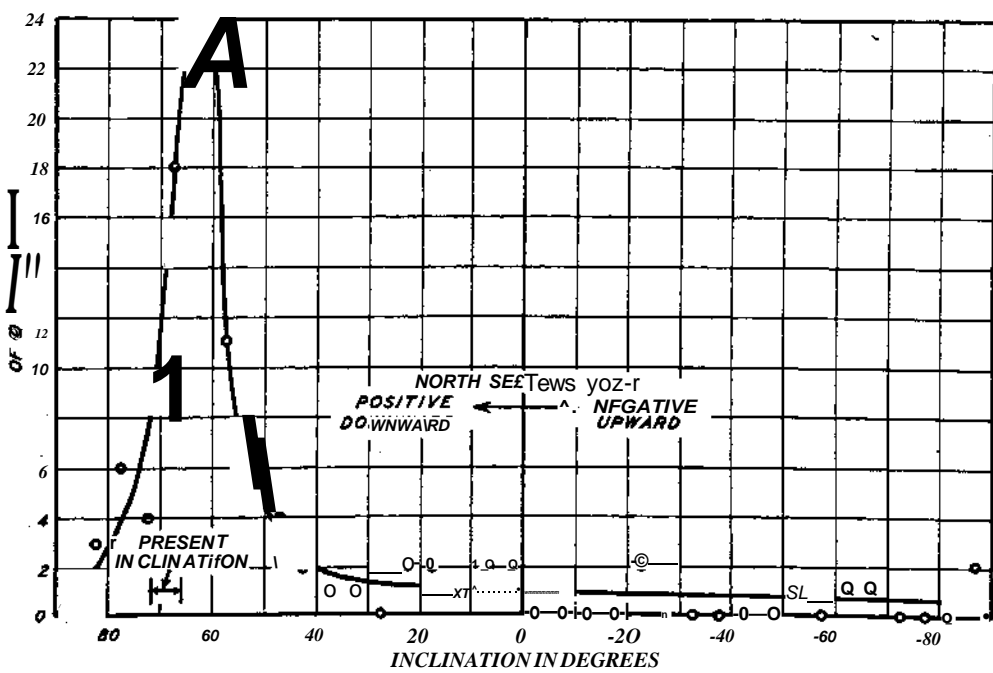


FIG. 2. Frequency distribution of inclination measurements on rock samples

part of the bed where an observation was made. The closeness of this relation is clearly brought out when a graphic reconstruction is made to restore the bed to its initial flat condition. Although the directions of magnetization in the fold are fanned out, and show differences in direction of as much as 127° , when the bed is made flat by a graphical transformation the magnetizations are all brought into general alignment so that these differences are usually less than 15° . This intimate relation of the directions of magnetization to the attitude of the bed is evidence that the bed was uniformly magnetized initially, that as it was folded it was merely flexed into its present configuration without yielding to shearing forces, and that since the time of the folding, 200 million years ago, the magnetizations at each point throughout the fold have not changed their initial directions.

There is still some question whether the reconstructed direction of magnetization of the fold at Pinto can be taken as a measure of the direction of the earth's field in Silurian time. The beds were laid down perhaps 150 million years before they were folded* and in that interval of time, long after deposition, they could have acquired their present reconstructed direction of magnetization. Further studies are needed to settle this point. There is evidence, however, that these beds acquired their magnetization, not in a field of a very local nature such as might surround a bolt of lightning or an intensely magnetized ore body, but in a field that was uniform over a large area, because beds of the same age at Hancock, 50 miles away, show the same reconstructed direction of magnetization. The surprising thing about the reconstructed direction of polarization is that the south-seeking pole is downward, contrary to the polarization of the present-day magnetic field, in which the north-seeking

pole is downward in the earth's northern hemisphere.

Along with this demonstration of stability, it has been possible to throw some light on the instability of samples stored in the laboratory. Some workers have considered that if a sample changes its direction of magnetization in the laboratory, it is inherently unstable and hence cannot be relied upon for deducing past directions of the earth's field. In the present work it has been observed that samples from the Silurian, as well as some of the younger samples from the western United States, also change when stored in the laboratory, and it has been possible to show that these changes are greatly accelerated by almost insignificant alternating magnetic fields. Stray fields of this kind exist in every laboratory building, but not in primitive geological areas. The mechanism by which such changes take place is not yet clear and requires further study.

New program. A new phase of the program of investigation now centers on determining in detail the regional character of the magnetization of Silurian rocks. It has been an astonishing experience to find that at two sites in the neighborhood of Washington there are rocks having their south-seeking poles directed downward. If it can be demonstrated that this anomalous magnetization prevails over large areas in a particular span of geologic time, a potent new tool will be at hand for studying some of the problems of earth physics.

Work on this phase of the program was initiated, under a special grant from the Carnegie Corporation of New York, by a reconnaissance study of Silurian rocks at Birmingham, Alabama, and the same anomalous magnetizations that were found at the Maryland sites were noted in a restricted series of beds. Below these beds two other major changes in magnetization of the Silurian rocks at Birmingham were



Folded Rose Hill formation, Pinto, Maryland

noted. Adequate fossils for precise dating were lacking at this site, and thus there is some question whether the rocks are strictly contemporaneous with those from Maryland.

Also, as part of the new work, an extensive sampling of the Hancock Silurian beds was made in the last few days of the report year. The data obtained indicate that in the 500 feet of deposition exposed at this site the anomalously magnetized beds occupy a middle section of approximately 200 feet, there being 100 feet above and 200 below which are magnetized much more nearly in the direction of the earth's present field.

Instrumentation. Experience with many different sediments has made clear the necessity for two basic improvements in instrumentation: greater sensitivity in the measuring apparatus, and a versatile core-drilling rig that will make it possible to sample exposures where the rocks are so massive that small oriented slabs cannot be removed easily by hand. A new remanent magnetometer has been built and shows promise of giving greater sensitivity and ease in making measurements. Since it contains many standard electronic parts, construction is relatively simple. A light-weight drilling rig is also ready for field use and, it is hoped, will make possible the study of many exposures that heretofore have had to be abandoned.

SEISMIC INVESTIGATIONS

As reported last year, a series of seismic observations from Solomons, Maryland, northwest to a distance of 300 km was carried out. When the times of the first arrived pulses were plotted as a function of their distance, it was seen that these times do not fall on a straight line, but that, beyond 130 km, each succeeding group of points appears to represent a wave

of greater velocity than that of the preceding set of points. This indicates an increase of velocity with depth, and it was possible to deduce (with the auxiliary confirmation of some arrivals later than the first or second ones) several different velocities and a multiple-layered structure down to the Mohorovičić discontinuity at a depth of some 40 km. The velocity of compressional waves in the material near the surface appeared to be 6.1 km/sec, while that below the Mohorovičić discontinuity was 8.1 km/sec.

One of the more interesting aspects of the data was a group of strong second arrivals, observed at distances of from 90 to 120 km, which came in about 2 seconds after the first arrivals. The best explanation at hand, based on the earth model deduced from all the other data, was that this arrival could be a shear-type wave (S) generated from the partial conversion of a compressional-type wave (P) at the interface between the bottom of the upper layer of rock and the top of the first intermediate layer at a depth of 10 km, the wave being called P10S. It was necessary to collect more observations in order to make sure that this peculiar wave was not a chance interference; observations were obtained at intervals of 5 km in this intensity region. The arrivals were as clearly discernible as those previously observed and seemed to furnish convincing evidence that the phase was real and due rather to the earth structure than to a fortuitous interference. This wave, if it could be proved a converted P-S wave as interpreted, might be an exceedingly useful one, because a generated S wave must rise at a steep angle from the interface, and from the difference in the arrival between it and the first arrival, the depth of the layer could be deduced with considerable precision. Thus the desired type of exploring probe would have been attained. The validity of this Interpret,!

tion was tested, however, using a new method of recording the seismic waves, and these tests showed during the past report year that this conspicuous arrival is in reality a compressional wave (P wave), not a shear wave (S wave), and hence is not a P₁ and will not serve as the desired "probe."

It thus became necessary to reconsider the data. In the meantime investigations had been made on the amplitude of the seismic waves due to the routine calibration of the amplifiers at each observation point, with a single electric pulse generator. From these data it was found that the amplitude of this second arrival rose somewhat abruptly and then dropped *off* with distance. The observations of the abrupt rise were somewhat hampered by the existence of unconsolidated sediments ending at about 80 km from the source of explosion and by the presence of a city (Washington, D. C.)—a locality of vigorous seismic unrest—at the distance of interest. The rather abrupt appearance of this wave and its P-wave characteristics seemed to indicate that it was a critical reflection as first predicted by Knott in 1899. Trial solutions showed that if the velocity below the Mohorovičić discontinuity was taken as 8.1 km/sec (as measured), and a depth of some 33 km and a velocity of 7.1 km/sec just above the discontinuity were assumed, good agreement was obtained with the data, which, though consistent in themselves, need further checking with shots so placed that the seismic waves may travel in directions other than the ones normally observed. It is hoped that, with the aid of the Navy, the Department may establish its own "quarry." For this a water-filled unused stone quarry, far from any habitation, is needed. A typical geophysical problem is here presented: the interpretation of a set of data, with the open question of how

closely this agrees with the actual properties of the earth. More measurements are necessary.

In the latter part of July 1948 the Department was notified that the Tennessee Valley Authority was about to set off 500,000 pounds of explosive for the purpose of obtaining crushed rock for a large dam on the South Holston River at a site 5 miles from Bristol, Tennessee. This turned out to be the first of three large shots. The other two were of 1,500,000 pounds and 800,000 pounds, fired in October 1948 and February 1949, respectively. They furnished an opportunity to obtain precision seismographic observations out to 1500 km. Excellent seismograms were obtained, roughly in two lines radiating northeast to Maine and northwest to Wisconsin from the dam: to the northeast, 15 seismograms from 250 to 1200 km, and to the northwest, 9 seismograms from 90 to 800 km. One other seismogram was obtained by the New Mexico School of Mines at a distance of 1500 km west. The success of these measurements was due in large part to the co-operation of the Tennessee Valley Authority. Some of the observations were made by colleagues at Columbia, and several stations were occupied by the Office of Naval Research.

The first result obtained from these observations was the measurement of the travel of the compressional (P) wave through the ultrabasic rock just under the Mohorovičić discontinuity. This ray of the wave first arriving at distant observation points takes a downward path from the explosion through the intermediate layers. It is then refracted at the Mohorovičić discontinuity and extends almost parallel to this level and not far below it, and near the observation point it is refracted once more into the intermediate layer and proceeds to the surface. If the crust were uniform and the rock just under it homogene-

ous, then the arrival times at the various stations would lie along a straight line in a time-distance plot. But the actual observations, accurate to 0.02 second, diverged as much as ± 1 second from the straight line of the mean travel time. This indicated a nonuniformity which could be due to differences in crustal velocities, in crustal structure, in total crustal thickness, or in the propagation velocity under the Mohorovičić discontinuity. For the final shot the stations were distributed from 90 km out to 900 km, and they gave evidence of a critical reflection similar to that which was observed from the shots from Solomons. The indicated depth is greater, however, under the region northwest from South Holston. The tentative conclusion is that the data indicate among other things a greater depth of the Mohorovičić discontinuity under the Appalachian Mountains, particularly northeast and northwest of South Holston at distances out to 500 km. This cannot be regarded as a definite result, however, until more work has been done on the upper crustal layers near South Holston.

THE EARTH'S ATMOSPHERE

THE EARTH'S ELECTRIC FIELD

Thunderstorm Investigation

During the thunderstorm season of 1947, four airplane flights were made over thunderstorms, but records from only three of these were satisfactory for analysis. These results, as given in last year's report, were regarded as provisional. After the close of the 1947 season, instruments were improved and the airplane was stripped of armament, so that higher altitudes could be reached and maintained.

During the 1948 thunderstorm season, again with the co-operation of the U. S. Air Forces, successful flights were made

during July, August, and September from the Clinton County Air Base, Wilmington, Ohio, guided by the V-beam radar installation at near-by Jamestown. During October, additional storms were surveyed utilizing Tinker Field Air Base, Oklahoma City, Oklahoma, as the center of operation. During the season 21 thunderstorms were surveyed and 65 traverses were made over the centers of the storms. Results of the season's operations may be summarized as follows:

The electrical conductivity of the atmosphere increases with altitude up to the limit surveyed (48,000 feet) in a manner consistent with that found on the balloon flight of *TL Explorer II*. This variation may be expressed closely by an equation of the general form $A = A_0 - f^2 A^2$, where Xh and A_0 are the positive conductivities (at height h and at ground level, respectively). A is a constant with a mean value of 2.0×10^{-8} , if h is expressed in feet, but varied on 6 flights from 1.6×10^{-8} to 2.4×10^{-8} . The mean value of A_0 is 0.7×10^{-4} esu, but it varied on 6 flights from 0.4×10^{-4} to 1.4×10^{-4} esu.

The conductivity over the thunderheads surveyed appeared to be similar to that found at the same altitude away from the strong fields of the thunderhead.

The vertical current passing through the storms surveyed varied from a small fraction of an ampere to more than 6 amperes.

The arithmetical mean of the total current taken over the 21 storms is 1 ampere. The arithmetical mean, regarding each of the 65 traverses as independent, also amounts to 1 ampere.

The direction of the current on all 65 traverses was such as to return negative charge to ground. This is opposite to the sign of the air-earth current over fair-weather areas.

The sign and magnitude of the current, if the storms surveyed on this project can

be regarded as typical, are such as just to maintain, by thunderstorm activity, the negative charge of the earth, which is demonstrated on a world-wide basis by an air-earth current of the same sign over all fair-weather areas. These studies provide the experimental basis for a satisfactory explanation of this long-standing puzzle of the maintenance of the earth's negative electrical charge.

Atmospheric Conductivity in Peru

The atmospheric-electric data recorded at Huancayo during the winter of 1947 have been thoroughly studied with the object of determining the causes of the large diurnal variation in atmospheric conductivity which has been recorded at Huancayo for some years. There is a variation by a factor of about 3 between the maximum and minimum values.

The negative conductivity shows a greater variation than the positive. This has been found to be due to a diurnal variation in negative small ion mobility, which varies in such a way as to augment the effect of the variation in negative small ion content. The mobility of the positive small ions remains constant, so that the diurnal variation of positive conductivity is due entirely to variation of positive small ion content, which, in fact, is greater than the variation of negative small ion content. Average values of mobility from all data are: $\mu_+ = 2.3$ cm²/volt/sec, $\mu_- = 2.9$ cm²/volt/sec. Reduced to sea-level pressure, these values are: $\mu_+ = 1.5$ cm²/volt/sec, $\mu_- = 2.0$ cm²/volt/sec.

Values of positive small ion content were found to fit the formula of ion balance: $q = \alpha n_1 n_2 + \beta n_1 n_3 + \gamma n_1 N_1 + \delta n_1 n_2 n_3 / N_1$, where q is ionization, n_1 positive small ion content, n_2 negative small ion content, n_3 negative intermediate ion content, N_1 negative large ion content, N_2

neutral condensation nuclei content, and $\alpha, \beta, \gamma, \delta$ are the appropriate combination coefficients. These data furnish the following values of the combination coefficients: $\alpha = 0.9 \times 10^{-6}$ cc/sec, $\beta = 4.6 \times 10^{-6}$, $\gamma = 0.4 \times 10^{-6}$, and $\delta = 3.3 \times 10^{-6}$ in the period 5^h to 10^h and 10.5×10^{-6} in the period 14^h to 18^h, with intermediate values from 10^h to 14^h.

These figures show that the variation in small ion content can be attributed to the following causes in the proportion shown: variation in ionization 25 per cent, variation in intermediate ion content 12 per cent, variation in large ion content 63 per cent.

There is strong evidence that the change in μ_2 is due to the presence of more than one type of intermediate ion and an increase in the proportion of the less mobile types from morning to afternoon. On the other hand, values of μ_2 derived from day- and night-time data are surprisingly consistent.

These results are calculated without correcting the measured values of q for the fact that sources of alpha rays are excluded from the inside of the chamber. This correction will increase them by a factor of about 5/3.

If a correction is made for the exclusion of alpha-ray sources, both the total ionization figures and the percentage due to alpha rays are found to be unusually high. The table on the following page summarizes the results.

UPPER ATMOSPHERIC RESEARCH

Work directed toward exploratory geophysics of the upper atmosphere has progressed substantially along the lines indicated in the report for 1947-1948. Major effort has been devoted to the design, assembly, and testing of electronic devices and components required for the conduct

	DAY		NIGHT	
	Ionpairs/ cc/sec	Per cent	Ionpairs/ cc/sec	Per cent
Alpha rays in air...	30.0	76	38.4	80
Gamma rays in air	1.4	4	1.8	4
Gamma rays from ground and in- herent ionization of chamber.	2.8	7	2.8	6
Cosmic rays	5.0	13	5.0	10
Total	39.2	100	48.0	100

of special experiments utilizing electromagnetic waves for exploration of the upper atmosphere. An experiment to determine the precise time of certain sunrise characteristics of the ionosphere has revealed new facts concerning the structure of the upper atmosphere. Improved laboratory facilities at the Derwood Experimental Laboratory have materially raised the efficiency of operation.

Panoramic Recording of the Ionosphere

Development of a prototype high-speed ionospheric recorder has continued with the following objectives: (1) ability to explore the ionosphere at intervals of a few seconds in order to record rapidly changing phenomena; (2) maximum flexibility of operation over the entire frequency range, 1-20 Me, or any portion thereof; and (3) completely self-contained standards of time and frequency assuring dependable performance under a wide range of operating conditions.

Although technical details of instrumental improvements are included in other reports, it is of interest to note that a technique has been developed which makes it possible to approach the ultimate theoretical limit of resolution. When an ionospheric recorder is swept over a wide band

of frequencies in a few seconds, the difference in frequency between a given transmitted pulse (and the corresponding receiver tuning) and the ionospheric echo from an earlier pulse becomes a limiting factor. This "detuning effect" is dependent on rate of change of frequency between pulses, velocity of propagation of radio waves, and maximum height range of the instrument. The new development automatically compensates for the frequency change between pulses, with the result that scanning speeds can be increased and receiver performance improved through better ratios of signal to noise.

Construction of two ionospheric recorders for operation in arctic and equatorial regions has continued. Many of the components have been completed and others are well advanced. All important instrumental design problems have been solved, although some mechanical problems such as antenna structures for field installations are still being studied.

The sunrise experiment of May 5 to June 10, 1949 has revealed new facts concerning properties of the outer atmosphere. It has also demonstrated the adaptability of a high-speed recorder for the precise timing of events within the ionosphere. Recordings were made daily from 04^h 30^m to 05^h 45^m at intervals of 5 or 10 seconds. Analyses were performed to determine the precise time at which a characteristic "sunrise effect" was observable in the F region. Although the time of sunrise in the F region was at least 1% hours before ground sunrise, no increase of F-region ionization or other characteristic "sunrise effect" was observed until approximately 20 minutes after ground sunrise. This corresponds to a grazing angle of 5° or a solar zenith angle of 85°, representing a limiting angle at which the F region must be inclined to the sun's rays before a sunrise effect is observed from the ground. Nearer to the

time of ground sunrise it is inferred that the ionizing energy of the sun's rays is completely absorbed in the already illuminated atmosphere.

Several additional occurrences of ionospheric "clouds" and rapid fluctuations in ionization were recorded during the above-mentioned observing period. The dates of occurrences corresponded to magnetically quiet periods, in contrast with the magnetically disturbed interval of the original report on this phenomenon. The cause of such ionospheric "clouds" is still open to conjecture, although some evidence appears to be accumulating in favor of horizontal wind motions rather than clouds of extraterrestrial origin.

Duplex-Channel Differential Recorder

The basic principles and fundamental objective of the duplex-channel differential recorder were described in the report for 1947-1948. It will be recalled that the basic idea underlying the new development involves simultaneous transmission of radio waves on two channels separated by a small increment of wave frequency. The instrument is adjusted to record a selected characteristic of the ionosphere. The device "locks on and follows" the time variations of this characteristic and records the data on a pen-and-ink chart for immediate and greatly simplified analysis.

The computer section of this instrument, which incorporates complicated electronic counting and timing devices, has been finished and tested. Detailed circuit diagrams and descriptions of operating sequence have been prepared. Other basic circuits for all except the radio frequency components have been tested in preliminary assemblies. Operation of this instrument as an integral unit is anticipated early in the ensuing report period.

Experimental Cosmic-Ray Research

Large ionization chamber. The large ionization chamber installed at the Derwood Experimental Laboratory has been operating for several months, providing sufficient statistical data to determine the level of statistical fluctuations with the upper half of the chamber unshielded. As anticipated, large variations were found; these are doubtless due to changes in the local radioactive content of the air. This confirms the expected necessity of completely shielding the instrument with lead to eliminate these variations, which are large enough to obscure the solar-flare effects that the meter was designed to detect. Lead shielding is now being installed on the upper half of the meter.

Neutron chamber for cosmic rays. It is planned to use two available Compton-Bennett ionization chambers to record variations in cosmic-ray neutron flux. One chamber will contain $B^{10}F_3$ with enriched boron 10, and the other $B^{11}F_3$ with enriched boron 11, both isotopes now being available from Oak Ridge. Neutrons, slowed down by hydrogenous shields, are captured in boron 10 with emission of alpha particles, the ionization of which is recorded. No neutron capture occurs in the boron 11; thus by recording the difference in ionization between the two chambers, all ionization except that due to neutrons is canceled out. It is expected that such a neutron intensity recorder, especially at a high-altitude station, may be much more sensitive to changes in cosmic-ray intensity associated with solar flares, magnetic storms, and other causes than are the Compton-Bennett meters, which mainly measure meson intensity.

Worldwide network of Compton-Bennett meters. Continuous recording of cosmic-ray ionization in Compton-Bennett meters was effected at Godhavn (Green-

land), Cheltenham (Maryland, U. S. A.), Huancayo (Peru), and Christchurch (New Zealand).

A new control box for Compton-Bennett meter no. C-4 was completed. This meter, badly damaged in transit from Teoloyucan (Mexico), has been thoroughly overhauled, assembled, and filled with argon. Comparisons between meter C-4 and meter C-i are now in progress at Cheltenham to insure that quantitative changes in intensity at Climax and at Cheltenham due to solar

flares may be reliably compared. Since there is little difference in latitude and about 11,000 feet difference in elevation, quantitative comparison of solar-flare effects at these two stations should provide some indication of the nature of cosmic-radiation changes associated with solar flares. Dr. Walter O. Roberts, Director of the High Altitude Observatory at Climax, Colorado, has kindly co-operated in providing space for meter C-4, and will endeavor to insure its continuous operation.

THEORETICAL AND STATISTICAL STUDIES

COSMIC-RAY RESEARCH

Mechanism for the Solar-Flare Effect on Cosmic Rays

Work was continued in collaboration with Dr. M. S. Vallarta on a mechanism to explain the marked increase in cosmic-ray intensity observed during a few solar flares. As mentioned in previous annual reports, the rate of change of the magnetic moments of sunspot pairs appears sufficient to provide protons with enough energy, by a process analogous to that of the betatron, to reach the earth at latitudes where the cosmic-ray increases during the solar flares were observed. Except in unusual circumstances, protons of this energy, about 7 Bev, would escape from the sun only at regions very near the poles, and not at regions nearer the sun's equator, where flares are observed. The magnetic moment, however, of a pair or group of sunspots seems to provide the necessary and unusual circumstance permitting the particles to escape. To be certain whether the magnetic moment of sunspot pairs makes it possible for protons of the energy involved to escape through the sun's general magnetic field requires the integration of the equations of motion of a charged particle in the combined magnetic field of the sun

and of the sunspot. The equations of motion were set up in Cartesian co-ordinates and several points along a trajectory were obtained by numerical integration using two different numerical schedules, one of which was kindly furnished by Dr. J. von Neumann to insure that the numerical schedule could be handled by the Eniac electronic computer at Aberdeen, Maryland.

From results of a few numerical integrations, obtained with ordinary mechanical computing machines, it was found that only a very short interval of integration (arc length along a trajectory) could be used if the errors of integration were to be kept sufficiently small. This was due to the fact that the trajectory of the particle is roughly helical, with radius small compared with the solar radius. Consequently, to compute a trajectory long enough to determine whether the particle escapes from the sun would have taken, even for the Eniac, a prohibitively large number of integrations. Since the motion of the particle is approximately helical, the equations of motion have been transformed into helical co-ordinates. Parameters in the equations allow for the expected changes in the pitch and radius of the helix along the trajectory. In this system of co-ordinates it is expected

that, for a given accuracy, a much greater interval of integrations can be used than was the case for the integrations carried out in a Cartesian system. Work on the numerical schedule for the integration is now being started. By the fall of 1949 the results of the preliminary integrations should determine whether the problem can be solved in a reasonable time on the Eniac. The possibility of investigating the tunnel problem on the magnetized model of Malmfors and Alfvén was examined also. The model, because of its limited magnetic moment and the lower limit of useful electron energy for the beam, could only simulate the actual problem for a sun with magnetic moment much less than 10^{34} gauss cm^3 , the figure indicated by the cosmic-ray latitude variation, or for protons of energy much greater than the 7 Bev indicated by the latitudes at which solar-flare effects on cosmic-ray intensity were observed.

*Search for Systematic Variations in
Cosmic-Ray Intensity*

It has been pointed out by M. S. Vallarta and O. Godart that periodic variations of small amplitude are to be expected if the sun has a total steady magnetic moment of 10^{34} gauss cm^3 , as required to explain the knee of the latitude variation in cosmic-ray intensity. They have also indicated that a periodic 27-day variation in intensity would occur if the solar magnetic moment were sufficiently inclined to the axis of rotation, since in that case the solar cut-off energy would vary with the period of the sun's rotation. As a consequence of the inclination of the sun's axis of rotation to the ecliptic, a 6-month periodic variation was predicted whether or not the rotational and magnetic axes coincide. Data from several Compton-Bennett meters, obtained over a period of ten years or more,

were subjected to analyses to determine whether any of the predicted variations could be regarded as statistically significant. The 27-day variation was found to be quasi-periodic like the 27-day recurrence phenomenon in terrestrial magnetic activity. The amplitude of the periodic 27-day wave was too small to be regarded as statistically significant. The 6-month variation had maxima in reasonable agreement with the predicted times of maxima, although the amplitude was too small to be regarded as statistically significant. The results obtained therefore do not confirm, although they clearly do not deny, the existence of the periodic variations predicted on the basis of a permanent solar magnetic field.

Cosmic-ray data covering a period of about ten years were analyzed for a sidereal diurnal variation. Some indication of a sidereal wave was obtained, but its statistical significance has yet to be tested. The influence of the earth's magnetic field, however, on the interpretation of an apparent sidereal variation has only been worked out (Vallarta and others) for cosmic rays arriving vertically at the geomagnetic equator. This work shows that coincidence telescopes would be much better suited for interpretation than are Compton nondirectional meters.

GEOMAGNETIC STUDIES

An examination was made of the worldwide pattern of abrupt magnetic-field changes during the simultaneous initial phases, or "sudden commencements," of intense magnetic storms. According to the Chapman-Ferraro theory of magnetic storms, the electric currents causing the sudden commencement flow well beyond the atmosphere. These currents, which produce a field at the earth like that of an external dipole, and the effects of the

electromagnetic shielding of the ionosphere have been estimated by Price. Examination of the two sudden commencements which occurred during the Polar Year 1932-1933 has yielded results indicating agreement with theory, and the fields of two more sudden commencements are now being examined in an effort to draw more certain conclusions from the study. This work was begun with the co-operation of Sr. Mateo Casaverde, of the Instituto Geofísico de Huancayo, while he was a guest of the Department.

The westward motion of the magnetic dipole best fitting the earth's main field was investigated. This dipole is displaced from the center of the earth about 300 km in the direction of the East Indies. Since 1840, its motion, as shown from spherical harmonic analyses, has been westward at the rapid rate of about 0.25° of longitude per year. From the indications of declination charts, this motion seems to have been continuing at about this rate since AD 1600. This westward shift and the accompanying shifts of the irregularities in the earth's field have an important bearing on theories of the earth's main field; an internal structure deep in the earth is retarded, in its rotation about the axis, more than the crust is retarded. This gives us clues to dynamical properties of the core. This work is being continued with special reference to the results of varve measurements, which provide magnetic data over a longer time scale.

The weekly staff discussions on geophysical problems mentioned in last year's report were continued with active participation of other colleagues in the Washington area. During the fall of 1948, Professor V. C. A. Ferraro, of the University of the South West, Exeter, England, gave a series of lectures, followed by discussion, on theories of magnetic storms and aurora and of the ionosphere. The focus of attention

was the formulation of new problems susceptible of theoretical or experimental approach with particular reference to the electrical state of the upper atmosphere.

Hourly Frequency of "Sudden Commencements"

A statistical examination of the hourly frequency of geomagnetic "sudden commencements" was initiated in order to check the rather striking results obtained by Newton from his analysis of the Greenwich magnetograms. All available traces from several observatories in various geomagnetic latitudes and longitudes were examined, and it was found that only stations of latitude above about 30° produced curves which showed a systematic diurnal variation. When only sudden commencements which were followed by the larger magnetic storms were considered, this diurnal variation was more pronounced. A tabulation was also made of the ratio of those sudden commencements having a small preliminary impulse (Newton's sudden commencements) to all sudden commencements, and the results from seven observatories appeared to show that this ratio varies with geomagnetic longitude. Further data are being sought from the magnetograms of other observatories in strategic positions in order to reach a definite conclusion on this point.

Portable Magnetic Observatory

Active experimental study is being given to the development of an automatic, long-run, portable magnetograph capable of being set up in an isolated place and left to record the three geomagnetic elements without attention for several months. The basis of this proposed new instrument is the portable magnetograph developed during the war, with such modifications as appear necessary for the desired prototype

of a group of general-purpose recording instruments which can provide the information ordinarily provided only by a permanent magnetic observatory and staff.

LABORATORY PHYSICS

NUCLEAR PHYSICS

The major emphasis during the past year has been on the problem of proton-proton scattering in the relatively low-energy region of 200 to 500 kilovolts, using the one-million-volt electrostatic generator. This is the region which will be most sensitive to the exact shape of the potential well which is assumed in each analysis of proton-proton scattering; it will respond particularly to a possible long-range "tail" on the steep-walled potential well. This is a difficult region of energy for measuring proton scattering because of the difficulty in observing protons of low energy and in measuring accurately the number of protons in the incident beam during operation, with hydrogen gas necessarily present *in* the scattering chamber. A considerable measure of progress has been made during the year on these problems. Proportional counters have been built and operated which will reliably count protons having as low as 90,000 electron volts energy. It has been found that the proton beam current can be monitored by allowing the beam, after passing through the scattering volume, to bombard a lithium target; observing the number of alpha particles emitted in the disintegration of lithium of mass 7, the number of such particles will be proportional to the proton beam current. Preliminary scattering data have been taken in the region 300,000 volts. Efforts are now being directed toward reducing possible systematic errors in the data.

The high-resistance column used for measuring voltages on the one-million-volt generator has been overhauled during the

year. New Western Electric precision resistors were installed, and the assembled unit was carefully calibrated against the sharp gamma-ray resonances from boron, lithium, and fluorine bombarded by protons.

During the early part of the year, in cooperation with Dr. D. R. Inglis, of Johns Hopkins University, and Dr. E. M. Hafner, of Brookhaven National Laboratory, experiments were completed on the study of the proton groups emitted in the disintegration of carbon of mass 12, bombarded by deuterons. The yield and angular distribution curves of the protons were obtained for deuteron energies from 1 to 3.5 million electron volts. This work illustrated the complexity of the nuclear energy-level system in this region of the atomic table. As many as 21 levels of the intermediate nucleus (nitrogen 14) were observed.

BIOPHYSICS

The biophysics group continues to approach biological problems from the viewpoint of physics. The group is more convinced than ever of the benefits to be derived from cross-fertilization between the various disciplines. In addition to the three staff members of the group who are physicists by training, there are present among visiting investigators and fellows a cell physiologist, two biochemists, and an organic chemist.

To gain a more solid background for the biophysics program* a considerable effort has been made and is continuing in group study. In one series of seminars the book entitled *Dynamic aspects of biochemistry*, by Baldwin, was thoroughly covered. In

a second series, *Radiation effects*, by Lea, was presented. In addition, a number of outside visitors and lecturers presented other facets. Among the guests were Margaret Murray, B. A. d'Houssay, David Pressman, Alan A. Boyden, A. Szent Györgyi, William Libby, L. H. Grey, J. W. Boag, Britton Chance, and Robert Briggs.

The biophysics program is continuing to evolve. One of the principal preoccupations is the formulation of questions and modes of approach to central problems in biology in terms appropriate to men trained in physical sciences. The nature of some of the basic puzzles encountered by physical scientists in living matter has been examined. This educative process has given rise to focusing of attention on a number of points generally related to the problem of "early life." The role of metals which have the capacity for change of valence as active centers of enzymes and coenzymes, and the importance of trace elements as clues to biological processes, both currently and in the very distant past, are examples of the type of problem which has attracted interest and roused extended discussion. These studies of biological processes have brought the biophysics group into close contact with the men concerned with geochemistry, and the resulting discussions have outlined a new field of research relating to the early history of the earth.

While examining and formulating larger problems, the group has continued to make a considerable number of specific research contributions. The work during the report year has largely been concerned with metabolic and physiological studies using radioactive isotopes, and with studies of biological effects of radiation. Among the results of projects under way or reaching completion are the following:

Studies employing proteins labeled with radioactive iron (in collaboration with

Louis B. Flexner, of the Institution's Department of Embryology, and Gilbert J. Vosburgh, of the Department of Obstetrics, Johns Hopkins University and Hospital) have thrown new light on the turnover rate from the plasma of the guinea pig to the extravascular fluid. The observed rate was slow (0.3 to 1 per cent per minute) in comparison with that previously found for electrolytes. The substance employed was ferric betai-globulinate, produced by biological synthesis from inorganic radioactive iron. Another investigation was a determination of the turnover rate of chloride ion from plasma to extravascular fluid space. The value found was a rate of 60 per cent per minute, which, perhaps significantly, is the same as that observed earlier for sodium. The two results when taken together clearly cast doubt on the "pore theory" of capillary permeability.

A further study was a comparison of the permeability of *Escherichia coli* to sodium and potassium ions. It was found that the cellular membrane is completely permeable to both sodium and potassium and that the sodium ions within the water space in the cell are in equilibrium with those in the medium. This water space constitutes 75 per cent of the volume of the cells. There is no fixation of sodium in resting, growing, or highly metabolizing cells. Potassium ions can also diffuse freely across the membrane, but the picture of pure diffusion is complicated by metabolic processes which fix the potassium in a nondiffusible form.

The importance of potassium in biological processes has long been recognized. Potassium, sulphur, and phosphorus are the most important chemical constituents of the ash of most cells, each one contributing roughly 30 per cent of the total* Potassium is also a major constituent of fertilizers, since it is known to be necessary to plant nutrition. Whereas, however, the

roles of phosphorus and sulphur are well recognized, the actual biochemical function of potassium is not at all clear.

It is known, for example, that potassium, unlike sodium (which is chemically similar), is concentrated by cells, and that the potassium once taken up by the cell is not readily removed. Consequently, it has been inferred that the potassium is bound in some way to some component of the cell. Compounds or complexes with proteins, carbohydrates, and fats have been postulated, but none of these has ever been isolated or identified.

Experiments at the Department using radioactive potassium produced by the cyclotron have given some new light on this problem. It was found that the incorporation of one molecule of glucose by cells of *E. coli* caused the binding of two atoms of potassium. As the glucose was broken down further, the potassium was released. Other experiments indicated that the potassium was bound in the cell as potassium salts of the hexose phosphates which are the immediate products of glucose metabolism. This hypothesis was further supported by the observation that during the period when two potassium atoms were bound, one phosphate group was transferred to the glucose molecule. Thus, potassium apparently participates in the major process by which the cell obtains energy from its food supply.

Furthermore, it was possible to obtain new information concerning the metabolism of glucose by observing the uptake and loss of potassium. Some of the results could be interpreted to show that a large fraction of the glucose is consumed by direct oxidation rather than by the more familiar and better-understood phosphorylative mechanism. In a system as delicate and complex as a cell, any new method of observation is highly valuable, particularly when it does not interfere in any

way with the normal metabolism of the cell. Further work along these lines is in progress using potassium and rubidium, together with phosphorus and sulphur.

Another project under way is a study of the rate of uptake of radioactive fluoride in normal enamel. The work (in collaboration with Dr. F. A. Arnold, Jr., and Dr. R. C. Lichens, of the Dental Research Institute, National Institutes of Health) includes a study of the chemistry of fixation of topically applied fluorine. The biophysics group is especially in a position to be helpful in this project. The tracer fluorine employed cannot be produced in the nuclear reactor; a cyclotron is required.

Further study of P^{32} uptake in *Arbacia* eggs has provided additional knowledge regarding the chemical form of the phosphorus immediately after it enters the cell. Fertilized eggs were immersed in sea water containing very small amounts of phosphorus in the form of inorganic phosphate. On chemical analysis after a few minutes' exposure, the P^{32} in the *tggs* was found in at least three fractions: inorganic phosphorus, adenosine triphosphate, and hexose monophosphate.

Studies on the influence of the thyroid on calcium metabolism were continued by Dr. H. H. Darby, Research Associate of the Institution, who worked at the Department throughout the year and participated in many of the activities and discussions in biophysics. The influence of vitamin D on the damage done to the thyroid by thiouracil was checked. The work reported a year ago was completely confirmed.

An attempt was made to determine the part of the thyroid on which vitamin D does its work, and whether the parathyroid is involved. Many researchers believe that the parathyroid is involved in calcium metabolism. In none of the animals to which the thiouracil was fed was there any

histological change in the parathyroid, but an immense change in the thyroid was found. When the vitamin D was fed, no histological change was observable in the parathyroid, whereas large changes were seen in the thyroid itself. The central areas of the thyroid seem to respond better to the vitamin D than do the peripheral areas, and there appears to be a difference between the anterior and the posterior part of the thyroid. Co-operative research with Dr. Pauline Beery Mack, of Pennsylvania State College, on X-ray density measurements of the epiphysis has led to interest in this type of work at the National Institutes of Health, and, to a lesser degree, at the Naval Medical Research Institute.

The essential mineral elements for the proper functioning of a biological system have in the past been thought to be the more abundant substances, such as calcium, sodium, potassium, iron. At the same time, the literature has many statements that a particular element has been found necessary in extremely small amounts for some particular animal. Animal husbandry and research have traced certain disease conditions to the shortage of such trace elements and have cured these conditions by the addition of extremely small amounts of the element to the animal's food. Many years ago in New Zealand, Australia, and Scotland a disease of sheep was shown definitely to occur when there was a shortage of cobalt. It was cured by the addition of cobalt sulphate to the pastures on which these sheep were fed. No reason was given for the necessity for this cobalt.

Recently in the continuation of the search for more B vitamins (the water-soluble vitamins), vitamin B13 was discovered and in the chemical breakdown was shown to contain cobalt. It was separately found that cobalt was required by the bacteria in the sheep's rumen, not

primarily by the sheep. Another significant finding at this time was that this active biological material B12, the pernicious anemia factor which had previously been thought to be found only in liver, was also found in the excreta of cows and fowls. A combination of the work in Scotland and Australia on cobalt and the B12 work in the United States and England now was possible, especially since radioactive cobalt was available from the Department's cyclotron. With this in mind, sheep were fed radioactive cobalt, and a collection was made of the excreta. This was then analyzed both for the cobalt and for B₁₂ using microbiological tests. The cobalt was found in the excreta in more than one chemically bound form, but one of the forms was that of B12.

The B12 molecule was then traced to its synthesis in the bacteria which occur in the rumen or first stomach of the sheep. It is interesting here to note that the cow is also a ruminant and that the fowl has a double type of stomach in the gizzard and the crop. Any two-compartmented stomach like this will give a much longer period for food to be acted upon by bacteria than will a single stomach. In these animals, therefore, the bacteria are playing a significant role in the production of necessary nutrients for the host animal. Other bacteria, including *E. coli*, were shown to take up cobalt and synthesize it into B12.

The fundamental characteristic that first attracted investigators to this substance was that it seemed to be necessary for the building of proteins. In fact, the first name given to it by the English workers was "animal protein factor." Since there is necessarily an immense amount of protein building of all types in the developing embryo, it was of interest to follow a B12 molecule from the digestive tract of a hen into the egg. Radioactive cobalt was given hens by mouth and was found to be dc-

posited in the yolk, in the albumen, and to a very much less extent in the shell and its inner lining. In this manner it was possible to show that such a trace element as cobalt, which was fed at the level of a few micrograms per day, appears in the egg and plays a significant role at a level of a hundredth of a microgram per gram. The whole field of biological significance of trace elements is of challenging interest. Especially important are those elements that are bivalent and can help in forming intermediary compounds, even if these are highly transitory and have so far escaped discovery in the chemistry of the test tube, which differs markedly from the chemistry of the body.

A series of studies of biological effects of radiation has been carried out by Dr. W. R. Duryee, of the National Cancer Institute, working at this laboratory. Through observations on transparent living amphibian ovarian eggs, he has been able to develop new and objective criteria of radiation damage. These involve chromosomal fragmentation, nuclear damage, and changes in nature of colloidal suspensions within the nuclei. Using these criteria, he has been able to show that by subjecting amphibia to temperatures of 6° C, appearance of radiation damage can be postponed for as much as two weeks. Using a microinjection technique, he could render normal cells abnormal by injection of radiated cytoplasm, whereas injection of nonirradiated cytoplasm did not produce nuclear damage.

The lethal effect of ultraviolet radiation on bacteria is well known, but the mechanism is not at all clear. During the past year it was found that after irradiation the

bacteria (*E. coli*) were highly sensitive to many factors in the environment which had no effect on normal bacteria. Under certain conditions the bacteria would recover from the effects of the radiation and remain viable. A study of these conditions indicated that the enzyme systems had been thrown out of balance. It is probable that this unbalance was caused by cellular poisons produced by photochemical reactions within the cell.

In December 1948 it became clear that a number of young nuclear physicists had suffered radiation injuries leading to lens cataracts. In view of the widespread interest in this tragic occurrence, the Department felt it desirable to foster a survey and assessment of the situation. It seemed particularly worth while to conduct the investigation in such a way as to avoid duplicating efforts and thus to prevent unnecessary trouble for the men involved.

Acting in collaboration with Professor P. Gerald Kruger, of the University of Illinois, and with the encouragement and support of the Division of Medical Sciences of the National Research Council, survey questionnaires were sent to the various high-voltage laboratories of the country. A three-day session was held in Washington, in January 1949, which was attended by all individuals known to be afflicted. This was followed by ophthalmological examination at Johns Hopkins. The Department subsequently prepared a report of the findings of the meeting for the National Research Council, which was distributed to all high-voltage laboratories. It is hoped that this effort may aid in the prevention of future injuries.

OPERATIONS AND STAFF

CO-OPERATIVE WORK OF THE DEPARTMENT

Co-operation has been continued with individuals and organizations in this country and abroad in accordance with the Institution's policy.

Research work on the earth's crust and cosmic rays has continued under Navy contracts N7onr-290 and Nyonr-^0,, respectively, with advantage to the Government as well as to the Institution. These contracts provide for co-operative activity and the loan of equipment, but conform to the policy of no direct cash subsidy to the Institution for added or existing staff or facilities. A new task order under the first contract provides for one member of the staff to engage in co-operative research with the Navy for a limited period on atmospheric-electric problems. Some members of the staff have continued to assist various offices of the Government as consultants; one has been on full-time leave of absence for the entire year, and another since April 1, 1949.

Although the observatories at Huancayo, Peru, and Watheroo, Western Australia, have been owned and operated by the Peruvian and Australian governments since July 1, 1947, the Department retains a lively interest in their activities and has been privileged to co-operate in matters of program policy and operational detail. Messrs. Casaverde and Fernandez of the Huancayo staff were in residence in Washington as Fellows of the Institution for six months each, to receive training at our laboratory and others in the Washington area.

Sounding balloons were supplied to Professor G. Bernardini in Italy for research work there.

The Department has continued to have the advantage of collaboration with Dr. Louis B. Flexner, of the Department of

Embryology, in the biophysics program, and with representatives of the National Institutes of Health, National Bureau of Standards, Applied Physics Laboratory of the Johns Hopkins University, Brookhaven National Laboratory, Wilmer Institute, Department of Obstetrics of the Johns Hopkins University, and Catholic, Georgetown, George Washington, Howard, and Tulane universities.

The biophysics group has continued its responsibility for the operation of the cyclotron and the production of radioactive isotopes, which have been distributed without charge to some forty different groups in this country and abroad.

Dr. M. S. Vallarta, of Mexico, has continued to collaborate with the Department in the investigation of sudden increases in cosmic radiation associated with solar flares. Dr. John R. von⁴ Neumann, of the Institute for Advanced Study, has also assisted in this work. In addition, four observatories, previously mentioned, have continued to operate the Compton-Bennett cosmic-ray meters.

Joint experiments in the seismic program of the Department and the Geophysical Laboratory were carried out in close collaboration with Columbia University and New Mexico School of Mines, as well as with Navy and Army groups. Again grateful acknowledgment is made to the Navy (Office of Naval Research and Bureau of Ordnance) for under-water explosions scheduled for the convenience of our observers, and to the Tennessee Valley Authority for the unusual opportunity offered by the three large blasts at the South Holston Dam site.

We are greatly indebted to the U. S. Geological Survey for valuable assistance in selection of localities and sites for the rock magnetism studies.

PUBLICATIONS

In addition to the scientific papers appearing in current literature, two volumes of the Researches of the Department have been published, containing results of cosmic-ray and earth-current observations; they are listed under "Major publications" in the bibliography.

It is intended to publish, as Publications of the Carnegie Institution of Washington, the magnetic results from the Watheroo and Huancayo observatories (which were transferred to the respective local government agencies on July 1, 1947) for the years 1945 to 1947, the results through 1945 having already been published. The preparation of these two final volumes is now under way.

ADMINISTRATION AND OPERATION

A special experimental license W3XAU was obtained for observations on meteors.

Five panel trucks are now on loan from the Navy for use in the seismic investigations.

A modest quantity of surplus electrical material was obtained through the District of Columbia Educational Agency for Surplus Property.

The *Journal of Geophysical Research*, edited by the Director, with the help of Mr. Walter E. Scott, received support from the Institution. This journal is the continuation of the *Journal of Terrestrial Magnetism and Atmospheric Electricity*, edited for many years by Dr. John A. Fleming.

The Department continues to have the advantage of visits from guest investigators for varying periods. These are included in the appended list, which also shows the regular members of the staff, most of whom were employed for the entire report year.

SEMINARS

The recent work of Urey, Brown, and Libby has clearly demonstrated the rich discoveries that can be made by applying modern physical techniques to geophysics. Several staff members of the Department visualized the possibility that a survey of geological problems on the basis of familiarity with new techniques of physics and chemistry would bring to light new and significant problems and opportunities. It was particularly obvious that a great gap in man's knowledge is the period between the origin of the earth and the Cambrian. Accordingly, with the collaboration of the Geological Survey and the Geophysical Laboratory, a seminar was organized which had for its principal theme "Milestones in the Pre-Cambrian." The subjects discussed, during March 4 to May 13, 1949, were: Harrison Brown, Isotopic analysis of the universe; R. B. Roberts, Origin of the earth; G. Gamow, Origin of the universe; W. D. Urry, Radioactivity and the age of the earth; W. D. Urry, The radioactivity of potassium; H. E. Tatel, Radioactivity and the earth's cooling; M. A. Tuve, The mass spectrometer; H. C. Urey, Separation of isotopes by chemical processes; P. H. Abelson, Separation of isotopes by physicochemical methods; H. C. Urey, Temperature measurements on fossil shells by use of isotopic analysis; D. B. Cowie, Dating in the earth's immediate past by use of C^{14} ; E. Ingerson, The Pre-Cambrian; E. Ingerson, Isotopic separation by geologic processes; R. B. Roberts, Creation of the earth's atmosphere; W. W. Rubcy, Origin of the ocean; H. H. Darby, The origin of life (as formulated by Gparin); M. A. Tuve, An alternative mode for the origin of life; L. H. Adams, The nature of the earth and its origin; N. L. Bowen, Formation of the earth's crust; W. W. Rubcy, Mountain building; W. H.

Bradley, The problem of the ocean basins.

Some of the interesting potential research problems arising out of the talks and discussions were: (1) The possibility of detecting evidence of life earlier than the fossils by studies of trace-element patterns in sedimentary rocks or Pre-Cambrian graphite. (2) The possibility of determining a geological level corresponding to the time of appearance of living matter on earth. Since the oxygen of the atmosphere is derived from biological activity, a drastic change in the earth's atmosphere must have occurred at that time. Simultaneously, isotopic abundances must have shifted slightly. (3) The possibility of determining whether the granites are of sedimentary or of magma origin by observations of the trace-element patterns in such rocks. (4) Fractionation of hydrogen isotopes as a measure of hydrogen escape from the atmosphere.

During the period September 24 to De-

cember 20, 1948, Dr. V. C. A. Ferraro, of England, gave a series of lectures on "Geomagnetic storms and the upper atmosphere."

LECTURES GIVEN BY VISITORS

September 3, 1948, "The cause of the earth's magnetism," by E. C. Bullard.

September 24, 1948, "Gravity and the isostatic structure of the earth," by W. Heiskanen.

October 20, 1948, "A mechanism to explain increases in cosmic-ray intensity during some solar flares," by M. S. Vallarta.

December 21, 1948, "Cosmic-ray investigations," by Marcel Schein.

February 23, 1949, "Cosmic-ray investigations in Italy," by G. Bernardini.

March 4, 1949, "Isotopic constitution of meteors," by Harrison Brown.

May 19, 1949, "Work with C¹⁴," by William F. Libby.

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- COWIE, D. B. See FLEXNER, L. B.; SMITH, R. E.; VOSBURGH, G. J.
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- See TORRESON, O. W.
- GREIG, J. W. See TUVE, M. A.
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- See HEYDENBURG, N. P.
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MAJOR PUBLICATIONS

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STAFF AND ORGANIZATION

SCIENTIFIC STAFF

Staff Members:

- Geophysics:* L. V. Berkner, S. E. Forbush, O. H. Gish (retired September 30, 1948), E. A. Johnson (on leave of absence for governmental research), W. J. Rooney, H. E. Tatel, O. W. Torreson, M. A. Tuve, E. H. Vestine, G. R. Wait, H. W. Wells.
- Laboratory and Biophysics:* P. H. Abelson, D. B. Cowie, N. P. Heydenburg, R. B. Roberts, M. A. Tuve.
- Guests, Associates, Fellows, and Visiting Investigators:* P. Bhattacharya, India; E. O. Bowles, American University; M. Casaverde, Geophysical Institute of Huancayo, Huancayo, Peru; S.K. Chakrabarty, India; H. H. Darby; Miss E. Dollman, Brookhaven National Laboratory; W. R. Duryee, National Institutes of Health; G. Fernandez, Geophysical Institute of Huancayo, Huancayo, Peru; V. C. A. Ferraro, University of the South West, Exeter, England; P. S. Gill, Tata Institute of Fundamental Research, Bombay, India; J. W. Graham, Johns Hopkins University; E. J. Habib, Catholic University; Miss

E. Hill, National Cancer Institute; R. Hossfeld, Catholic University; D. R. Inglis, Johns Hopkins University; F. Irreverre, Experimental Biology and Medicine Institute, U. S. Public Health Service; W. C. Knox, National Institutes of Health; T. Murphy, University College, Dublin, Eire; J. N. Nanda, India; A. T. Ness, U. S. Public Health Service; R. Nieset, Tulane University; W. D. Parkinson; Mrs. I. Z. Roberts, National Cancer Institute; F. L. Talbott, Catholic University; M. S. Vallarta, Instituto de Fisica, Universidad de México, D. F., and Comisión Impulsora y Coordinadora de la Investigación Científica, México, D. F.; P. Wang, National Bureau of Standards; W. D. Whitehead, Jr., University of Virginia; F. W. Wood, Australia.

OPERATING STAFF

- Administrative:* M. B. Smithy, W. F. Steiner.
- Office and Clerical:* Mrs. J. H. Campbell, J. J. Capello, W. N. Dove, H. D. Harradon, W. C. Hendrix, Miss H. E. Russell, A. D. Singer.

Instrument Shop: B. J. Haase, L. A. Horton,
J. G. Lorz.

*Research Assistants, Laboratory Assistants, and
Technicians:* Miss E. Aldous, S. J. Buynitzky,
J. B. Doak, P. A. Johnson, C. J. Ksanda, C. A.
Little, Jr., P. F. Michelsen, P. L. Moats, A. E.
Moebs,* W. C. Parkinson, R. W. Reuschlein,
Miss M. Sands, W. E. Scott.

* Resigned.

Computers: Miss E. Balsam, Miss I. Lange.

Maintenance: C. Balsam, C. W. Burger,* C.
Domton, S. W. Malvin (retired December 31,
1948), E. Quade, M. A. Quade.

Part-Time and Temporary Employees: There
were 18 part-time and temporary employees
engaged during the year, usually for short
periods, to assist in the office and laboratory
work.

SPECIAL PROJECTS: TERRESTRIAL SCIENCES

FRANK T. GUCKER, JR., Indiana University, Bloomington, Indiana. *Studies of specific heats of aqueous solutions up to high temperatures.* (For previous report see Year Book No. 46.)

This is a continuation of the study of specific heats of salt solutions up to 85° C, carried out in collaboration with Dr. Frank W. Lamb, during which there was found a maximum in the apparent molal heat capacities of three typical uni-univalent electrolytes at about 60° C., and a decrease at higher temperatures. This is exactly opposite to the increase above 65° predicted by F. Zwicky, on the basis of the theory of electrostriction and of Bridgman's P-V-T data for water, and shows that either the theory or the data require revision. An extension of the experiments up to the critical point (218 atmospheres and 375° C. for pure water) seemed desirable in order to supply data in a region where none are available, and where a theoretical interpretation may be simpler, since most of the anomalous properties of water become less pronounced at higher temperatures. The experiments require a new type of calorimeter, suitable for work at high temperatures and pressures. This year, with the collaboration of Mr. Jean M. Christens, a suitable bomb calorimetric apparatus has been designed and nearly completed, and the extensive auxiliary equipment necessary for these measurements has been assembled.

A differential system was chosen, with water as the reference substance in one of the twin calorimeters, and solution in the other. The heat capacity of the solution is measured relative to that of water, which has been studied carefully up to the critical point by Osborne, Stimson, and Ginnings at the National Bureau of Standards. Each calorimeter is provided with a single small

tube connected to the bottom, through which it can be evacuated and water or solution can be introduced. In order to avoid the formation of any vapor during an experiment, with consequent absorption of a large amount of heat, a constant hydrostatic pressure of about 220 atmospheres is maintained, measured by means of an accurate dead-weight gauge. A strong, thin calorimeter is obtained by constructing the shell of Inconel, in two nearly hemispherical halves, held together by means of a threaded ring at the center. A platinum lining prevents corrosion by the salt solutions under the severe experimental conditions. An electrical immersion heater with metal fins distributes the heat inside the calorimeter without the use of a stirrer, and a thick plating of silver on the outside of each calorimeter helps to equalize the surface temperature and to reduce radiation. The electrical heating is regulated to raise the temperature of the two calorimeters at practically the same rate, and the difference in temperature is measured by means of a 16-junction thermel connected to points distributed over the surface of each calorimeter.

The calorimeters are held within a steel case which can be evacuated to about 10^{-4} mm. of mercury to prevent thermal conduction. Within the case is a heavy copper adiabatic jacket surrounding the calorimeters, provided with electrical heaters to maintain the desired uniform temperature, measured by multiple thermels between it and the calorimeters and also by a platinum resistance thermometer. A series of thin, highly polished aluminum shields re-

duces the radiation between the calorimeters and the copper jacket, and a second series of these shields reduces the radiation to the outer case, which is near room temperature. The large evacuated space reduces the hazard from failure of the calorimeters, and a second steel case surrounds the whole apparatus as a further precaution.

The apparatus, with its bombs of known volume, dead-weight pressure gauge, and platinum resistance thermometer, may be used to measure the compressibility, ther-

mal expansibility, and vapor pressure of solutions, as well as their specific heats. The auxiliary apparatus also would be useful in the measurement of the dielectric constant of water over a wide range of pressure and temperature. It is hoped that such measurements can be carried out, since the pressure and temperature coefficients of the dielectric constant are needed to calculate the limiting slopes of the apparent molal heat capacities, volumes, compressibilities, and expansibilities of solutions.

DIVISION OF PLANT BIOLOGY

Stanford, California

C. STACY FRENCH, *Director*

The attack on photosynthesis has been concentrated primarily upon those parts of the initial steps in the photosynthetic process which involve the photochemical splitting of water molecules, rather than upon the ensuing reactions by which carbon dioxide is reduced. Thus the general program of one group of investigators has centered about the absorption of light by plant pigments, the possible transfer of the absorbed energy from one pigment to another, and the correlation of these events with the ultimate chemical changes produced in living plants.

A broad survey, extending over a period of years, of the specific chloroplast pigments which are found in the various groups of photosynthetic plants has just been completed. From this work it has become evident that the pigment complexes characteristic of the main plant families were developed early in the course of evolution, and have remained constant in spite of the great changes in the external form of plants.

Experiments with seedlings initially grown in the dark have shown that during their subsequent exposure to light the formation of chlorophyll is much more rapid than had previously been realized. Even with moderately weak light an appreciable fraction of the protochlorophyll is converted to chlorophyll *a* in a few seconds. From measurements of the effectiveness of different wave lengths of light on this transformation in corn and bean seedlings, it was found that only the light which is absorbed by protochlorophyll, and not that absorbed by any other pigments present, can lead to chlorophyll formation.

It is now considered likely that chlorophyll *b*, which develops later than chlorophyll *a*, does not originate from chlorophyll *a*. A study of the correlation of pigment formation with the development of other parts of the photosynthetic apparatus has been started.

The first detectable chemical effect resulting from absorption of light by the pigments of the photosynthetic apparatus of plants is the splitting of water, which results in oxygen evolution and the reduction of some unidentified intermediate. For the experimental investigation of the reaction, it is possible to replace the unknown natural intermediate by certain common chemicals, such as a reducible dye. By ejecting a suspension of chloroplasts at high pressure through a fine needle valve, one obtains colloidal suspensions of material the particle size of which is much smaller than that of the so-called grana. This material is in a form suitable for chemical studies of the water-splitting step of photosynthesis. The activity of such a preparation is about one-third that of the original chloroplast suspension. Nearly all the original activity can be restored, however, by treatments which cause a partial reaggregation of the individual colloidal particles. Thus there are at hand some means for correlating morphology and function at a size level between the molecular and the microscopically visible ranges.

The suggestion that controlled cultures of algae may become a valuable source of foodstuffs has received rather surprising and widespread attention. This interest in a process deviating from traditional meth-

ods of food production is no doubt due to the compelling nature of the present problems of world food supply and the concern which has been aroused by popular discussion of the social factors involved in these problems.

Some of the more important constituents of the products formed by *Chlorella* have been established: of the carbohydrates, starch and cane sugar have been isolated; of the lipids, the fatty acids have been identified; and it has been found that all the ten essential amino acids are present. From an industrial viewpoint, however, a great deal remains to be learned regarding the practicability of producing *Chlorella* on a large scale and the use that may be made of its products. These problems are essentially of an engineering nature. For their further investigation an arrangement has been effected between the Carnegie Institution of Washington and the Research Corporation. The latter has, in turn, given financial support to the Stanford Research Institute to make an intensive study of the process and the products on a pre-pilot plant scale, with the cooperation of the Carnegie Institution of Washington.

The records of the transplant and crossing experiments on *Potentilla glandulosa* discussed in last year's report have been further analyzed. The genetic linkage between various morphological characters and certain physiological variations upon which the natural distribution depends has been thus established. Species which are widely distributed in various environments are able to fit these diverse conditions because such species consist of many physiologically and genetically distinct races. Each of these races fits a different set of environmental conditions. A better understanding of evolution should be attained by the study of the **genic-physiological** relationships of suitable plant groups.

The effective prosecution of such experiments is dependent upon the utilization of species which show a wide range of form and adaptation to different environments and which grow rapidly. The suitability of the genus *Mimulus* for such work is being tested by transplanting, by crossing, and by morphological observation.

The success of the Edinburgh plantings of a number of the strains of range-grass hybrids developed by the Division has led to an increase in the number of strains tested there by the Scottish Society for Research in Plant Breeding and to the undertaking of many similar plantings to supplement those made by the Soil Conservation Service of the U. S. Department of Agriculture. Thus co-operative experiments on forty-six strains have been arranged with a number of widely scattered laboratories. This has been done to test the climatic adaptability of *Poa* hybrids over a wide range of climatic conditions such as are found in the state of Washington, southern California, North Carolina, Scotland, Wales, Holland, Denmark, Norway, and Sweden. No universally outstanding strain has been produced, but several of the selected strains show excellent performance in a number of environments. Under different environments different strains are observed to give superior growth. Fortunately, many of these improved strains breed true from seed, thus being easy to distribute. A detailed cytological investigation is being conducted on the mechanism of seed and of pollen formation and on the way in which these processes are influenced by the environment. Some of these grass hybrids of particular interest have been selected and are being grown in preparation for a quantitative experiment on their growth response in controlled laboratory environments.

The taxonomic treatment of western

North American Poas rounds out the comprehensive gathering of knowledge of the distribution, cytology, genetics, physiology, and evolution of this economically important genus of plants.

In close co-operation with the staff, a number of visiting scientists have been

carrying out related investigations with different species of plants. By drawing upon their experiences and new materials, the Division's program, which traces the complex evolutionary patterns by which plants evolve, has been both broadened and strengthened.

PERSONNEL

BIOCHEMICAL INVESTIGATIONS

Staff: C. Stacy French, *Director*, Harold W. Milner, James H. C. Smith, Herman A. Spoehr, Harold H. Strain
Fellows: Violet M. Koski, Fergus D. H. Macdowall
Research Assistants: Marie L. G. Koenig, Nancy S. Lawrence, George H. Towner
Mechanic: Frank Schuster

Systematics and Genetics, Royal Agricultural College, Uppsala, Sweden, National Swedish Research Fellow

Stanford graduate students associated with the Division: Robert K. Vickery, Jr., George H. Ward

Research Assistants: Robert W. Ayres, Helen K. Sharsmith, Mary H. Wagner

Gardener: Wesley B. Justice

EXPERIMENTAL TAXONOMY

Staff: Jens C. Clausen, Paul Grun, William M. Hiesey, David D. Keck
Fellow: Herbert G. Baker, Lecturer in Botany, Department of Botany, University of Leeds, England
Guest Investigators: Pierre Dansereau, Directeur du Service de Biogéographie, University of Montreal, Guggenheim Fellow; Hedda Nordenskiöld, Institute of Plant

RESEARCH ASSOCIATE

Ralph W. Chaney, Professor of Paleontology, University of California, Berkeley, California

SECRETARY

Wilbur A. Pestell

CUSTODIANS

William E. Larson, Jr., James W. Groshong

BIOCHEMICAL INVESTIGATIONS

CHLOROPLAST PIGMENTS

HAROLD H. STRAIN

Pigments and evolution. For millions of years, life on the earth has required a continuous supply of organic matter. Throughout the long geologic ages, most of this organic material has been produced by the photosynthetic activity of green plants. In all plants, this remarkable photochemical action hinges upon the utilization of sunlight by the pigments contained within the microscopic chloroplasts of the plant cells. Through this reaction, the chloroplast pigments harness the atomic

fires of the sun for the production of oxygen and organic matter on the earth. In this way, the chloroplast pigments form an indispensable part of the complex organizational machinery required for the maintenance of virtually all living things.

In the search for clues to the evolution of the photosynthetic apparatus and to the evolutionary development of plants and of animals, much time has been devoted to determination of the pigments of present-day plants. Plants belonging to most of the major botanical families have now been obtained from various geographical habi-

tats, and their chloroplast pigments have been isolated and compared by the sensitive chromatographic adsorption method. These plants were collected from such diverse environments as Hawaii, the high Sierra Nevada of California, the western North American desert, the coastal regions of California, mountain lakes and streams, and the sea near California, Hawaii, and North Carolina. Some of the plants were native to arctic regions, others to tropic and to temperate regions. Some of the cultivated plants were native to such remote places as Japan, China, Malaya, India, the Mediterranean region, Africa, Madagascar, the Pacific islands, and North and South America. Most of the marine plants were obtained from the intertidal zone, but some of them were dredged up from depths of 50 to 100 feet.

The pigments of all these plants showed no significant variation with habitat. There was, however, a unique correlation between the occurrence of certain pigments and the taxonomic families to which the plants belong. In conformity with earlier results, all the higher plants yielded the same principal pigments, usually in about the same proportions. In a few organisms, the proportions of the several xanthophylls varied significantly. Whereas lutein was usually the principal xanthophyll, violaxanthin occasionally predominated, and in one plant (*Fremontia californica*) zeaxanthin was at least equal in amount to lutein.

Among a large number of algae, the presence of certain chlorophyll and carotenoid pigments was also found to be characteristic of the principal taxonomic groups. As there is some question concerning the classification of certain algae, knowledge of the pigments provides another guide for establishment of their relationship. For example, the fresh-water *Vaucheria* contains pigments also found in

the Heterokontae, the yellow-green algae, of which some five unicellular species grown in pure cultures have now been examined. The similar fresh-water *Dichotomosiphon* and the marine *Derbesia* contain pigments characteristic of the siphonalean green algae of the order Chlorophyceae. Such observations on the functional pigments of the plant cells provide significant links between the fields of chemistry, physiology, taxonomy, and phylogeny.

There has been wide acceptance of the view that the so-called complementary pigments of algae have developed in response to the spectral quality of the incident light. Algae growing in deep water and receiving a preponderance of blue-green light are presumed to have developed pigments which complement the weak absorption of the chlorophyll for green light. But in the algae dredged up from deep water, the pigments were found to be characteristic of the plant group; they were not determined by the conditions under which the plants grew. If there has been any effect of light on the development of the pigment systems, the change must have occurred before evolution of the plants known today.

All these diverse facts indicate that the pigment systems characteristic of the major botanical families were established early in the evolution of the plant world. Although plants have varied enormously in form and in adaptation to different environments, the pigment system shows no corresponding variation. Even though the pigment system is remarkably unstable in killed plant material, in living organisms it is one of the most constant physiological systems ever developed.

As man and animals depend upon organic substances of particular molecular structure that stem from photosynthesis,

the pigments can be regarded as the mold in which the molecular building bricks of the organic world are formed. At this molecular level, pigments may possibly establish the pattern of asymmetry that permeates the entire organic world, and thus they may set a limit to the variability of plants as well as of animals.

One may well ask the question, Why is the system of chloroplast pigments subject to so little variation? A partial answer lies in the fact that variations of the pigment system induced by injury or by mutation usually block the photosynthetic process. Consequently, plants thus changed will be eliminated rapidly from the native populations, because they will have lost their capacity for self-nourishment and independent existence. If this intimate connection between the pigment system and the photosynthetic process is the true explanation for the constancy of the pigments, then this dependence, too, must have been established millions of years ago, before the establishment of the plant groups that are known today. But there may be other causes for the constancy of the pigment system, and these are being sought in further studies of the pigments and their reactions.

Enzymatic oxidation of the chlorophylls. Through the use of adsorption methods, a rapid enzymatic oxidation of the chlorophylls to other green pigments has been discovered. This oxidation reaction varies with the conditions under which the leaves are killed, and with the plant material. Of the plants tested, it was most rapid in the young leaves of barley and of potatoes. It occurs rapidly when leaves of these plants are permitted to stand with organic solvents in the presence of oxygen. It does not occur in the absence of oxygen or in leaves that have been exposed to heat.

The products of the enzymatic oxidation of the chlorophylls are spectrally similar to the unaltered pigments, but are somewhat more adsorbed than the chlorophylls in columns of powdered sugar, so that, with experience, they are easily isolated. Each chlorophyll yields a single oxidation product, and in this respect the enzymatic oxidation differs from the spontaneous oxidation of the chlorophylls in methanol. This oxidation in methanol (known as allomerization) yields several oxidation products, one of which is identical with the product of the enzymatic oxidation. The enzymatic oxidation takes place in the presence of small amounts of water; the allomerization in methanol, by contrast, is inhibited by small amounts of water.

Chlorophylls themselves yield interconvertible isomers when the pigments are heated. The oxidation products do not yield the analogous isomers. Apparently the oxidation of the chlorophylls alters that portion of the pigment molecule involved in the isomerization reaction.

Improvements of the chromatographic adsorption method. In spite of its remarkable sensitivity and its wide applicability to chemical separations, the chromatographic adsorption method has not found wide application in industry. One of the limitations to separations by this method on an industrial scale is the discontinuous nature of the procedure. Now, however, a modification of the column has been devised so that certain adsorbed substances can be forced to migrate horizontally as well as downward. As a result, the flow of liquid through the column can be made continuous while the components of the mixture are collected in the respective portions of the percolate. Experiments to test applications of this continuous procedure are under way.

THE PHOTOCHEMICAL ACTIVITY OF
DISINTEGRATED CHLOROPLASTSH. W. MILNER, N. S. LAWRENCE,
M. L. G. KOENIG, AND C. S. FRENCH

The studies on the photochemical splitting of water by chloroplast material have been continued with much the same ultimate objectives as were described in the report last year. Progress in this investigation has been made in several lines, one of the most interesting of which has been the finding of certain large increases in the activity of the material upon reaggregation of very finely divided colloidal solutions of chloroplast material.

The dispersion of chloroplasts into extremely small particles is now accomplished much more simply and effectively with a new device. In last year's report the use of supersonic vibration for this purpose was described. It now appears that more effective results may be obtained by extruding the suspension of chloroplasts in water containing 15 per cent methanol through a fine needle valve under high pressure. The equipment for this consists simply of a round steel bar 3 inches in diameter with a x -inch round hole 4 inches long in its center. Into this is fitted a steel plunger with a rubber and a leather washer. The bottom of the hole in the steel bar is tapped to receive a steel needle valve. In use, the whole assembly is placed in a hydraulic press. When the pressure on the liquid rises to 20,000 pounds per square inch, the needle valve is opened slightly and, with the pressure held at this level, is adjusted to maintain a flow of about 5 cc. of liquid per minute. Under these conditions it is possible to obtain about three-fourths of the chloroplast material in a state of fine dispersion which will withstand centrifuging for one hour at 12,000 times the force of gravity without

sedimenting. By this treatment the activity of the chloroplast material is reduced to about one-third of its initial value. These preparations appreciably exceed those obtained by any other method so far investigated in fineness of particle size and in total yield, and they are prepared with greater ease.

The stabilization of such preparations, which has long been the greatest technical difficulty, has been considerably improved through the discovery of the stabilizing effect of 15 per cent methanol in the aqueous suspension medium. By the use of this concentration of methanol it is possible to retain half of the original activity of these preparations for a week by storing the material at -5° C. Thus a single preparation can be used for experiments extending over several days. Equimolar concentrations of ethanol are equally satisfactory for stabilization at very low temperatures, but are inferior to methanol at higher temperatures. The stabilizing influence of methanol was found during an attempt to use it as a means of fractionally precipitating the proteins in this mixture. Even in concentrations as high as 95 per cent and in the absence of salts, methanol or ethanol does not cause the formation of a precipitate except after standing for several hours at room temperature.

In solutions with 15 per cent methanol, however, the addition of small amounts of salts causes precipitation of chloroplast material. In water solutions without methanol a higher salt concentration is required to produce a precipitate. The appearance of this precipitate at salt concentrations of 0.1 M or lower is accompanied by a great increase in activity under illumination, sometimes by as much as two or three times, above that of a similar suspension without the salt. This activity is never

greater, however, than that of the intact chloroplasts. It appears that the aggregation caused by the salt takes place in such a way that several active centers catalyzing the reaction are brought closer together.

This activation differs from the chloride effect described in the work of other laboratories in that the new effect depends upon a reaggregation of the particles. Furthermore, other salts such as sulfates give even greater effects than do chlorides. This activation connected with precipitate formation is produced by a treatment of the material with salt at relatively high concentrations in aqueous methanol, and is not found when traces of salts are added to the reaction mixture, as in the case of the chloride effect.

This activation phenomenon, which has caused considerable difficulty in the assay of fractions obtained by salt precipitation, has, however, proved to be of considerable interest for its own sake. This effect may be related to the arrangement of the lipids present in the material. The participation of the lipids became evident when it was found that extracting the dispersion of chloroplast material with petroleum ether abolished or greatly reduced the salt activation effect. This extraction removes about 2 or 3 per cent of the total lipid which is present. After the petroleum ether extraction, the capacity of the dispersions for activation by salt may be re-established by treating the material with a small amount of ethyl ether, then evacuating to remove the ethyl ether completely.

One difficulty in separating the active components from the inactive substances by fractional precipitation has been inability to obtain complete redispersion of the precipitates. This also prevents comparable activity measurements on the precipitated and dispersed material. The use

of detergents in combination with adsorption techniques offers some hope of overcoming these difficulties.

THE OXIDATION-REDUCTION PROPERTIES OF CHLOROPLASTS

F. D. H. MACDOWALL

The photosynthetic reaction is now considered to be composed of two main steps: the splitting of water, which results in the evolution of oxygen and the formation of a reducing substance; and the reduction of carbon dioxide by the substance formed in the first step. Illuminated chloroplasts, even though removed from living cells, cause the reduction of a number of substances but not the reduction of carbon dioxide. A greater negative electric potential is required for the reduction of carbon dioxide than for that of the substances which are reduced by isolated chloroplasts. It was therefore of interest to find out what reducing power, as expressed in terms of electric potential, may be reached in suspensions of illuminated chloroplasts.

Measurements were made of the potentials of bright platinum electrodes immersed in suspensions of chloroplast material containing various added reducible dyes. Illuminated suspensions always showed a lower potential than nonilluminated ones. The lowest observed potential, referred to the standard hydrogen electrode, was -0.25 volt. It was found that the potential of such suspensions was lower in freshly prepared material than in that which had stood for a short time. It thus appears that chloroplasts in intact leaves may be able to produce an appreciably greater negative potential than that attained by isolated chloroplasts, perhaps even a potential effective for the reduction of carbon dioxide.

DEVELOPMENT OF THE MECHANISM FOR THE
EVOLUTION OF OXYGEN

JAMES H. C. SMITH

When leaves grown in the dark are illuminated, they develop the ability to liberate oxygen from carbon dioxide. We do not now know at which stage during the course of illumination the ability to liberate oxygen originates, nor at which stages other parts of the photosynthetic processes have their beginnings.

One of the first observable changes connected with the development of the photosynthetic apparatus during the illumination of dark-grown seedlings is the conversion of protochlorophyll to chlorophyll *a*. According to current concepts of the structures of these two compounds, this conversion is a hydrogenation of protochlorophyll. It seemed possible that the hydrogenation of protochlorophyll might be involved in the splitting of water, which at the same time would result in the evolution of oxygen. Therefore, the first experiment performed to correlate the events that lead to the development of the complete mechanism for photosynthesis was a determination of whether oxygen is evolved during the transformation of protochlorophyll to chlorophyll *a*.

Two very sensitive methods were used to test for the evolution of oxygen during this photochemical transformation, namely, the emission of light by luminescent bacteria, and the quenching of the phosphorescence of tryptaflavine adsorbed on silica gel. Under the conditions of the tests, the evolution of oxygen was not detected by either method. From these experiments there is no evidence that the transformation of protochlorophyll to chlorophyll *a* produces oxygen. Furthermore, etiolated leaves seem to be incapable of evolving even traces of oxygen immediately upon being illuminated.

Green leaves and etiolated leaves have also been compared with respect to their ability to liberate oxygen after being placed in a vacuum in the dark and then illuminated. Under these conditions, in the absence of carbon dioxide, green leaves quickly evolve a limited quantity of oxygen but etiolated leaves evolve no oxygen. These facts suggest that during greening the leaves develop a potential reservoir for photosynthesis from which they can liberate oxygen by photochemical action.

The oxygen liberated by illumination of the evacuated leaves was estimated by measuring the quenching of the phosphorescence of tryptaflavine adsorbed on silica gel. A phosphorometer of simple design was constructed for this purpose. This instrument was capable of detecting oxygen pressures of the order of 10^{-5} mm. of mercury. Since the quenching is proportional to the logarithm of the oxygen pressure, the method is more sensitive in the lower ranges of oxygen pressure.

THE NATURE OF THE TRANSFORMATION OF
PROTOCHLOROPHYLL TO CHLOROPHYLL

V. M. KOSKI AND J. H. C. SMITH

It has been known for some time that most higher plants do not form chlorophyll in the dark and that the rate of chlorophyll formation which takes place in the light is affected by temperature. Therefore, the biosynthesis of chlorophyll must involve both thermochemical and photochemical reactions. A more detailed analysis of the effect of light and temperature on the greening of leaves has demonstrated that the formation of protochlorophyll is a thermochemical reaction and the transformation of protochlorophyll to chlorophyll is strictly a photochemical reaction.

Protochlorophyll accumulates in plants grown in the dark at room temperature,

but it is not formed either in the light or in the dark at 0° C. These observations make it clear that protochlorophyll is formed by a thermochemical process.

Since light is involved in the transformation of protochlorophyll to chlorophyll *a*, the question arises as to whether this transformation is a mixture of thermochemical and photochemical reactions or whether it is a strictly photochemical reaction. This was tested by measuring the rate of the reaction at different temperatures and different light intensities.

The rate of transformation of protochlorophyll in dark-grown corn leaves was measured at two temperatures, 5 and 18° C, and at three light intensities, 30, 120, and 240 foot-candles. The rates at the two temperatures were identical for each of the three light intensities and were roughly proportional to the light intensity. Because the rate of the transformation is independent of the temperature and proportional to the light intensity, it may be concluded that the rate-determining step is photochemical.

The rate of transformation is very rapid. Light energy of 240 foot-candles from a 40-watt 4500 White Westinghouse fluorescent lamp converts 56 per cent of the protochlorophyll in 10 seconds.

The transformation reaction was also measured with monochromatic light as the source of illumination. Light of wave lengths 640 and 650 m μ , each having a band width of 5 m μ , was used. An analysis of the rates showed the transformation to obey strictly the mathematical expression for second-order reactions.

As a result of these experiments it may be concluded that in etiolated corn leaves the transformation of protochlorophyll to chlorophyll *a* is limited by photochemical action, and that the rate of transformation is determined by the amount of light absorbed by the protochlorophyll.

In the previous Year Book it was reported that in continuous light, transformation of the protochlorophyll present in etiolated seedlings produces chlorophyll *a*. As greening progresses, chlorophyll *h* appears. Thereafter, chlorophylls *a* and *b* increase in constant proportion to each other. Similar results have now been obtained from experiments with intermittent light. These relations make it highly improbable that chlorophyll *h* is derived from chlorophyll *a*.

THE ACTION SPECTRUM FOR THE FORMATION OF CHLOROPHYLL

J. H. C. SMITH, V. M. KOSKI, AND C. S. FRENCH

The possible transfer of energy between pigments and the chemical utilization of light energy absorbed by plant pigments can be investigated in a system similar in many respects to photosynthesis. This system is the photochemical mechanism by which protochlorophyll is changed into chlorophyll by the action of light. This reaction is, in a limited way, analogous to the photochemical steps of photosynthesis in that it takes place in the leaf and the active pigment is very closely related to chlorophyll. It is simpler than photosynthesis in that protochlorophyll, the pigment concerned in the absorption of light, is also the substance which is transformed by the action of the light, and in that the product of the reaction, chlorophyll, is a known chemical substance. The process of greening of dark-grown leaves involves a system of better-known components and fewer chemical steps than does photosynthesis, and yet it is a system closely associated with photosynthesis and one in which the utilization of light energy can be more easily investigated. Under certain conditions it is possible in this system to measure a photochemical transformation which is not complicated by a number of thermochemical steps.

The specific question which this analogous reaction can help to answer is one relating to the possible participation of several different pigments in causing a single type of photochemical change to take place. It has been well established that photosynthesis may be powered by light energy absorbed by a number of different pigments. In all these cases chlorophyll *a* is always present, and it is considered likely that the energy absorbed by the other pigments is in some way transferred to chlorophyll *a*, which then initiates the first steps of photosynthesis.

In the greening of etiolated plants there is also the possibility that nonchlorophyllous pigments such as carotenoids may participate by the absorption of light and the transfer of energy to protochlorophyll. A series of experiments carried out a few years ago in another laboratory can be interpreted in this way. Whether or not light absorbed by chlorophyll can be used to cause further chlorophyll formation has long been of interest. The older methods are not adequate to settle this question.

The development in this laboratory of procedures for the extraction and analysis of chlorophyll and protochlorophyll, as well as the construction of a device for the production of high enough intensities of monochromatic light throughout the visible spectrum, has made possible a greatly improved reinvestigation of these long-discussed questions.

By means of these new techniques the effectiveness of various wave lengths of light in producing given effects in biological material can be accurately measured. If the absorption spectra of the pigments contained in the biological material are known, it is then possible to compare the action spectrum with the absorption spectra of the pigments and thus to see clearly which pigments are photochemically active.

Precise measurements have now been made of the action spectrum for the transformation of protochlorophyll to chlorophyll in normal and albino corn and in normal bean seedlings. The results make it evident that the carotenoid pigments not only are ineffective, but reduce the action of the blue light by absorbing it. Thus they act as a light filter at short wave lengths. The results with normal corn and bean leaves are in distinct contrast with the older work on oats, in which it was found that the carotenoids, even though present in abundance, did not cause internal screening. In albino corn, which contains very small quantities of carotenoids, blue light was found to be the most effective part of the spectrum.

Another experiment was performed in which about one-half the protochlorophyll present in the leaf was converted into chlorophyll, and the leaf was then illuminated with light having a wave length of 6800 Å, which is strongly absorbed by chlorophyll, but very weakly absorbed by protochlorophyll. This illumination at 6800 Å gave no significant increase in chlorophyll content. It is therefore evident that red light absorbed by chlorophyll is not involved in the transformation of protochlorophyll.

It may be said in conclusion that only light which is absorbed by protochlorophyll is active in the transformation of this pigment to chlorophyll. The action spectrum obtained for this transformation in albino corn seedlings, which contain little other pigment than protochlorophyll, gives therefore a measure of the absorption spectrum of protochlorophyll in living etiolated leaves, a result which cannot be obtained directly because of the low concentration of this pigment in such leaves.

SPECTROSCOPIC EQUIPMENT

C. S. FRENCH, J. H. C. SMITH, AND
GEORGE H. TOWNER

The recording spectrophotometer mentioned in last year's report has been considerably improved, but is not yet ready for routine use. The major change has been the incorporation of a cam which is run by the wave-length drive mechanism to take care of most of the necessary corrections. The remaining small corrections are now handled by the curve follower, an arrangement which provides for considerably greater accuracy. A means has been devised for the automatic plotting of this secondary correction curve. Comparison of the spectral absorption curve for a pure sample of chlorophyll *a* as recorded by this machine with that measured manually by another instrument indicates that the maximum error in measuring the per cent transmission is now within 3 per cent. The stability and reproducibility are somewhat better than this figure. The apparatus is now being rebuilt for greater precision, increased stability, and freedom from scattered light. From the experimental work to date, it appears that its eventual performance should be well within 1 per cent for the measurement of absorption spectra, and within a few per cent for the spectroradiometry of very weak light beams, such as the fluorescent light from leaves or algae.

A large-aperture grating monochromator has been built for the measurement of the effectiveness of different parts of the spectrum in the formation of chlorophyll in dark-grown leaves. The results of these experiments are discussed in a separate section of this report. This monochromator is based on a diffraction grating kindly loaned by Mr. Harold Babcock, of the Mount Wilson Observatory. This 4 by 4 inch grating, with 15,240 lines per inch,

is used with two large camera lenses designed for aerial photography. The lenses have an aperture ratio of $f/2.5$ and 12 inches focal length. The angle between the two optical axes is 30° , the grating being placed at the intersection of the two axes and rotated about this point by a screw-drive mechanism. This screw drive is provided with a double thread and a positioning pin moved by a cylindrical correcting cam so that for each revolution of the drive wheel, 100 angstroms of the first-order spectrum is swept across the exit slit. The wave lengths of light in the spectra of both the first and second orders are thus strictly proportional to the amount of rotation of the drive wheel and can be read continuously by means of separate counters for the two orders. Readings of these counters are correct within 5 angstroms. The first-order dispersion is 50 angstroms per millimeter. The maximum transmission of the instrument in the first-order spectrum is in the red, and in the second order it is in the blue. The light source used in conjunction with this instrument is a high-pressure capillary mercury lamp. This combination produces adequate intensity in a 50-angstrom band width for studying the formation of chlorophyll in leaves at all points throughout the visible spectrum.

Another monochromator for use in the recording spectrophotometer has been made. This instrument has a similar collimator lens and a 4 by 4 inch transmission replica grating obtained through the courtesy of Professor R. W. Wood, of Johns Hopkins University. This grating, with 14,000 lines per inch, has a high transmission in the first-order blue part of the spectrum. After passing through the grating, the light is reflected from a movable plane mirror which controls the wave length conning out of the instrument.

From this plane mirror the light goes to a spherical mirror of 63 cm. focal length. The image of the spectrum on the exit slit is thus about twice the size of the entrance slit. The linear dispersion in the plane of the exit slit is therefore 27 angstroms per millimeter. The drive wheel of this instrument is also arranged so that one rotation corresponds to 100 angstroms. These two instruments may be coupled together with selsyn motors, thus acting as a double monochromator to sweep the spectrum at a constant rate. Either of them can also be used independently for photochemical purposes.

THE URONIDES OF LEAVES

H. A. SPOEHR

Higher plants are composed primarily of carbohydrates. Cellulose, probably the most abundant organic substance occurring in nature, is the chief component of the structural and supporting parts of higher plants. Starch, one of the most common plant constituents, serves the plant as an important storage food material. Both cellulose and starch are formed by the linking together of a large number of sugar units of one type, namely D-glucose. Also of very wide distribution in all plants are the uronides. This group of carbohydrates is more complex than cellulose or starch in that its members are composed of more than one type of sugar unit, combined with either glucuronic or galacturonic acid. This greater complexity in composition of the uronides greatly increases the difficulty of gaining an understanding of the structure and properties of this class of compounds. Further difficulties arise from the colloidal character of the uronides and their occurrence in the plant in association with other carbohydrates, which makes clean separations extraordinarily difficult.

The phytochemical relations of the uron-

ides and their physiological function in the plant are still largely unknown. Yet their very complexity of structure and of chemical reactivity invites speculation concerning their role in the physiology and chemistry of the plant. It has been generally assumed that the uronides are primarily cementing elements in the structural fabric of plant tissue. But it is possible that in leaves they play a more dynamic role in the metabolism of the plant, as intermediates in the formation of other compounds, and even in the photosynthetic process. Before any such hypotheses can be tested with leaf material, considerably more exact information is required concerning the isolation, purification, and chemical composition of the leaf uronides.

Uronides have been isolated from several species of leaves, including sunflower, spinach, plane tree, and more particularly flax. It has been found advantageous to use leaves which are free of starch. The uronide content has been found to range from 8 to 15 per cent of the dry leaf material. The uronides are extracted with water, precipitated with ethanol, purified by repeated solution in water, and reprecipitated with alcohol. The product obtained in this manner is principally in the form of calcium salts with about 25 to 30 per cent uronic acid. On further purification, a water-soluble polyuronic acid is obtained, composed principally of galacturonic acid. The method of hydrolysis in which 90 per cent formic acid is employed, and which gave very satisfactory results with compounds composed of mannuronic acid and of hexoses and pentoses, is of no value with compounds containing galacturonic acid. This is apparently due to the fact that mannuronic acid readily forms a lactone which crystallizes well and the acid can be isolated in this form, whereas galacturonic acid does not form

a lactone. Although polygalacturonides can be hydrolyzed with 90 per cent formic acid, on concentration of the acid hydrol-

ysate the galacturonic acid rapidly undergoes condensation or polymerization to form insoluble compounds.

EXPERIMENTAL TAXONOMY

JENS CLAUSEN, DAVID D. KECK, WILLIAM M. HIESEY, AND PAUL GRUN

Broadly interpreted, the experimental taxonomy program has as its threefold objective an understanding of the various kinds and degrees of kinships between plants, of the relations between plants and their environments, and of the evolutionary processes that have produced the diversity between plants and their fitness to the environment. Progress toward this objective becomes more and more a co-operative effort, not only within the staff itself but with outside individuals and institutions.

These investigations advance along an extended front, and during the year the groundwork has been laid for new approaches. Previously, the transplant studies had shown that widely distributed species are able to occupy their diverse environments because they are composed of many physiologically and genetically distinct races, each fitted to live in a different sector of the area occupied by the species as a whole. On the other hand, the experiments on the Madiinae and other plants had revealed the presence of various kinds of barriers to free interbreeding that have arisen during the process of speciation, giving rise to species and other units in various stages of differentiation. The processes through which plants become fitted to their environments remain to be explored. It is known that this fitness depends upon their physiological characteristics, which in turn are governed by genes, but the details of this interaction are unknown. Exploration of the genic-physiological relationships of appropriate plant groups may therefore hold an important key to our understanding of evolution.

Possibly no single organism can be found that fulfills all the requirements of a program in physiological and ecological genetics with these objectives, and probably several groups of plants are needed, each bringing to light features not so readily discovered in others. During the year progress has been made in the search for such groups of plants by testing the suitability of several different genera. These tests meanwhile serve to extend our knowledge of plant relationships, as all have interconnected objectives and aim toward the solution of related problems.

PERSONNEL AND GUEST INVESTIGATORS

During the year a larger group than ever before has participated in this program. Dr. Paul Grun, of Cornell University, joined the staff in March 1949. Dr. Herbert G. Baker, Lecturer in Botany at the University of Leeds, England, spent the year from October 1948 with the group on a Carnegie Institution Fellowship, investigating the evolutionary patterns in some plants having both an Old and a New World distribution.

Dr. Pierre Dansereau, Director of the Service de Biogéographie at the University of Montreal, was with the Division from March through August 1949 on a Guggenheim Fellowship. He was drawing upon the program in experimental taxonomy for material to include in a book on the taxonomy of the vascular plants, in which the aim is to correlate cytotaxonomy, ecology, and experimental methods with conventional taxonomy. He also familiarized

himself with the plant communities of the Pacific coast region, especially of the transect marked by the Division's stations, as a background for the correlation.

Dr. Hedda Nordenskiöld, of the Institute of Plant Systematics and Genetics of the Royal Agricultural College, Uppsala, Sweden, was a visitor in the spring of 1949 under a National Swedish Research Fellowship, collecting and studying western American forms of the wood rush, *Luzula*, to correlate with studies on that genus in western Europe. Two Stanford graduate students, Mr. Robert K. Vickery, Jr., and Mr. George H. Ward, have utilized the greenhouses and other facilities in researches intimately connected with the Division's program. These various visitors have brought many new points of view into the group.

POTENTILLA GLANDULOSA

This species of the rose family has many contrasting ecological races and is well adapted for genetic experiments, as was brought out in Year Book No. 47 (1947-1948). It is also a good subject for transplant experiments, since the segregating and cloned second-generation hybrids between races from lowland and high-altitude climates have proved excellent for the study of principles in ecological genetics. During the year the statistical analysis of the long-term selection experiments has been completed with the efficient aid of Dr. Helen K. Sharsraith, and a manuscript on these experiments is now in preparation. The physiological properties of the races and hybrids have not been investigated, however, as this species is difficult to clone and grows slowly, and hence is less well adapted for physiological studies than for the other approaches to ecological genetics.

HYBRIDIZATION IN ACHILLEA

The members of the *Achillea millefolium* complex occur almost throughout the northern hemisphere and have evolved an unparalleled series of contrasting climatic races. They clone easily and are excellent for physiological investigations (Year Book No. 45, 1945-1946, pp. 112-117), but they have only a few distinctive morphological characteristics that can be used as markers in a genetic investigation. The first seven Fi hybrid combinations between contrasting races were made in 1948 and grown in 1949, as follows:

Achillea borealis, $72=27$ chromosomes:

San Joaquin Valley race, 36° N. X Kiska Island coastal race, 52° N.

San Joaquin Valley race X Kiska Island alpine race

San Joaquin Valley race X California coastal race

Achillea lanulosa, $n=18$ chromosomes:

Port Orford, Oregon, coastal race, 43° N.
X Vera Cruz, Mexico, montane race,
7000 ft., 20° N.

Port Orford coastal race X California redwood region race

Port Orford coastal race X Great Basin race

Achillea millefolium X *borealis*:

Northern Iceland coastal race, 65° N. X
San Joaquin Valley race, 36° N.

The most contrasting cross is between the giant San Joaquin Valley race of *A. borealis*, which is 150 cm. tall, winter-active, and from a continental subtropical climate in California, and the diminutive (8 cm. tall), winter-dormant, alpine, subarctic race of the same species from treeless Kiska Island in the Aleutians. It is expected that recombinations of the genes in the second generation of the hybrid of parents from such contrasting climates will produce offspring of great physiological variation.

EXPLORATORY STUDIES IN MIMULUS

A new plant being tested for its applicability to experimentation is *Mimulus guttatus*, the common monkey flower. It and its close relatives compose a highly variable group of plants that has evolved races all over western North America west of the Rocky Mountains from Alaska to Baja California, and from sea level up to 11,000 feet. Most of the races of *guttatus* are perennials, but some are annuals. Both perennial and annual forms may flower as soon as four months after sowing. They clone easily and cross easily, most forms are self-compatible, and a single pollination may result in hundreds of seeds. Cultivation is difficult, however, for this plant occurs naturally in moist soils or even in running water, although it can be grown to maturity without trouble under greenhouse conditions. Its adaptability to garden culture is now being tested.

Four Fi hybrids between races of the *Mimulus guttatus* complex from very different climates along our station transect were grown this year, namely, coastal X Sierran foothill; coastal X alpine (*Mimulus Tilingii*); coastal X subalpine Great Basin; Sierran foothill X alpine (*M. Tilingif*). The crossings were easily made except those involving the alpine form, which has been known as a distinct species; and the hybrids appear to be fertile.

Since October 1948 Mr. Robert Vickery has carried on these investigations as a project for his doctor's degree through a co-operative arrangement between the Division and the Department of Biology of Stanford University. The experimental work is being done at our stations. Thirty-nine populations of 5 species of the *guttatus* complex have been grown in addition to 57 strains of 23 other miscellaneous species, and in all 7 of the 10 sections of the genus have been represented.

Seventeen races and forms of the *Mimulus guttatus* complex, mostly from our transect, have been crossed during the year in all possible directions. These strains came from the immediate coast, the coastal mountains, and a series of localities over the Sierra Nevada from the foothills on the west to 10,000 feet elevation near the crest and to the plateau of the Great Basin beyond, and included the closely related annuals *M. nasutus* and *M. laciniatus*. A Chilean form of *M. luteus* was also included.

In addition to these crossings, designed to explore thoroughly the interrelationships within a single section of the genus, these 5 species and 9 others, representing 7 sections, were less intensively hybridized to ascertain the genetic relationships among species of various sections and the limits of crossability between species.

Two major objectives motivate the *Mimulus* investigations. One of these is to determine through experimental means the evolutionary relations between species of distinct sections of the genus, between closely related species of the same section, and between ecological races of one species. The other objective is to ascertain the suitability of *Mimulus*, specifically the members of the *guttatus* complex, for experiments on ecological and physiological genetics. To this end some no selected individuals of races and hybrids of this complex were cloned and transplanted to the three altitudinal stations, and it is hoped that a few key individuals can be tested next spring in the controlled greenhouses of the Earhart Plant Research Laboratory of the California Institute of Technology. The two major objectives dovetail and supplement each other.

POA INVESTIGATIONS

The background of the range-grass breeding program, employing members of

the genus *Poa*, and aiming at both practical and basic scientific objectives, has been detailed in previous Year Books (cf. No. 46, 1946-1947, pp. 95-103). The purpose is to explore the possibilities of creating new species of range grass having new sets of qualities and suited to new environments, by combining the inheritances of existing species. It entails the production and study of hybrids between species belonging to different sections of the genus and from very contrasting environments. The parental species and many of the hybrids set most of their seed without fertilization, and thus reproduce as apomictic clones rather than through ordinary genetic segregation.

The *Poa* hybrids often had as one parent a wild strain obtained from the extensive collections in the Pullman Nursery of the U. S. Soil Conservation Service. From the beginning of these experiments we have received the wholehearted co-operation of the Soil Conservation Service through Dr. A. L. Hafenrichter, chief of the nursery division of the Pacific Coast Division, and members of the staff. Accordingly, the *Poa* hybrids are under test not only at the three altitudinal transplant stations of the Institution, but also in a series of nurseries of the Soil Conservation Service extending from southern California to northern Washington. The members of our staff are following the progress of the tests in the nurseries of the Service in addition to those at our own stations. The most extensive of these plantings are at the Pullman Nursery, where about 5000 individuals of some 70 different forms have been grown this year.

Intercontinental transplant experiments. The apomictic clones of *Poa* offer exceptional advantages for transplant experiments on a geographically widely extended scale because they can be propagated by seed. In response to a request for seed of

winter-active grasses, samples of the new *Poa* hybrids and their parental species were sent to Dr. J. W. Gregor, in charge of grass research, of the Scottish Society for Research in Plant Breeding, at Corstorphine, Edinburgh. More than 2000 individuals of 15 strains were grown in an experimental plot with 8 replications, and Dr. Gregor has supplied an analysis of detailed notes and measurements on these strains made throughout the season of 1948.

Dr. Clausen, who was in Europe in 1948 attending the Eighth International Congress of Genetics in Stockholm, had the opportunity of seeing this experiment and conferring with Dr. Gregor on the results. Plants that had come from places having warm summers and severe winters did not grow well in the cool, moist summer climate of Edinburgh, but plants from cool coastal regions made a better showing. The summer climate of Edinburgh is not duplicated at any of the other stations where these grasses are being tested, and it appears to bring out differences between those plants that require heat for their best growth (continental-type plants) and those that thrive under cool conditions (plants fitted for the west coasts of the continents). These characteristics are transmitted from the parents to their hybrids. In view of the favorable early results, it was decided to augment the experiment by testing additional strains and hybrids.

Through personal contacts made in Europe by Clausen it was also possible to arrange for a co-operative intercontinental transplant experiment on an extensive scale. The seeds were shipped to be sown in 1949, and the plants will be ready for measurement in 1950. About 40 largely apomictic species and hybrids of *Poa* are included in this experiment, and they are being tested in 13 environments that range from southern California, at 34° N., to

central Sweden, at 61° N., from sea level to 10,000 feet, and from coastal to continental climates, and that include various seasonal combinations of temperature and moisture.

The places at which these clones of *Poa* are now being tested and the collaborators in charge of them are as follows:

Western United States:

San Fernando, California, Dr. Paul Lemmon, Soil Conservation Service Nursery; coastal, 34° N. latitude.

Central California, Carnegie Institution stations, at sea level, 4600 feet, and 10,000 feet altitude, 38° N. latitude.

Pullman, Washington, Mr. John L. Schwendiman, Soil Conservation Service Nursery; continental, 2400 feet altitude, 46° N. latitude.

Bellingham, Washington, Mr. W. E. Chapin, Soil Conservation Service Nursery; coastal, 48° N. latitude.

Eastern United States:

Raleigh, North Carolina State College, Dr. Ben W. Smith; continental, 36° N. latitude.

Northern Europe:

Aberystwyth, Wales, Dr. T. J. Jenkin, Welsh Plant Breeding Station; very coastal, 52° 30' N. latitude.

Edinburgh, Scotland, Dr. J. W. Gregor, Scottish Association for Research in Plant Breeding; coastal, 56° N. latitude.

Kapelle (Rotterdam), Holland, Dr. A. J. Th. Hendriksen, of D. J. van der Have, seed growers; subcoastal, 52° N. latitude.

Ötoftegaard, Denmark, Dr. H. N. Frandsen, Danish Associated Farmers' and Cooperatives' Experiment Station; intermediate, 55° 40' N. latitude.

Uppsala, Sweden, Dr. E. Akcrberg, Ultima Branch of the Swedish Seed Association (Svalöf); continental, 59° 50' N. latitude.

Volbu, Norway, Dr. Paul Solberg, State Experiment Station for the Mountain Districts, Løken; intermediate, 1500 feet altitude, 61° N. latitude.

Western Asia:

Seeds were earlier sent to Amman, Transjordan, Jubeiha Experiment Station (Mr. M. A. Haddad); continental, 3200 feet altitude, 32° N. latitude.

Each of these experiment stations is interested in discovering new plants for grazing purposes, and we are interested in determining the ranges of tolerance of the hybrids and their parents. These two objectives can be achieved through the experiment as planned, for the responses of each form indicate fairly accurately its range of tolerance, and each station is free to retain, multiply, or use for crossing any form that is well fitted for its own environment.

Two of the hybrids are intended for a selection experiment. They are sexual and therefore will segregate. In the very different climates of this experiment, selection will be made for the best strains. It would be of importance in the study of evolution to determine whether seeds of a single hybrid can give rise to new strains that fit contrasting climates.

Most of the stations have arranged to take brief notes on the performance of the plants through the various seasons, from which the seasonal periodicities of these forms can be determined. It is furthermore hoped that a member of our staff may be in a position to take more detailed and more fully comparable notes at a time when the plants are mature.

Physiology. As another step in this unique experiment, it is hoped that during the coming year key forms may be tested under controlled conditions in the greenhouses of the California Institute of Technology, where Dr. Hiesey will co-operate with Dr. F. W. Went. Through these tests it should be possible to discover some of the environmental factors that determine the physiological responses of these *Poas* in various climates and to correlate the

responses with the heredities of the plants. Such tests will also indicate to what extent the experimental results under the controlled conditions of this unique new laboratory can be translated into predictable field performances in various climates.

Cytology. One other approach to the study of the Poas included in the intercontinental experiment is through cytology. Dr. Grun is now studying the influence of the environment on chromosome distribution in selected clones of *Poa* in the transplant series of gardens, and it is hoped he will be able to study similarly the forms to be grown under the different controlled conditions at Pasadena. It is known that environmental conditions do affect chromosome pairing in some species. By using the cytologically unbalanced *Poa* species and hybrids, it may be possible to analyze further the patterns of environmental influences which cause variation in the behavior of chromosomes.

Embryology. Plants such as *Poa* that produce seed without fertilization can follow various methods in developing their seed, but how and where the asexual embryos arise can be determined only by detailed microscopic studies. Dr. Axel Nygren, of the Institute of Plant Systematics and Genetics of the Agricultural College of Uppsala, Sweden, who is making such studies on the embryology of European species and hybrids of *Poa*, expressed interest in studying our hybrids also. His request was welcomed, because such studies on the development of the embryo would complement Gran's investigation of the male sex cells of the same hybrids. Dr. Nordenskiöld prepared and shipped material for Dr. Nygren's preliminary studies while she was at our laboratory. Additional materials will be available to Dr. Nygren in the plot of the intercontinental transplant experiment at Dr. Akerberg's neighboring institution in Uppsala, so that

these plants will serve a double purpose.

Genetic studies. Our understanding of the inheritance patterns in *Poa* has been advanced through the study of some 8000 F₂ and F₃ individuals at Stanford, in addition to the 5000 plants tabulated at Pullman. All together, 175 progenies have been analyzed. These represent offspring of 97 F_i individuals from 22 different crossings and 9 kinds of interspecific hybrids, as listed in table 1.

All hybrids of which a sufficient number of progenies were grown produced not only apomictic but also sexual offspring in somewhat variable proportions. In all crossings but one, both parents were apomictic. In the exception, the paternal parent was sexual, but this combination produced at least as large a proportion of apomictic offspring as the others. Among 97 F₂ progenies, 43 were apomictic to a greater or less degree, and 54 were purely sexual, as listed in table 1. This proportion between apomicts and sexuals might suggest a very simple genetic formula for the inheritance of apomixis in *Poa*, but it is not so simple as that, for the apomictic F_i individuals are apomictic to very different degrees. This is indicated by their F₂'s, in which the proportion of maternal-type offspring ranges from 10 to 90 per cent of the total

The derived apomicts, in turn, produce asexual offspring of their own type and, in addition, aberrants which probably arise by means of the still active sexual process (table 1). This behavior resembles that of their naturally occurring apomictic parents. Unlike the parents, however, which produce weak aberrants that are quickly eliminated in competition, the derived apomicts may produce aberrants that are either stronger or weaker than themselves. Since some of these aberrants may, in turn, prove to be apomictic again, this source of

variation must be considered in breeding work.

A very vigorous, fertile, and adaptable apomict has arisen in this manner from a weak apomict hybrid of *Poa ampla* X *pratensis alpigena*. The particular Fi parent of this new apomict was itself apomictic, but it was a nonflowering dwarf at Stanford, though fairly vigorous at Mather and Timberline, where it ripens seed. Most of

south of the habitat of either of its grandparents. It is one of the apomicts now being tested in all the environments of the intercontinental transplant experiment.

Another promising apomict, developed from a sister Fi of the *ampla* X *pratensis alpigena* cross just mentioned, had quite a different history. This apomictic line was of an agriculturally highly desirable type, combining some of the best characters of

TABLE 1

NUMBER OF PROGENIES OF INTERSPECIFIC HYBRIDS OF POA

HYBRID COMBINATIONS	F ₂ PROGENIES		F _a PROGENIES				TOTALS
			FROM ABERRANTS IN APOMICTIC F ₂ 'S		FROM SEXUAL F ₂ 'S		
	Apomictic	Sexual	Apomictic	Sexual	Apomictic	Sexual	
<i>ampla</i> X <i>pratensis</i>	20	15	7	2	6	7	57
<i>ampla</i> X <i>compressa</i>	1	5	4	4	14
<i>ampla</i> X <i>arida</i>	1	1	2
<i>Canbyi</i> X <i>pratensis</i>	1	3	4
<i>scabrella</i> X <i>pratensis</i> ...	14	25	..	2	20	23	84
<i>scabrella</i> X <i>compressa</i>	1	1
<i>scabrella</i> X <i>arida</i>	2	1	3
<i>scabrella</i> X <i>ampla</i>	2	2	4
<i>scabrella</i> X <i>gracillima</i> ...	3	3	6
Totals.....	43	54	7	4	30	37	175
	97		11		67		

its offspring are uniformly weak and vegetative at Stanford like itself, but a few of its aberrants are vigorous and flower freely. One of these is the new apomict, which is both very fertile and highly apomictic. It is vigorous like *ampla*, rhizomatous like *alpigena*, rust-resistant, and apparently better adapted for contrasting climates than either parent of the original hybrid. This derivative of a plant from the Palouse Prairie in Washington and another from Lapland thrives at Stanford and also at the two mountain stations, at a latitude far

each parent. It was only 10 per cent fertile, however, and some of its aberrants were as vigorous as itself or even more so. In an attempt to produce a more fertile line. Fa populations were grown from four of its best aberrants, which had presumably risen sexually. One of these was considerably improved over its Fi parent. It is 70 per cent fertile—twice as fertile as its *ampla* grandparent—and it is 85 per cent apomictic, a proportion comparable with that found in wild Poas. Moreover, its aberrants are weak, so they will be eliminated

in competition with the vigorous apomictic type. In the gene exchange it also gained longer rhizomes, an advantage in many climates. These examples illustrate selection methods that can be applied in improving plants that already are apomictic.

The situation is different in the sexual fraction of the F_1 interspecific hybrids in *Poa*. They produce segregating, variable F_2 progenies. Tests on a series of individuals from such F_2 populations showed that some F_2 plants had become apomictic like the grandparents, but others had remained sexual. Among 67 F_3 progenies from sexual F_2 's, it was found that 30 were more or less apomictic and 37 were still sexual (table i). The degree of apomixis in such F_1 's varies as in the apomictic F_2 progenies, a fact which suggests that the segregation for this characteristic is still fairly intricate. From the breeder's point of view it is most fortunate that a sexual period of recombination of genes or blocks of genes may intervene before the inflexible hereditary pattern of apomixis is regained.

An example of the effect of such recombination is afforded by a sexual hybrid out of *Poa scabrella* from coastal southern California X *P. pratensis* from the Athabasca region of Canada. This sexual F_1 inherited winter activity and the bunchgrass habit from its *scabrella* parent, and increased tillering and slight summer activity from *pratensis*. Its periodicity limits it to environments with mild winters, where it outperforms its *scabrella* parent because of its longer period of activity. At Mather, where the winter is long and cold, it is forced into winter dormancy and is scarcely able to survive. A highly variable F_2 was grown from this plant at Stanford, all the individuals of which were winter-active. Four of the best F_2 plants, which had the longest rhizomes and were most summer-active, were selected for progeny tests. The best of these shows fertility in-

creased from 25 to 90 per cent, has long rhizomes, is active most of the summer, is apomictic, and survives the Mather winter successfully. This strain is also included in the intercontinental transplant experiment.

The apomictic, nonsegregating *Poa* progenies are on the whole much more vigorous than the sexual, segregating ones. Many of the variable offspring of the latter are so weak that they are unable to survive even in the experimental field. This difference in performance is understandable when we remember that these are hybrids between very distinct species. Each parental species has a balanced combination of genes selected in the long process of evolution. In different species, however, these balances are produced by different sets of genes. The F_1 hybrids have received an unbroken set of genes from each parental species, and in so far as these sets can produce a harmonious physiological development when together, the F_1 is successful. The apomictic offspring of the F_1 are also successful, for they actually represent an unaltered clone containing an intact chromosome set from each parental species.

By contrast, the offspring of the segregating, sexual sister hybrids arise from an exchange of genes. This causes great variation, because the offspring receive various proportions of the parental genes. Probably a number of the exchanged genes govern physiological processes that run counter to each other, as, for example, photosynthesis and respiration. If such processes become unbalanced, growth may be adversely affected. Many offspring of these sexual hybrids actually die in the seedling stage, and others make almost no growth or are very susceptible to disease. This is the same pattern of weak and diseased offspring already observed in many other interspecific hybrids previously investigated. Occasionally, however, a vigor-

ous, well adapted plant arises through such interspecific gene shuffling, and if that event coincides with the reinstatement of apomixis, a new apomictic species or an agronomically important new grass may appear.

Taxonomy of Poa. The experimental program has dealt with relatively few species in *Poa* and principally with the members of but two of its sections. In part because of the presence of incomplete apomixis, and in part because of a wealth of minor morphological characters that appear in a bewildering array of combinations, *Poa* is a highly variable genus, and the specific and even the sectional lines within it are rather indistinct. This has meant that a sound taxonomic treatment of *Poa* was required for presenting the experimental results.

Poa is of almost world-wide distribution, but for the present purpose Dr. Keck has made a systematic study of the 50 or so species occurring in the western United States, where it is the largest genus of grasses represented. The resulting treatment, which is helping to clarify the relationships in this complex group, has utilized the cytological and experimental results thus far available.

CALIFORNIA PLANT COMMUNITIES

Dr. Keck has been co-operating with Dr. P. A. Munz, of the Rancho Santa Ana Botanic Garden, on a classification of the plant communities in California as a prelude to the writing of a new type of regional flora for California. The plant communities reflect the climatic differences within a region in much the same way as do their component species and climatic races, a fact which suggests that similar environmental factors govern the distribution of all. The results of experiments on California plants are to be utilized in the writ-

ing of the manual, and, conversely, the preparation of the manual is a means of discovering groups of plants that are in need of experimental attention.

ARMERIA STUDIES

H. G. BAKER

The genus *Armeria*, the thrift or sea pink of the Plumbaginaceae, is almost restricted to Europe and the Mediterranean borders of North Africa. The only species which ranges outside this area is *A. maritima* (Mill.) Willd. The wide, discontinuous distribution of this species includes stations in northern Asia and North and South America, as well as in Europe. There is evidence which suggests that the disjunct nature of this distribution is connected with extensions and contractions of the range of the species during the Pleistocene epoch. These isolated populations form promising material for the study of local adaptation and of the relation of this to the particular breeding mechanisms that are found in *Armeria*.

Discontinuity in the distribution of *Armeria maritima* is particularly striking in North America. Here forms are scattered from Newfoundland and the Gaspé Peninsula through the Canadian and Alaskan Arctic, and along the Pacific coast as far south as southern California. A single, very isolated population of *A. maritima* var. *labradorica* Lawr. occurs at an altitude of about 12,000 feet on Hoosier Ridge in the Colorado Rockies, more than 1500 miles from the nearest known population of its closest relatives in the vicinity of Hudson Bay.

Apart from those in the far north, populations of each of the European forms of *Armeria maritima* contain balanced proportions of two kinds of plant, which appear to differ only in the morphology and physiology of their pollen and stigmata.

The flowers of one kind of plant bear stigmata that somewhat resemble miniature maize cobs, and anthers containing pollen grains (type A) with coarsely reticulate ornamentation. The stigmata of the other kind of plant are clearly papillate, and the pollen grains (type B) show an ornamentation of fine spines. Both kinds of plant are self-incompatible but cross-compatible, for type A pollen germinates only upon "papillate" stigmata, and type B pollen only upon "cob" stigmata. Such populations are said to be dimorphic.

The races which inhabit northern Norway and the Kola Peninsula, northern and northeastern Asia, and North and South America contain only one kind of plant and are therefore monomorphic. These plants have type A pollen and papillate stigmata, and are self-compatible.

More than 1500 individuals of *Armeria maritima* are being grown in a uniform garden and in the greenhouses for the purpose of analyzing their heritable differences and of correlating these with the environments from which they came. These represent 41 natural habitats in Europe, the Faeroes Islands, Iceland, Greenland, and North and South America, and include some of those which geographically are most disjunct.

Three major groups are recognizable in the cultures of this species. The first comprises the plants from western Europe. These are dimorphic, obligatorily outbreeding, with large, showy flowers, and their populations are very variable. Several distinctive races have developed in this group, and they occupy sea cliffs, pebble beaches, and salt marshes. Farther inland, they occur in high montane habitats and rarely in the lowlands. Within this group, populations from the Shetland and Faeroes Islands and Iceland in the North Atlantic may constitute a distinct oceanic racial complex.

The second group, inhabiting arctic Eurasia and northern North America, is composed of monomorphic, self-compatible plants that have small, inconspicuous flowers containing relatively few pollen grains. Within this group, var. *lahradorica* Lawr. is in culture from localities ranging from the Gaspé Peninsula, through Labrador and by Hudson Bay, to Great Bear Lake in the Northwest Territories and the remarkably disjunct Colorado station, together with representatives of the more highly arctic var. *sibirica* Lawr. from Greenland.

The forms of the third major group of *A. maritima* inhabit the coasts of Pacific North America and northeastern Asia. They have conspicuous flowers like European races, but are monomorphic and self-compatible. They show less variation within populations than does the European dimorphic complex, although there may be considerable difference between populations. The plants from the north, from Puget Sound northward and westward along the coast to Alaska and Sakhalin, have, among other distinctive characters, ciliate leaves. They have been recognized as var. *purpurea* Lawr. Rarely in the Puget Sound area and more commonly southward to southern California, the populations usually have glabrous leaves and belong to var. *californica* Lawr. Unlike the European dimorphic members of this species, the members of this group have not evolved forms adapted to salt-marsh conditions or to alpine environments.

It is possible to intercross all the forms of *Armeria maritima* which have been cultivated, irrespective of their geographic origin. Such crossings are successful only when the appropriate kinds of pollen and stigmata are brought together, even when self-compatible and self-incompatible forms are being intercrossed. For example, pollen of the self-compatible race from the

California coast (type A/papillate) will germinate upon the stigmata of only one kind of the European self-incompatible var. *typica*, namely type B/papillate. On the other hand, the Californian form can be successfully pollinated only from the other European kind (type A/cob). Crossings already performed between European var. *typica* and Canadian var. *lahradorica* suggest that the genetical determination of this breeding mechanism is relatively simple, and resembles that of dimorphic heterostyled genera such as *Primula*.

These rules apply also to the production of interspecific crosses. By following them, successful crossings have been achieved between *Armeria maritima* and each of the following species: *A. plantaginea* Willd., *A. canescens* Host., *A. pseudarmeria* Lawr., and *A. Welwitschii* Boiss. The last crossing is notable because it is intersectional and indicates that the boundaries of intercrossability may reach far into the genus.

In addition to these experiments, material for pollen analysis of the Plumbaginaceae was obtained in some of the major American herbaria.

INVESTIGATIONS IN THE GERANIACEAE

H. G. BAKER

Geranium. The section *Robertiana* of the genus *Geranium*, as defined by Knuth, is probably unnatural. Two of its species, however, *G. Robertianum* L. and *G. purpureum* Vill., are certainly quite closely related, and they have been the objects of this study. *Geranium purpureum* is diploid ($2n=32$) and strictly annual. Its distribution is essentially Mediterranean, although fingers reach through France to south-western England and Ireland, and also into eastern Africa and Macaronesia. *Geranium Robertianum* is annual or biennial and is distributed widely through Europe (except in the north) and along

the mountain chains of Asia to China. Diploid forms ($2n=32$) have been found in the British Isles, but all forms examined from continental Europe are tetraploid ($2n=64$).

Seventeen representative forms of *G. Robertianum* from coastal and inland British populations, and 3 races of *purpureum*, have been in cultivation in Leeds, England, for periods up to 5 years. A dozen of the cultures of *Robertianum*, 3 of *purpureum*, and 7 of hybrid origin were planted in the garden at Stanford and have provided information concerning their performance in a completely different environment. A race of *Robertianum* from Ithaca, New York, which differs from any known in Europe, has been added to the cultures, and it is hoped to extend these further to include races from northern India. Further crosses have been made this year between races of both species, whereby it will be possible to study the genetic barriers between them.

Er odium. The abundance of plants of *Erodium*, the filaree, is particularly striking to a visitor in California, and some of the problems which they pose are very challenging.

Three species of this genus have long been known to be important members of the introduced flora of California and to have considerable value as forage plants. In all probability, *Erodium cicutarium* L'Her. reached California with early settlers from southern Europe, and was plentiful in the Central Valley by 1844. *Erodium botrys* Bertol. and *E. moschatum* L'Her. followed later. Until 1943, two distinct forms had been confused under the name *E. botrys*, and very recently one of them has been separated by J. T* Howell as *E. obtusifolium*. This form may have been introduced from North Africa.

Forms of these four species have been

grown, studied, and intercrossed, and, in addition, probable natural hybrids have been detected in a mixed natural population of *botrys* and *obtusiplicatum* on the Stanford campus. These putative hybrids appear to be fertile, although *botrys* and *obtusiplicatum* frequently occur together, and there appears to be little difference in their ecological requirements. There is

some evidence that the prevalent self-pollination of these species may account for the restricted amount of hybridization between them in many localities. Seed has been gathered for the cultivation of *E. cicutarium* from a series of habitats, which should give some indication of the part played by direct environmental modification in the polymorphism of this species.

PALEOBOTANY

RALPH W CHANEY

The nearest American relatives of the Chinese redwood, *Metasequoia glyptostrochoides*, are *Taxodium distichum*, the swamp cypress, and *Sequoia sempervirens*, the coast redwood. Superficially similar in foliage and other characters, these two species differ fundamentally in their climatic and topographic requirements. The swamp cypress lives in regions of summer rainfall in the eastern United States, and is limited to lowlands, largely to river swamps. The coast redwood occupies areas with winter rainfall and summer drought, and is confined to the shores of the Pacific where summer fog is prevalent; primarily a tree of well-drained river flats, it is also abundant on valley slopes to an altitude of 3000 feet. *Taxodium distichum* ranges north into regions of continental climate, where there are wide seasonal extremes in temperature, with several cold winter months; *Sequoia sempervirens* does not live beyond the limits of a marine climate characterized by low daily and annual ranges in temperature, where freezing is seldom prolonged. In view of these differing regimes of rainfall and temperature, it is natural to find that the coast redwood has an evergreen habit whereas the swamp cypress is deciduous.

During the middle part of the Tertiary period, from twenty to forty million years

ago, fossil representatives of *Taxodium* and *Sequoia* were widely distributed over the western United States, as was the now Asiatic genus *Metasequoia*. In the John Day Basin, where the most complete sequence of Tertiary floras is to be found, *Metasequoia* was at the outset the most abundant of these conifers; at Twickenham its leafy shoots make up over three-fourths of the specimens recently collected, with katsura (*Cercidiphyllum*) and birch (*Betula*) also abundant; both these trees are modern associates of *Metasequoia* in central China. The gradually rising Cascade Range, which involved the piling up of lava flows and pyroclastics during this later Tertiary volcanic climax, appears to have altered the environment in eastern Oregon both as to climate and as to topography. Judging from the Miocene vegetation, this barrier to the west was at least partially responsible for reduced precipitation and greater extremes of temperature. With the blocking of streams, many lakes and swamps were formed. In the Dayville region, *Taxodium* was a predominant tree, together with oaks, hickory, and others now found living with it in the eastern United States. *Metasequoia* survived there in limited numbers, apparently in better-drained habitats on adjacent slopes. *Sequoia* is never found in direct association, but is well represented in volcanic

ash deposits of the near-by Blue Mountains, in association with other conifers and with angiosperms which suggest a higher elevation. Within a distance of forty miles there are Miocene records of the occurrence of these three genera of the Taxodiaceae, now widely scattered and in diverse habitats. The continued uplift of the Cascades and the consequent changes in environment on their eastward flanks have eliminated a majority of the Tertiary tree genera from the John Day Basin and from other interior localities in western North America. *Sequoia* was first restricted to the coastward slopes, where it ranged as far north as Portland during Pliocene time, and is now limited to the coast of southwestern Oregon and California. Current studies of its living conditions at the south end of its range, in Monterey County, suggest that if a trend toward drier climate were to continue into the future, the coast redwood might make its last stand in the coastal mountains of central California, in a habitat much like that which can be reconstructed for the region of last occurrence of Miocene redwoods in the John Day Basin.

With the change to a drier climate, *Taxodium* and many of its associates have disappeared from western North America. A study of its modern distribution in the southeastern United States, using funds made available by a grant from the American Philosophical Society, has added many significant data regarding the occurrence and associates of *Taxodium distichum*, and is providing a basis for comparisons with the limited forests of *Metasequoia glyptostroboides* in central China. The rainfall regime there is the same, and the Chinese redwoods are likewise deciduous; but the mountainous setting introduces major differences which can be interpreted only after further field work has been completed. In view of the present unsettled

political situation, plans for work in China during 1949 have been postponed. Fortunately there was a continuation of field studies in the *Metasequoia* forest by Chinese botanists during the summer of 1948; there is now a basis for determining that this relict tree occupies a zone of ecological transition between the temperate forest of higher altitudes and the subtropical forest below. Additional collections from the Tertiary of Oregon and British Columbia, and extensive studies of material already available, are indicating that many of the fossil occurrences of *Metasequoia* in western America represent ecological transition zones similar to that in the mountains of Hupeh and Szechuan. This region will continue to provide critical data regarding Tertiary climate and topography, containing as it does one of the most significant forest survivals ever discovered.

Under the auspices of the Save-the-Redwoods League, and with the assistance of the University of California College of Agriculture, a large number of seedlings of *Metasequoia glyptostroboides* have been propagated. These are being distributed over a wide area in western North America, from southeastern Alaska to British Columbia along the coast, and at numerous localities at varying altitudes in western Washington, Oregon, and California. Seedlings have been sent also to Mexico and Guatemala, where they will be planted in upland areas now occupied by survivors of the Arcto-Tertiary Flora. This Flora, which appears to have had its origin in Alaska and other northern lands in Cretaceous and early Tertiary time, formerly included *Metasequoia* as one of its most abundant members. The subsequent migration of this forest southward, and the gradual elimination of *Metasequoia* and other genera, is a matter of substantial record. It is now proposed to plant

Metasequoia wherever there are existing constituents of the Arcto-Tertiary Flora. In addition it will be replanted in as many of its localities of Tertiary occurrence as possible, leaving out—at least for the present—the areas east of the Cascades and Sierra Nevada where existing conditions are now too arid to offer much hope for the growth of a tree with such high moisture requirements. At this time there is no basis for confidence that the redwood of Asia will survive Alaska winters, though its deciduous habit carried it through the exceptionally cold winter of 1948-1949 as far north as Portland, Oregon. In any event we shall gradually learn whether the factors which resulted in the extinction of *Metasequoia* in North America during

later Tertiary time are still operating, or whether this tree may again become a resident of a continent from which it disappeared some fifteen million years ago.

Dr. Daniel I. Axelrod has continued his study of Pliocene floras of Nevada and California. Several of these represent vegetation of a more arid type than is elsewhere found in the fossil record of North America. As a result of these investigations, knowledge of the origin and relations of desert and steppe vegetation is being greatly enlarged. A group of papers by Dr. Axelrod under the general title "Studies in Late Tertiary Paleobotany" has been approved for publication by the Carnegie Institution in its Contributions to Paleontology.

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DEPARTMENT OF EMBRYOLOGY

Baltimore, Maryland

GEORGE W. CORNER, *Director*

The Department of Embryology continued its work during the year without serious interruptions or distractions. Changes in the group were few. Mrs. Faith Wilson LaVelle, Fellow of the American Association of University Women, left the laboratory in July 1948, having practically completed her research project under the direction of Dr. R. K. Burns. She received the degree of Doctor of Philosophy from Johns Hopkins University in June 1949, offering a thesis based on her work in this Department, which will be published in the Contributions to Embryology. Dr. Jerome S. Harris was appointed to a fellowship of the Carnegie Institution, which he relinquished for part of the year to serve as intern in obstetrics in the Johns Hopkins Hospital. Dr. Edward C. Gillespie came following an internship in the Johns Hopkins Hospital, Department of Obstetrics, to spend a year in research with Dr. S. R. M. Reynolds. Dr. Charles L. Schneider, of Wayne University Medical School, Detroit, came for several months for the same purpose. Dr. Jorgen U. Schlegel, a member of the Department of Anatomy of the University of Copenhagen, spent the year here on a fel-

lowship of the Rockefeller Foundation. Dr. R. Mahanti, Professor of Anatomy at Orissa University, Cuttack, Hindustan, was guest of the Department for several months, observing technical procedures and studying research problems. Professor M. H. Toosy, of the Medical School at Lahore, came for four months by request of the Embassy of Pakistan. Dr. E. Carl Sensenig, Associate Professor of Anatomy in the Medical College of Alabama, again worked in the Department in the summer of 1948. Dr. L. J. Wells, Associate Professor of Anatomy in the University of Minnesota, returned for several weeks early in 1948 to complete the preparation of a monograph on the development of the human diaphragm begun during his tenure of a Guggenheim Fellowship in the Department in 1946-1947. Toward the end of the period of this report, Dr. Árpád Csapó, an experienced pupil of Professor Szent-Györgyi in the biochemistry of muscular tissues, arrived from Budapest via Sweden to be a fellow of the Carnegie Institution and to work in consultation with Dr. Reynolds and Dr. Corner on the physiology of uterine muscle.

PROGRAM OF INVESTIGATIONS

Morphology and experimental embryology. Dr. C. H. Heuser, Curator of the Embryological Collection, prepared during the year about a dozen valuable early human embryos in serial sections for addition to the collection. He continued his studies on the morphology of early human and baboon embryos, with special regard to the formation of the yolk sac. Dr.

Heuser also devoted much time to the task of preparing for press an article ("Developmental horizons in human embryos," stages xix to xxiii) which was left unfinished by Dr. G. L. Streeter at the time of his death.

Under Dr. Heuser's direction Dr. M. H. Toosy continued the study of an interesting double monster, one of the earliest

known specimens of this anomaly in man.

Dr. R. K. Burns devoted his time during the year to the completion of two extensive review articles on the embryonic differentiation of the reproductive tract in vertebrates, and to supervising the preparation for publication of papers by Dr. Faith Wilson LaVelle (on the development and response to hormones of the reproductive tract of the hamster) and Robert J. Faulconer (on the embryology of the ostium of the Müllerian duct in man).

Dr. Joseph Gillman, Research Associate, Johannesburg, South Africa, persisted in his efforts to obtain very early baboon embryos of known age. The difficulties and uncertainties familiar to workers in this field are such that the youngest baboon embryo yet obtained for the Collection by Dr. Gillman is 13 days old. Efforts to obtain the critical stage of 10-11 days are being continued.

Dr. Elizabeth M. Ramsey, following the completion of her studies on the uterine vessels of the rhesus monkey in pregnancy (see below under "Published research") began intensive study of the same problem in man. Through the co-operation of the Department of Obstetrics of Johns Hopkins Hospital, several pregnant human uteri have been sent in directly from the operating room and have been injected by Dr. Corner with India ink through the uterine arteries. These valuable specimens, to which additions may be expected from time to time, have been placed at the disposal of Dr. Ramsey.

Dr. J. U. Schlegel was occupied during the year in perfecting a technique for the demonstration of blood and lymph vessels by the use of an injected dyestuff which stains the endothelial lining of the vessels while it is passing through their walls. The substance used is fluorescent and thus becomes visible in ultraviolet light, so that the vessels are seen as shining channels in

otherwise unstained tissues. The method is aimed at demonstrating the arteriovenous anastomoses in the endometrium recently described on the basis of observations by other methods, by Schlegel, Dalggaard, and Okkels at Copenhagen. It offers also a ready means of determining the time necessary for the passage of the solution of dyestuff from the blood into the lymph stream. The method was described in a preliminary report at the 1949 meeting of the American Association of Anatomists.

Mrs. Dorcas H. Padget made further progress during the year on her studies of the development of the cerebral veins, begun several years ago under a grant to Dr. Corner from the Life Insurance Research Foundation.

Dr. E. Carl Sensenig during his annual summer visit made an investigation of the early development of the spinal meninges in the human embryo, and presented a preliminary report on this subject at the 1949 meeting of the American Association of Anatomists.

Very early human embryos and associated corpora lutea. Dr. Arthur T. Hertig, Research Associate, has actively continued the program of collection of human embryos in association with Dr. John Rock, and with the co-operation of Dr. William J. Mulligan and Dr. F. A. Pemberton, at the Free Hospital for Women, Brookline, Massachusetts and the Boston Lying-in Hospital. During the year, uteri and oviducts from 17 fertile patients subjected to hysterectomy for various reasons, and for whom one or more coital dates were recorded at about the presumed time of ovulation before the operation, were searched. Four embryos were recovered, three of which are of extremely early age. All these are now in the collection of the Department of Embryology, as follows:

No. 8698. A dividing ovum in the 2-cell stage. This is the youngest human cm-

bryo, developing under normal circumstances, ever seen. The specimen was recovered from the oviduct at operation 60 hours after coitus. An ovary with the corpus luteum was also removed. Dr. Hertig estimates the endometrium as of the early part of day 17 of the standard cycle, i.e., day 3 after ovulation. (A closely similar 2-cell egg, inseminated and cleaved in vitro in the laboratory of Dr. John Rock in Boston, is also in the Carnegie Collection; see Year Book No. 47, 1947-1948, p. 117.) Dr. Corner estimates the corpus luteum as about 48 hours old. The two symmetrical blastomeres of this wonderful specimen are enclosed in the zona pellucida. One polar body is clearly visible, the other if present is obscured. The specimen has been adequately photographed and will be cut into serial sections by Dr. Heuser.

No. 8630. An abnormal 5-cell segmenting ovum from the cavity of a uterus corresponding to day 19 of the standard cycle, or about day 5 after ovulation. The blastomeres are multinuclear and show other evidences of abnormality and delayed development.

No. 8663. A normal blastocyst from the cavity of a uterus corresponding to day 19 of the standard cycle or about day 5 after ovulation. This is the second youngest known normal human embryo at present writing. The specimen, which is probably a little shrunken, measures 0.134 by 0.115 mm. and has a cavity 0.057 mm. in diameter. It closely resembles the embryos of other mammals at a similar stage of development. There is an inner cell mass composed of 15 or 20 cells. The rest of the blastocyst wall is composed of a single layer of large cells. It has been perfectly sectioned by Dr. Heuser.

No. 8672. A 13-day ovum measuring 2.34 mm. in diameter, implanted on the posterior wall of the left lateral sulcus of

the uterine cavity. The tissues of this specimen, in the embryonic disk as well as in the trophoblast, are in poor cytological condition, and the chromatin of mitotic figures in dividing cells is clumped. The embryo was probably dying. It shows early unbranched chorionic villi, a bilaminar germ disk, and an early definitive yolk sac which has been formed by an hourglass constriction of the large space enclosed within "Heuser's membrane" (coelomic or exocoelomic membrane of various authors). In this respect the specimen sheds light on the problem of the peculiar origin of the yolk sac in man, long debated in this laboratory and by those outside it who have followed the advancing knowledge gained from the Hertig-Rock and other early human embryos.

The corpus luteum. Dr. Hertig reports that the work on the histology and histochemistry of the corpus luteum of pregnancy, referred to in the last Year Book, has been carried on during the year by himself, Mr. Roger F. White, Dr. John Rock, and Miss Eleanor C. Adams and will soon be ready for publication. About 40 selected human corpora lutea of pregnancy and 50 nonpregnant specimens have been used.

Dr. John Rock, of the Free Hospital for Women, Brookline, Massachusetts, has continued to give his advice and help in the program of collection of early embryos. His own studies on living human eggs are made in association with that program, though supported by other organizations, chiefly the American Cancer Society through the Committee on Growth of the National Research Council. He reports having recovered 158 human ova from 49 patients; 123 ova were Inseminated in vitro and cultured for 48 hours in serum. Three of these eggs divided in vitro, two of them reaching the stage of 2 blastomeres and one of them dividing into 3 blastomeres.

Two patients received pituitary extract treatment before the ovaries were removed. Their ovaries yielded 9 ova, recovered from follicles over 1 cm. in diameter, all containing spindles of the second polar division, as do fully mature follicular ova in most mammals that have been studied. It appears that these ova were caused to mature by the pituitary extract.

Embryonic pathology. In recent years it has become known that one of the common contagious fevers, rubella or German measles, a disease which is of trivial concern to the patient, may be the cause of dire consequences when the patient is a woman who happens to be in the first $\frac{1}{2}$ months of pregnancy. In such a case the infant is frequently born blind, with congenital cataracts, often with abnormally small eyes (microphthalmos), and sometimes even with microcephaly and mental retardation. Congenital deafness and anatomical abnormalities of the heart may also occur. Some infants of mothers who acquire German measles at the stated period of gestation may no doubt escape damage, and possibly there is a difference in the virulence of epidemics. At any rate, the discovery of this hitherto unsuspected relationship has caused so much concern among physicians that some of them have advised the termination of pregnancy when the mother has had German measles early in gestation. The Department of Embryology has received three fetuses from such preventive abortions, and has been entrusted with the preliminary study of two more from another laboratory. Dr. Corner and Dr. Heuser have undertaken the investigation of these specimens. The examination will be laborious and may for various technical reasons yield little information, but the gravity of the problem seems to make it a duty to see what can be learned from these fetuses.

Physiology of the uterus. Dr. S. R. M.

Reynolds and a group of fellow investigators have been at work on various problems in the physiology of the uterus, making particular use of the tokodynamometer, to which reference has been made in recent reports. Dr. Reynolds himself put a good deal of effort, with members of the Johns Hopkins Hospital obstetrics staff, into testing improved models of the tokodynamometer looking to a design suitable for wide practical use. He and the clinical observers have begun to use the instrument in a comparative study of the uterine factors involved in the induction of labor by the use of various oxytocic drugs. With Dr. Louis M. Hellman and Dr. Bruce A. Harris of the Hospital staff, he has completed the collection and analysis of data on the first stage of labor, which show the step-by-step development of the expulsive muscular force of the parturient uterus and the developing dominance of the fundus. Dr. Hellman has employed the tokodynamometer in studying the effects of pituitrin given during labor, with a view not only to explanation of its action, but also to improvement in the practical use of pituitrin in labor.

Dr. Reynolds has given much thought to the problem of the mechanical factors involved in maintaining the circulation of blood through the umbilical cord from the placenta to the fetus. Inasmuch as the anatomical structure of the umbilical cord is an important element in the problem, an anatomicophysiological study of the cord has been begun, in which the structure and relative size of the fully distended umbilical vessels will be studied, together with the status and function of "Wharton's jelly," the connective tissue of the umbilical cord. An extensive program of research on this important and hitherto neglected phase of the fetal circulation is planned.

Dr. Jerome S. Harris, working with Dr. Reynolds and Dr. Edward C. Gillespie,

completed a study of the changing form of the human uterus as it enlarges during pregnancy. Using repeated X-ray pictures, he observed two phases of the development of the gravid uterus. Until the 20th week of gestation, growth occurs in all three dimensions, but thereafter little growth occurs in thickness, and after the 30th week the width actually diminishes, and enlargement continues in length only. Dr. J. Tyler Baker, of Easton, Maryland, in the past a frequent contributor of embryonic material to the laboratory, was influenced by Dr. Reynolds' publications to compile a large series of direct measurements of the enlarging uterus obtained with obstetrical calipers. Though his data are not so precise in individual cases as measurements from X-rays, the large number of cases permits statistical treatment. The results of detailed analysis agree with Gillespie's data in showing that the 7th month is a critical time with respect to the provision of space in the uterus and favorable conditions of uterine blood circulation for the infant.

Dr. Gillespie also undertook experimental studies on the effect of alloxan diabetes on sexual receptivity in rabbits, on fertility, and on the growth of the uterus and the fetuses.

Dr. Charles L. Schneider spent 3. few months observing the work of Dr. Reynolds' group and conducting experiments on the experimental production of toxemic conditions in pregnant rabbits.

Mrs. Lyla T. Bradin continued her studies, previously reported, on the relation between the length of the phases of gestation in different species and the relative maturity of the fetus at birth. A preliminary report on the times of appearance of the various ossification centers in a number of small animal species, with respect to the rate of growth and the changing form of the uterus, was presented

at the 1949 meeting of the American Association of Anatomists.

General physiology of the embryo. Dr. Louis B. Flexner and his associates made further progress during the year in their program of correlating the physiological with the morphological development of the embryonic tissues. With Virginia B. Peters, Dr. Flexner studied the growth in volume of the nerve cells of the brain cortex (of the guinea pig) and the relation of the volume of the cell to that of its elements, the nucleus and cytoplasm, taken separately. By such observations a critical period of change in the development of the guinea pig's brain cortex is found to occur at the 41st to the 45th day of gestation. Dr. Leonard Gallant, of the Henry Phipps Psychiatric Clinic, Johns Hopkins Hospital, and Dr. David B. Tyler, working with Dr. Flexner, find that electrical activity of the cortex first occurs at about the same time.

Dr. Josef a B. Flexner and Dr. Louis Flexner are studying the permeability of the cerebral blood vessels to sodium and its changes during development of the fetal brain.

Dr. David B. Tyler has been investigating the enzyme systems that effectuate metabolic processes, particularly those concerned with carbohydrate metabolism, in the fetal brain, and the changes in their activity during development. He is applying to fetal tissue (rat's brain) the method usual in such work on adult tissues, i.e. that of counteracting the various enzyme systems in the tissues by the use of chemical substances known to interfere with the action of specific enzymes. Striking quantitative differences in the sequence of the chemical steps in the metabolism of carbohydrates, occurring at different stages of development, are thus being observed and subjected to interpretation with the aim of locating the sites of action of the metabolic

enzymes and the history of their differentiation in the embryonic tissues.

co-operative activities. Some of the more extensive co-operative activities of the Department have already been referred to, namely, Dr. Heuser's study of early human embryology in association with Dr. Hertig and Dr. Rock, and the joint work of Dr. Reynolds and associates with the Department of Obstetrics of Johns Hopkins Hospital. Dr. Roger B. Scott, of the Department of Gynecology, Johns Hopkins Hospital, continued to use the monkey colony and operating-room facilities in his experimental studies on the production of endometriosis.

One of the most important co-operative services of the Department of Embryology has not been specifically mentioned in recent reports, though it continues unceasingly. This is the examination of specimens sent in by physicians, mostly abortive embryos and fetuses which come with urgent requests for information as to the cause of death or of abnormalities, the age of gestation when aborted, and other questions, some answerable and some unanswerable at the present stage of knowledge. This work is carried on chiefly

by Dr. Heuser, and to a less extent by Dr. Corner. In addition to the specimens sent for such study, there are frequent requests for information on matters concerning human and animal embryology and concerning the physiology of the reproductive system and its disorders.

Dr. Corner is acting as general adviser and consultant to the American Academy of Ophthalmology in the planning and preparation of a motion-picture film explaining the embryology of the human eye. Dr. George K. Smelser, of Columbia University, has been made a welcome guest of the laboratory in order to facilitate his work as technical adviser on the film.

In April 1949 Dr. Lawson Wilkins, of the Department of Pediatrics, Johns Hopkins Medical School and Hospital, gave two talks at the Department of Embryology on developmental and functional disturbances resulting from sex hormone deficiencies in human subjects. In June Dr. Alfred Yost, of the Collège de France, spoke at the laboratory on his experimental researches on the relation of the sex hormones to embryonic differentiation of the reproductive system.

PUBLISHED RESEARCH

HUMAN EMBRYOS OF EIGHT AND NINE DAYS

In Year Book No. 42, for the year 1942-1943, mention was made of the acquisition, through Drs. Arthur T. Hertig and John Rock, of two human embryos, nos. 8155 and 8171, thought to be about 8 and 9 days old respectively. These specimens were in due course successfully sectioned by Dr. Heuser and the sections were photographed by Mr. Heard for subsequent reconstruction on plastic sheets by Miss Eleanor C. Adams under Dr. Hertig's supervision in Boston. A detailed account of the two specimens has now been pub-

lished in the Contributions to Embryology, volume 33. These two specimens represent the stage of human development when the ovum has become well implanted and the uteroplacental circulation is just beginning to be organized. In the 8-day ovum, definitive trophoblast of both types is actively forming, and in the 9-day specimen further differentiation of the syncytiotrophoblast has resulted in the formation of a network of lacunar spaces for the reception of a small amount of stagnant maternal blood from enlarging capillary sinusoids eroded by the invading trophoblast. The embryo

in each of these ova is a bilaminar germ disk without axial differentiation. Amniogenesis has barely begun in the 8-day ovum, but is well advanced in the 9-day specimen. The so-called exocoelomic membrane ("Heuser's membrane"), which is not yet formed in the 8-day specimen, is present in the elder ovum and is described as being delaminated from the adjacent cytotrophoblast.

The two specimens here described lie between the 7-day and 9-day specimens described by the same authors in 1945 (Carnegie nos. 8020, 8004). The two younger specimens have solid trophoblast, but the two 9-day ova show stages of lacuna formation. The four present all the essential stages in amniogenesis, which consists in the progressive *in situ* delamination of amniogenic cells from the cytotrophoblast dorsal to the germ disk.

EMBRYOLOGY OF THE SKELETON; HISTOGENESIS OF CARTILAGE AND BONE

The late George L. Streeter was preparing, at the time of his death in July 1948, another section of his series of "horizons" or classificatory stages of human embryogenesis. This section was to cover horizons xix to xxiii, that is to say, the last phases of the embryonic period, as distinguished by Streeter from the fetal period of development. It was his intention to trace certain features of the development of each major organ system through the five horizons, and he had already prepared for publication a special article (see bibliography) on the histogenesis of cartilage and bone, in which he used the embryonic humerus as an index of differentiation. The following extract from Dr. Streeter's own summary will serve to indicate his line of thought:

"By confining attention to one bone and following it in all its stages one obtains a

more complete history of the consecutive factors that play a part in the development of the skeletal system. . . .

"In studying the humerus during its cartilaginous period, it was found that cartilage cells, as they grow older, pass through an orderly series of transformations. These include proliferation and growth of the cells, a characteristic vacuolization of their cytoplasm, formation and increase in amount and character of the intercellular substance, and terminal liquefaction or disintegration of the cells. By dividing these consecutive transformations into five arbitrary phases, it was possible to plot maps of the humerus showing the areas of distribution of the respective phases of cartilage that characterize the bone as it increases in size.

"It was found that there is always a growth center in the shaft in which the oldest cartilage cells are located. Adjoining it, above and below, are zones of successively younger phases, the youngest always at the ends of the bone.

"When the cells at the growth center reach the terminal phase of disintegration, the cells of the inner coat of the periosteum penetrate the primary osseous shell and make their way into the cartilage, which is thereon abruptly transformed into marrow.

"The time when this invasion occurs is arbitrarily adopted as the conclusion of the embryonic and the beginning of the fetal period of prenatal life. It occurs in specimens about 30 mm. in length."

EARLY DEVELOPMENT OF THE HUMAN VERTEBRAL COLUMN

The early development of the human vertebral column, and indeed of the mammalian spine in general, has received very little attention. Later stages of human development, after the appearance of car-

tilage and during ossification, have been more thoroughly studied, and the osseous stage has been extensively investigated particularly by roentgenologists, who need to understand the adult details and congenital anomalies seen in their diagnostic films. This situation has resulted in much confusion of description and terminology through the attempt to designate embryonic structures which are not fully understood or well defined, with reference to the adult structures of which they are forerunners. Dr. E. Carl Sensenig, now of the Medical College of Alabama, came to the Department of Embryology in 1944 on a special grant from the Carnegie Institution for the purpose of reinvestigating the early development of the vertebral column. His work was aided in several subsequent summers by the Joseph Henry Fund of the National Academy of Sciences. Results of the investigation appear in volume 33 of the Contributions to Embryology.

The embryology of the vertebrae is a very complicated matter, as might be expected in view of the peculiar demands upon the vertebral column for a combination of strength in protection of the spinal cord and in support of the body with flexibility in movement, firm anchorage for the ribs, and safe exit for the spinal nerves. The development of the individual units of this structure is indeed so complex that it baffles any attempt to discuss it in non-technical terms. For this reason it is impossible in a report intended largely for nonbiological readers to summarize the admirable monograph of Dr. Sensenig. It must suffice to say that he gives a carefully detailed description of the earliest mesoblastic (embryonic connective) tissue and its division into the primitive somites or sclerotomes. Each sclerotome is divided into a cranial and a caudal half by the appearance of a sclerotomal fissure through

the somite. Each of the vertebrae is formed (roughly speaking) by the fusion of the caudal half of one somite and the cranial half of the next tailward somite. This much has been known, for the vertebrates in general, since the description of Remak in 1855. Dr. Sensenig now fills in the details for the human species. Technical readers must study his work for themselves. They will note that he discards elaborate classifications of the stages of vertebral development because of the great overlap between successive periods, and finds that three periods, those of formation of membranous tissue, cartilage, and bone, suffice for descriptive purposes. He has not been able to trace the development of the nucleus pulposus with certainty, but leans to Luschka's theory of origin from the notochord.

Dr. Sensenig's observations on the origin of the ribs disagree with the reports of previous authors except von Bochmann, in that he finds that the caudal sclerotome-half as well as the cranial half contributes tissue to the developing rib. Part of the capitulum of the human rib is found to arise from the preceding vertebral segment. The same is true of the anterior zygapophysis of the vertebra. Sensenig states, contrary to the views of previous writers, that the early embryonic plane of separation between vertebral rudiments is not represented in the adult by the mid-point of the vertebral disk, but rather by the caudal surface of the disk where it articulates with the centrum or body of the vertebra. He gives a detailed account of the myocoeles (cavities of the somites), showing that in man they do not regularly connect with the coelomic cavity.

Two types of chondrification in vertebral development are described. In one type, which is slightly earlier in appearance, cartilage formation occurs directly in the loose areas of the primitive centrum. In other

parts of the vertebra, chondrification passes through a typical precartilag stage.

EMBRYOLOGY OF DEFECTS OF KIDNEYS AND URETER

Variations in the number and arrangement of the ureters, with or without associated abnormalities of the form of the kidneys, are not unusual in adult human subjects. Because they offer a variety of difficulties when encountered in the diagnostic clinic and the operating room, a voluminous clinical literature has grown up on the subject. The embryological development of an anatomical anomaly, when it can be ascertained, often makes the adult conditions more comprehensible. Such anomalies are rarely seen in the embryonic stage, however, for the number of embryos subjected to detailed study is infinitely smaller than the number of persons examined by physicians. The Carnegie Collection contains one early embryo (no. 6516, 9.6 mm. CRL) in which a double ureter is developing on one side of the body. After the study of this specimen was begun by Dr. Lawrence H. Wharton, Jr., while he was a student in Johns Hopkins Medical School, Professor Sidney I. Kornhauser, of Louisville, Kentucky, very kindly made available another embryo (H9S of the University of Louisville collection). These specimens are among the youngest thus far reported that exhibit anomalies of the ureter. In the Baltimore embryo the left ureter is duplicated, and there are two renal blastemata (early kidney-forming tissue masses) corresponding to the two left ureters. The Louisville specimen has developed an accessory rudimentary ureteric bud on the right side, anterior to a normally located ureter and blastema. Although the factors which caused these variations from the normal cannot be established without a larger

series of cases, and indeed probably not without experiments on embryos of other more available species, the specimens described by Dr. Wharton bring out the fact that the formation of a normal kidney depends upon the occurrence of certain critical conditions in time and space, beginning with the formation of a normal mesonephric duct which gives rise to a normally situated ureteric bud. During this same period, the metanephrogenic condensation must be organized locally, and the bud must make contact with it to initiate further differentiation of the blastema, which in turn stimulates formation of the primitive collecting system from the pelvic part of the ureteric bud. Thus there are many opportunities for one or another failure of growth in rate or pattern, which may cause fundamental anomalies of the urinary organs.

ATRESIA OF THE OVARIAN FOLLICLE

In the ovaries of mammals many more egg-containing follicles are formed than can be used in the whole reproductive life of the individual female. The ovaries therefore contain at all times a number of follicles that are being obliterated before they reach maturity. The process of regression, which is called atresia, consists (generally speaking) in loss of the egg cell and the lining of granulosa cells, and in proliferation of the connective-tissue elements of the theca interna and theca externa until the cavity is obliterated. In the case of large follicles, absorption of the fluid and consequent shrinkage assists in the reduction of volume. The process of atresia differs considerably in detail from one species to another. For this reason it is useful to have a description of it as it occurs in a primate species. Little is known of the rate at which atresia takes place, or of its timing with respect to the ovulation cycle.

Dr. Somers H. Sturgis, now of Massachusetts General Hospital, undertook a study of atresia in the rhesus monkey while at the Department of Embryology in 1942 on a Rockefeller Fellowship which was interrupted by the war. Dr. Corner's collection of rhesus ovaries of known reproductive history, in complete serial section, was placed at his disposal. Sturgis begins his account by describing and illustrating three successive stages of atresia: The first is characterized by dissolution of the granulosa; the second ends with loss of identity of the egg cell, with full development of a zone of hyaloid tissue from the theca interna; and the third progresses to disappearance of all distinctive elements.

Sturgis was able to form an idea of the rate at which atresia proceeds by the study of certain peculiar examples of atresia occurring in small follicles that were partially luteinized. In other words, at the time when a normal mature follicle ruptured and was converted into a corpus luteum, a small follicle in the same animal responded by some chance to the same hormonal influences that were affecting the large follicle, so that while part of its wall was undergoing atretic changes, another part was converted into corpus luteum tissue. The atretic process could thus be tentatively dated from the cyclic history and from the corpus luteum tissue of determinable age. Sturgis concludes from his 3 specimens of partially luteinized atretic follicles that stage 1 of atresia probably develops in 24 to 48 hours, and is certainly over in 5 days; stage 2 requires about 3 weeks. The follicle then gradually regresses and probably reaches the terminal phase of relative quiescence in about 5 weeks after the initiation of atretic changes. These estimates refer to atresia beginning about, the time of ovulation and may not apply to the speed of the retrogressive

process under other circumstances. Evidence is presented showing that atresia of relatively large follicles starts just before ovulation, probably serving thus to prevent multiple ovulation in animals which, like rhesus, normally shed only one ovum at a time. The theca interna of these degenerating follicles appears to function for only a few days just before and just after ovulation. Sturgis conjectures that such activity may augment the production of estrogen at a time when that hormone is necessary for ovulation and for luteinization of the follicle that is destined to rupture.

THE CERVICAL MUCOSA OF THE RHESUS MONKEY

The exit canal of the uterus (cervix uteri) is very important in reproductive physiology, for through it the sperm cells must travel inward and subsequently the infant must pass outward at the time of birth. It guards the entrance of a passageway that opens ultimately into the peritoneal cavity, which must be barred against invading microorganisms while permitting the entrance of the sperm and the exit of uterine secretions and (in menstruating animals) the catamenial flow. For these various functions the lining of the cervix must be adaptable to changing physiological needs.

Dr. Clara E. Hamilton's article in volume 33 of the Contributions to Embryology describes the changes in the epithelial lining of the monkey's cervix uteri in the ovulation cycle, in pregnancy, in castrated and amenorrheic animals, and under various experimental conditions of hormone administration. The work was done at the University of Illinois under the direction of Dr. Carl G. Hartman, member of the Department of Embryology from 1923 to 1941. Although Dr. Hamilton's work was extramural as regards the Carnegie labora-

tory, her material came chiefly from Dr. Hartman's and Dr. Corner's collections.

The observations deal with the cellular morphology of the epithelium (height, position of nuclei, content of mucus, regression, and secretion). It was found that the cell height is increased by estrogen, whereas sudden drops in estrogen level cause secretory changes and subsequent regression of the epithelium of the canal and the cervical glands. No inhibition of the effect of estrogen on the cervix by progesterone or testosterone was found with the doses used. Progesterone but not testosterone enhances the mucus content of the cells. In the light of these findings, the characteristic changes of the cervical epithelium in the ovulatory cycle, pregnancy, and castration are explained.

BLOOD VESSELS OF THE PREGNANT UTERUS

This year saw the culmination of a laborious program of research, marked by the publication, in volume 33 of the *Contributions to Embryology*, of Dr. Elizabeth M. Ramsey's monograph on the vascular pattern of the endometrium of the pregnant rhesus monkey. It has long been evident that the blood vessels of the uterus, and particularly those of its endometrial lining, undergo highly important changes during pregnancy. The peculiar coiling of the arteries of the human endometrium, first noticed by William Hunter in 1774, which is found also in the Old World monkeys, has aroused great curiosity as to its possible usefulness in the implantation of the embryo and the subsequent development of the placenta. Peculiar problems are also presented by the endometrial veins, with respect to the source of nourishment of the implanting embryo. The pathways by which the menstrual blood leaves the intervillous space of the placenta have been much discussed, though without full agreement by the various investigators.

Dr. Ramsey therefore began some years ago, at the suggestion of Dr. Carl G. Hartman, an ambitious attempt to study and describe the vessels of the endometrium and the maternal part of the placenta in the rhesus monkey in order to provide a sequential account of their functional changes throughout pregnancy. In all, 13 female rhesus monkeys were bred to secure a series of pregnancies of known dates. At the chosen time in pregnancy each animal was anesthetized and the blood vessels of the uterus were injected with India ink or other injection mass. Serial sections of large blocks from the implantation sites were made, and the blood vessels and other relevant structures were painstakingly modeled by Dr. Ramsey, using the technique of serial reconstruction on transparent plastic sheets. This project illustrates the effective way in which a specialized research laboratory can deal with certain kinds of work requiring organized co-operation, for it has demanded not only Dr. Ramsey's skillful and persistent efforts, but so much expert service of the departmental staff, from the breeding of the animals through the injecting and sectioning of the uteri to the illustration of the final publication, that a list of those who contributed special skills in furtherance of Dr. Ramsey's research would be almost a list of the whole personnel.

The monograph must be read and the illustrations must be studied to gain an idea of the instructive findings of the research. Summarizing briefly, the coiled arteries are found at first to become more coiled at the implantation site, but afterward to become extended and straight. This uncoiling, which takes place at the beginning of the last third of pregnancy, coincides with the end of the period of uterine growth and the commencement of the period of stretching without growth

that has recently been investigated by Reynolds (see this and recent Year Books). At the same time, the number of arteries connecting with the intervillous space decreases.

The spiral arteries of the placenta undergo a very peculiar change early in pregnancy, by which their endothelial lining becomes greatly thickened by increase of its cells. Dr. Ramsey devotes much attention to this phenomenon, arriving at the conclusion that the proliferation of lining cells is truly a proliferation of endothelium, rather than an invasion by cytotrophoblast cells of fetal origin as it has been considered by some workers.

Dr. Ramsey finds, with respect to the veins of the endometrium at the implantation site, that as pregnancy advances the number of venous channels draining the base of the placenta diminishes, until much the greater part of the venous drainage is into the marginal sinus.

This evidence from a whole series of monkey placentas, graded by age from relatively early to late in gestation, helps to explain the failure of certain workers (e.g. Spanner) on human placentas to find basal placental drainage in the relatively late specimens at their disposal; but on the whole the findings confirm the general theory of placental circulation put forward by Spanner (1934 to 1940) and show that his "overflow" type of filling and emptying of the intervillous space operates in the rhesus monkey as well as in humans, despite minor structural differences.

PHYSIOLOGY OF THE UTERUS

Uterine contraction pattern during labor. Last year's report (Year Book No. 47) included a description of the strain-gauge tokodynamometer invented by Dr. Reynolds for the primary purpose of recording the movements of the human uterus dur-

ing the advanced stages of pregnancy and especially during labor. The instrument, applied without harm and without great inconvenience to the abdomen of the patient, simultaneously records the activity of the muscular wall of the uterus at three different positions, the fundus (i.e. the upper part of the uterus, which is the bottom of the uterine bag), the middle, and the lower end near the outlet. Thus it provides information not only about the strength, duration, and rate of the contractions of the uterus, but also about the relative strength and rate at the three levels. Gradients of force from one point of the uterus to another, and the origin, spread, and dissipation of the contraction waves, are recorded on graphs which can then be conveniently subjected to intensive study and analysis, of the progress of labor, its normal or abnormal character, and the effects upon the uterus of drugs administered to the patient. The instrument has been in active use in the Johns Hopkins Hospital maternity clinic since it first became available, and has been widely copied for use elsewhere.

The results of research with the Reynolds tokodynamometer carried out in the Johns Hopkins clinic have now begun to appear in published form. Dr. Reynolds, with the collaboration of Dr. Louis M. Hellman and Dr. Paul Brans, of Johns Hopkins Hospital, laid the foundation for subsequent clinical reports by describing in the *Obstetrical and Gynecological Survey* the patterns of uterine contractility observed in normal labors and in some of the common types of abnormality (premature and false labor, uterine inertia). Normally progressing labor, as indicated by progressive dilatation of the cervix, is characterized by a gradient of diminishing physiological activity from the fundus to the lower uterine segment. Deviation from

this pattern is associated with prolongation of labor or failure of dilatation of the cervix. The gradient of activity is associated with a high tension in the tissues of the uterine wall at the fundal end, exceeding that in the cervix by about 3 to 1. This favors stronger contractions in the fundus than in lower parts of the uterus and hence contributes to the orderly emptying action of the uterus at parturition. The effect of rupture of the membranes is such that the relative tension at the fundus increases with respect to that at the lower uterine segment. The delicate balance of forces which prevents the untimely emptying of the uterus is thus tipped suddenly, by spontaneous or artificial rupture of the membranes, in a direction favoring delivery of the uterine contents.

Work done by the uterine muscle in labor. The same authors have presented a method of estimating the work done by the different uterine segments during labor. Their article was appropriately published in a Festschrift article for Professor Dr. E. Röthlin, of Basel, an eminent investigator of the pharmacology of the uterus. Graphs recorded by the Reynolds tokodynamometer from a normal labor provided the data for analysis.

The work done by the uterine muscle during the contractions could be computed by planigraphic measurements of the area of the curve under the graph of each contraction. To avoid the impracticable task of analyzing all records with a planimeter, a formula based upon the integration of the intensity, duration, and frequency of the uterine contractions was established, whereby the work per hour could be calculated from the graphs. Such calculated values proved to be parallel to those computed from planimeter measurements, and when corrected by a constant factor could be used to study the work done by various segments and the effect

upon the work of drugs administered to the patient. The formula may therefore be applied with confidence in subsequent studies upon records made with the tokodynamometer.

Recording uterine contractions in animals. Dr. Reynolds and Dr. Irwin H. Kaiser (formerly Fellow in the Department of Embryology) have shown how a dynamometer employing the strain-gauge principle can be used in measuring the responses of uterine muscular tissue of laboratory animals. In an anesthetized rabbit, for example, the uterus is exposed by an abdominal incision and a blunt needle is inserted into its cavity. Pressure changes within the uterus are thus communicated through a rigid tube to the strain gauge. The resulting graphs can be analyzed for the force of the contractions and for the work done. With this apparatus Reynolds and Kaiser have been able to study the quantitative effects of oxytocic (uterus-stimulating) and antihistaminic drugs. The latter (Pyribenzamine and Benadryl) were found to be in general oxytocic, affecting both muscular tone and rhythmic contractility. The hormonal status (pregnancy, nonpregnancy) does not affect the responsiveness of the rabbit's uterus to Pyribenzamine as it does to the pituitary oxytocic substance.

Uterine circulation time. Many recent investigators have suspected that there is a relation between deficient blood circulation in the uterus and toxic conditions during pregnancy. Thus there is a need for measurement of the uterine blood flow. Dr. Edward C. Gillespie and Dr. Reynolds have developed a very ingenious method of making such measurements in experimental animals and have applied it to the rhesus monkey. The technique depends upon observing and measuring the rate at which a substance injected into the muscle of the uterus is cleared away from the site

of injection by the circulating blood in comparison with the clearance of a similar injection into the abdominal muscles. By use of a new high-speed jet injection ("Hypospray") it is possible to propel fluids under the skin almost without pain. The depth of penetration is controllable by the operator. Gillespie and Reynolds found that such injections of the radiopaque dyestuff Diodrast could be made twice weekly in pregnant monkeys without harm. In a typical series shown in their paper (106 days gestation) the clearance of 1 cc. of the dye from the monkey's uterus required 34 minutes, from the abdominal muscle 25 minutes.

Adaptation of uterine blood vessels and accommodation of the products of conception. As the fetus grows, the uterus in which it lies must enlarge with it. The process of uterine growth is not, however, one of mere stretching and enlargement until some extraneous agent causes the uterus to empty itself; it involves an elaborate series of adjustments of the uterus in form and function, resulting in exact adaptation to its contents. For some years Dr. Reynolds has been giving his attention to the various elements of this adaptive process. In volume 33 of the Contributions to Embryology he presents a detailed study of the part played by the blood vessels of the uterus in its accommodation to the products of conception. The data presented were obtained from observations and experiments on rabbits by a wide variety of technical methods involving chiefly injections with diffusible dyes and corrosion preparations. A summary of the results shows, first, that there are two distinct phases in the adjustment of the uterine vascular system to the shape and size of the conceptus. The first of these involves progressive stretching of the blood vessels about the spheroidal conceptus. A maximum degree of distention

is attained about the 22d day of pregnancy in the rabbit. At this time, the second phase of the vascular adjustments takes place after quick conversion of the conceptus to a cylindrical form. The period of rapid fetal growth which ensues involves only lengthening of the uterus. The effect of this upon the blood vessels in the uterus is to cause them, by virtue of their special arrangement, to be separated from one another in the latter part of gestation, without further increase in length. As a consequence, the hemodynamic work involved in moving blood through the uterine blood vessels is no greater when the fetus weighs 80 gm., near term, than at the time of conversion, when it weighs a fourth or less of that amount.

A special mechanism exists by which blood is shunted through the placenta at the time of uterine ischemia, prior to conversion of the conceptus from a spheroid to a cylinder. This shunting takes place by virtue of the existence of two more or less discrete vascular areas in the uterus arising from a common arterial supply. One area (placental) is along the mesometrial border of the uterus, the other (intramural) is over the lateral and ventral aspect of the uterus. The uterus, by virtue of the shape imposed upon it by the conceptus, is subjected to greatest tissue tension on its lateral aspect. That this tension offers resistance to the flow of blood is shown by resistance to passage of dyestuffs and other substances injected experimentally through vessels on the ventral surface of the uterus around spheroidal conceptuses.

Hydrostatic factors within the uterine tissues affect the flow of blood through the blood vessels within them. Although there is a pattern of hormonal changes within the maternal organism throughout pregnancy which affects at any given time the general level of tonus of the uterine muscle,

these changes are superimposed upon a morphological relationship by which the tension to which the tissues are subjected is a function of the factors of the internal pressure and the radius of curvature at any point. Hormonal factors exert themselves by changing the pressure within the uterus through their action on the myometrium, whereas the shape and size of the conceptus influence the tension within the uterus by affecting its radii of curvature.

The uterus in premature birth. The observations on the physiology of the uterus made by Dr. Reynolds and his co-workers during the past several years, all of which have been summarized in the Year Books, could not fail, of course, to yield results of interest to obstetricians. Dr. Reynolds has been in great demand as a lecturer before obstetrical societies. In one of his lectures, given at Denver in May 1948, he outlined the bearing of his findings upon the problem of premature birth, pointing out that the phenomenon of "conversion" or elongation of the uterus during the sixth month of pregnancy, and the associated changes in the circulation, constitute a critical period at which premature birth becomes a special danger. In such studies as he has been making, however, lies the best hope of finding ways and means to recognize impending premature birth and ultimately to ward it off.

ANATOMY AND PHYSIOLOGY OF THE OVARIAN ARTERIES

In the last two Year Books (Nos. 46, 47) reference was made to the observation by Dr. Reynolds that in the human ovary, as well as in the ovary of the rabbit (and presumably of other animals), the ovarian artery has a conspicuously spiral course as it runs along the hilum of the ovary. Evidence that this peculiar condition is dependent upon the presence of estrogen

secreted by the ovary, mentioned last year, has now been published by B. Delson and S. Lubin, of the Cumberland Hospital, New York City, in collaboration with Reynolds.

The same three authors, writing on the vascular patterns in the human ovary, note that the vascular system is more complex than in the rabbit. The branches of the main ovarian artery are helical, with gradually diminishing diameter. The functions of spiraling in these vessels are thought to be (1) adaptation of the vasculature to growth of the ovary and (2) provision of a mechanism for the reduction and regulation of blood pressure within the ovary. When ovarian function diminishes with age, the spiraling decreases. The occurrence of spiraling in the prenatal ovary is presumably stimulated by maternal estrogenic hormones, and its regression, which occurs during several months after parturition, is thought to be due to the removal of their influence.

The hypothesis that coiling of the ovarian artery affords a means of rapidly reducing blood pressure in the ovary as compared with that in the systemic arteries is subject to analysis by hydrostatic theory from measurements of the arteries. Dr. Reynolds points out in the Swiss journal *Acta anatomica* that there are several mechanisms which on physical grounds may be involved in such a reduction. One of these is the rapid decrease in diameter of the ovarian artery after it enters the ovary. Another is the effect which the coiling has in increasing the length of vessel to be traversed by the blood per unit distance along the hilum of the ovary. Reynolds then proceeds to show by hydrostatic theory that the coiling acts also to favor streamline or axial flow under conditions which would give rise to turbulent flow if the vessels were straight.

Angles of branching in the ovarian ar-

tery. In the ovary, more than in most other organs, there is much internal change of structure. The rapid growth and regression of the follicles and the corpora lutea, and the frequent occurrence of minor or major pathological structures (cysts), continually produce alterations in the pattern of the organ and of its constituent elements, particularly the blood vessels. In spite of this, the ovary seems to obey the general law that the blood flow is approximately equal in all parts of an organ. Older writers (Thoma, Hess) long ago laid down certain general principles which are involved in the equalization of blood flow in organs, basing them upon the ratio of the diameters of arterial branches to those of their main stems, and upon the angles formed between the stem and its branches. Dr. Reynolds, in the same article to which reference was made in the preceding paragraph, has sought to find a formula for the angulation of branches of the ovarian arteries. Using one of his corrosion preparations of the rabbit's ovary, in which the channels of the blood vessels are preserved as casts in solid plastic, he measured the angles of branching. The result confirmed a general principle set forth by Sir D'Arcy Thompson in his famous book *On growth and form*, which states that the normal pattern of the blood vessels is such that the circulation can be maintained with minimum effort and a minimum of wall surface. The essential factors in branching arteries which govern this relationship are (i) the diameter of the branch relative to that of the stem, and (2) the angle of branching. It follows from these facts that there must be a relation, expressible by a mathematical formula, between the diameters of arteries and the angles of branching. Dr. Reynolds finds that the following formula fits the specimen he has intensively studied: K (coefficient of arterial branching) =

diameter of branch divided by diameter of stem X cosine of angle of branching.

The article is illustrated with a beautiful three-color half-tone stereoscopic picture, removable for use in the stereoscope, from a photograph by Chester F. Reather.

PHYSIOLOGY OF MENSTRUATION

Prostigmine. In 1940 Soskin, Wachtel, and Hechter reported that the cholinergic drug prostigmine, when administered hypodermically to women suffering from delayed menstruation, will bring about menstruation within three days. Since a woman who is not menstruating (because of pregnancy) does not respond in this way, Soskin and his colleagues suggested that the finding could be made the basis of a test for pregnancy. They explained the induction of bleeding by prostigmine by certain elaborate pharmacological assumptions involving the mechanism of menstruation and its failure in amenorrhea. The finding has been widely advertised in pharmaceutical literature, and there have been numerous clinical papers supporting the observation, though few of them are scientifically critical. For this reason Dr. Corner tried prostigmine in monkeys, soon after the Soskin report appeared, administering it during the summer amenorrhea that is characteristic of rhesus monkeys in captivity in our climate. Menstruation was not induced. These unpublished experiments were greatly extended by Dr. Irwin H. Kaiser during his recent incumbency of a fellowship in the Department of Embryology. He found that prostigmine does not induce menstrual bleeding in spontaneously amenorrheic animals, or in castrated animals during estrogen treatment; nor does it alter hormone-withdrawal bleeding following courses of estrogen, estrogen with progesterone, or a sequence of estrogen-

progesterone-estrogen simulating the normal cycle.

These conditions under which prostigmine failed to induce menstruation in monkeys are not exactly similar to the kinds of amenorrhea in human patients in which it is claimed to be effective. They do not therefore of themselves invalidate the results on humans, but they do call for greater caution than has been used by some of the clinical writers.

Estrogen and the endometrial coiled arterioles. Many students of menstruation during the past twenty-five years have been tempted to assume that the coiling of the arteries of the endometrium is in some way causally related to the bleeding of menstruation, because (speaking approximately) the phenomenon of menstruation and the phenomenon of coiling of the endometrial arteries both occur only in the higher primates (man, apes, and Old World monkeys). In Year Book No. 47 reference was made to Dr. Kaiser's observation that a process resembling menstruation occurs in certain New World monkeys which do not have coiled endometrial arteries.

In a subsequent publication he now reports having produced experimentally in rhesus monkeys, which normally have coiled endometrial arteries, a condition in which they lack such arteries, and yet undergo bleeding of a type usually thought identical with menstruation. This was done by giving massive doses of estrogenic hormone to castrated rhesus monkeys. Discontinuance of the injections was followed, as expected, by menstruation-like bleeding. Post-mortem examination of the uterus under similar experimental conditions (i.e. after 4 weeks of massive dosage) revealed excellent proliferative development of the endometrium but virtual absence of coiled arteries.

In the *American Journal of Obstetrics*

and *Gynecology* for December 1948 Dr. Kaiser reviewed the newer concepts of menstruation. It has been known for some years that the menstrual blood flow results from a sudden drop in the concentration of ovarian hormone (estrogen) in the blood at the end of the menstrual cycle, but just why the hormone deprivation causes the blood vessels in one particular organ, the uterus, to break down with ensuing hemorrhage has been a matter of much conjecture and of largely unsuccessful experiment. A number of recent workers have set up new hypotheses involving details of the uterine vessels, i.e. the coiled arteries mentioned above; supposedly numerous arteriovenous anastomoses; an apparently inadequate lymphatic drainage. Dr. Kaiser's article reviews these hypotheses in the light of his own observations of the nonessentiality of the coiled arteries to the menstrual process. The problem remains one of the outstanding mysteries of human biology.

ENZYMES IN EMBRYONIC TISSUES

Dr. Josefa B. Flexner and Dr. Louis B. Flexner, working in part under an American Cancer Society grant recommended by the Committee on Growth of the National Research Council, have added another contribution to their series on biochemical and physiological differentiation during morphogenesis. They are currently interested in studying the turnover of phosphorus in the metabolic processes of growing tissues, in relation to the rate of growth and the degree of differentiation of the tissues in form and function. The present article deals with the presence and activity, in the fetal brain and liver of the guinea pig, of two enzymes that are concerned with phosphorus metabolism, namely adenylypyrophosphatase ("apyrase") and acid phosphatase.

The first of these (apyrase) is a substance or group of substances found in animal cells which has the property of releasing energy for use in bodily work from energy-rich phosphorus compounds such as phosphocreatine and adenosine triphosphate. A similar enzyme in muscle, adenosine triphosphatase (myosin), has been isolated and identified chemically. In other tissues the chemical nature of the enzyme is not so clearly defined, hence the use of a different name, adenylypyrophosphatase, or apyrase for short.

The authors find that a critical time with regard to apyrase in the brain cortex of the fetal guinea pig is reached at the 42d day of gestation. At this time the activity of the enzyme suddenly begins to rise from a relatively low level toward the high concentration which is found at the time of birth and in young animals. Much the same is true of the fetal liver. The activity in both tissues is still higher in older animals.

Acid phosphatase, which splits the phosphates in acid environments, is widely distributed in the body. It presents a challenge to the student of animal metabolism by reason of our ignorance of its function. In the fetal liver this enzyme was found by Flexner and Flexner to follow a curve similar to that of apyrase. In the brain cortex, however, it behaves differently, for here it is at a constant level of activity about twice as high as that found in the adult.

PERMEABILITY OF THE HUMAN PLACENTA

Frequent reference has been made in these reports to the researches led by Dr. Louis B. Flexner on the function of the placenta, and to the joint efforts to apply his methods to the human placenta in which Dr. Louis M. Hellman and Dr. G. J. Vosburgh, of the Department of

Obstetrics of the Johns Hopkins Hospital and Medical School, have been associated. These three, together with Dr. W. S. Wilde and Mr. N. K. Proctor, have now published another contribution in which they report on the permeability of the human placenta and the supply of water to the human fetus, as studied by the use of deuterium oxide (heavy water) as the tracer substance. Observations were made upon 7 patients whose pregnancies were being terminated by abdominal operation at various stages of gestation, because of serious conditions involving danger to the mother. Measured small amounts of deuterium oxide made isotonic with sodium chloride were injected into the mother's veins about 10 minutes before the obstetrician delivered the fetus and clamped the umbilical cord. The amount of the heavy water which passed from the mother's blood through the placenta to the fetus was ascertained in the case of the older viable fetuses from blood samples, and in the case of the younger, nonviable fetuses by direct extraction of the fetal tissues. The result, as expressed in a graph showing the rate of transfer of the water, agrees with the experience of Flexner and his colleagues with regard to the transfer of sodium through the human placenta, and of several other substances in animals. In view of the laborious nature of the experiments the authors feel that they are justified in presenting conclusions from only 7 patients. There was a fivefold rise in permeability of the placenta to water from the 14th week of pregnancy (the earliest case in the series) to a peak at the 35th week, and thereafter a sharp decline to term. At the peak, the very large quantity of 3.6 liters of water per hour crossed the placenta to the fetus. The placental transfer coefficient for water is five times as great

as that for sodium at corresponding periods of gestation. The human fetus receives across the placenta at the 14th week of gestation 700 times and at the 31st week 3800 times as much water as is incorporated in the growing tissues.

CAPILLARY PERMEABILITY

The use of substances such as heavy water and radioactive salts, which do not significantly differ from the ordinary water and salts of the body in their physiological activities, but are easily identified as they travel through the body by their weight or radioactivity respectively, is not of course limited to the study of special problems, such as the rate of placental transfer, in which Dr. Flexner first used such substances. He and his colleagues have in fact for some years been applying the tracer method to the more general problem of the permeability of the blood capillaries. As participants in the Cold Spring Harbor Symposium on Quantitative Biology in the summer of 1948, Dr. Flexner, Dr. Dean B. Cowie of the Department of Terrestrial Magnetism, and Dr. Gilbert J. Vosburgh of the Department of Obstetrics, Johns Hopkins University and Hospital, presented a summary of the joint studies in which they and others (A. Gellhorn, M. Merrell, R. M. Rankin, R. O. Scholz, W. S. Wilde) have taken part.

Among the four major problems to which they have contributed, the first is that of the rate of exchange of water, of sodium, of chloride, and of iron (in the physiologically important form of ferric betai-globulinate). These substances, marked by their weight (heavy water) or by being made radioactive, are injected into a blood vessel and the rate of their escape, as measured by the amount still in the blood stream after various time intervals, is determined. The results show in the

first place that the substances named pass through the walls of the capillary blood vessels into the surrounding tissues at different rates for different substances. The average capillary wall of the guinea pig is 23 times as permeable to water as to sodium and to chloride, and at least 100 times as permeable to water as to ferric betai-globulinate.

In man, 78 per cent of the blood-plasma sodium and 105 per cent of the plasma water is exchanged per minute with extravascular sodium and water. An amount of water equal to a man's entire weight passes out of his blood capillaries, and is replaced by an approximately equal amount, every 20 minutes. The capillary part of human blood circulation, seen in the light of these facts, is a system of fine tubules with permeable walls through which floods of water bearing salts and other metabolic substances are pouring at every moment throughout life.

A second problem which is largely solvable by the tracer method concerns a much debated hypothesis that whereas water and dissolved gases pass through the whole of the thin protoplasmic wall of the capillary, i.e. through the endothelial cells as well as the intercellular cement, the only important avenue for escape of the electrolytes is the cement substance, much as if one should say that water running through hollow tiles could leak through both the tiles and the joints between them, but salt only through the joints. Flexner, Cowie, and Vosburgh make a calculation based on an estimate of the area of the capillary wall to which 1 ml. of plasma is exposed, on the diffusion constant of a salt ($KC1$), and on the concentration gradient of the salt in the blood plasma. From these data the theoretical amount of potassium chloride which can diffuse across the interface per day can be worked out. The amount of radioactive chloride which actu-

ally moves across the capillary wall in a unit of time was previously measured (Cowie, Flexner, and Wilde, 1941). This proves to be about what would be predicted if the electrolyte were diffusing through the whole available wall, rather than through the far smaller area of the intercellular cement. The same deduction may be made for water and for sodium.

A third problem is to determine whether, as has been thought by some workers, the dissolved substances of the plasma pass the capillary wall "in bulk"; that is, do all go across in the same concentration that exists in the plasma, or do they cross the wall in different proportions? Results show that the latter supposition is correct. The fluid passing through the capillary wall is found to contain a far smaller proportion of ferric globulinate than of chloride to the amount of these respective substances in the plasma. The iron-protein compound is thus largely held back within the capillary while the water and salts freely interchange with the same substances in the tissues.

Finally, the method permits deeper insight into the physical processes involved in passage of the capillary wall by these substances, indicating that diffusion rather than filtration is the essential process.

Transcapillary exchange of iron; assay of iron. A separate paper by the same three authors, presenting the observations on which the above statements about the transcapillary exchange of iron were based, will be found listed in the bibliography appended to this report. In order to make accurate determinations of radioactive iron in biological material, an improved method of assay had to be developed. This also is cited in the bibliography.

THE AMNIOTIC FLUID

The fluid that exists about mammalian embryos and fetuses within the amniotic

membrane, serving to float the embryo and protect it from physical shock, presents interesting problems concerning its formation and the rate of exchange of its water with the water of the mother's blood, from which it is ultimately derived. The amniotic fluid has generally been considered to be rather stagnant. Some writers have thought that it consists mainly of urine discharged by the fetus. That dyestuffs may be made to pass into it from the mother's blood has been known. Flexner and Gellhorn applied the isotope technique to the problem in 1942, using guinea pigs, and found that the water of the fluid is replaced at the surprisingly rapid rate of about once an hour, whereas the rate of replacement of the sodium is about 50 times slower. The transfer of water and sodium to the human amniotic fluids has now been measured, using deuterium oxide (heavy water) and radioactive sodium (Na^{24}) as tracer substances. The observations required a team of specialists, namely two obstetricians, G. J. Vosburgh and L. M. Hellman, of the Johns Hopkins Hospital, Department of Obstetrics; a physicist, D. B. Cowie, of the Carnegie Institution's Department of Terrestrial Magnetism; and three physiologists, L. B. Flexner, W. S. Wilde, and N. K. Proctor, of the Department of Embryology. The tracer substances were injected intravenously into pregnant women who were undergoing operative procedures involving surgical exposure of the uterus. At measured intervals after the injection, samples of the amniotic fluid were drawn through the wall of the intact uterus with needle and syringe and were submitted to measurement of the deuterium oxide and the tagged sodium. Details of the method and calculations are given in the paper. It was found that the water of the human amniotic fluid is completely replaced on the average once every 2.9 hours. The sodium

is transferred about 5 times more slowly than the water. At term a fetus weighing 3.4 kg. is surrounded by approximately 1000 cc. of amniotic fluid which is exchanging water at the rate of 350 cc. per hour. This is by no means a condition of stagnation; indeed, it is astonishingly rapid. The fetal urine alone cannot account for so much water, which therefore is probably furnished directly through the amniotic membrane.

SEX RATIO OF ABORTIONS

Wherever human birth records are kept, it appears that among newborn infants boys are slightly more numerous than girls. In the United States the sex ratio for live births is 105.6 boys to 100 girls; for stillbirths, 124.1 to 100, and for the two groups combined, 106.0 to 100. The sex ratio at the time of conception in man ("primary sex ratio") is not accessible to direct observation. Dr. E. Carlton MacDowell, of the Department of Genetics, arrived some years ago at the sex ratio of mice at conception by an ingenious method requiring surgical exploration of the mother's ovaries, and found it approximately 1:1. If the primary sex ratio in man were also 1:1, the figure of 105.6 to 100 at birth would mean that a slightly greater number of female embryos than of male succumb during gestation. On the contrary, however, widely accepted reports made during the past hundred years have asserted that many more male embryos and fetuses than female succumb during gestation. The sex ratio of aborted fetuses has generally been reported still higher in the earlier months of gestation than in the later months and at birth. Four of the largest reported series of observations when combined give a sex ratio of 224 (males to 100 females) in the 4th month, 143 in the 5th, 121 in the 6th, and 114 in the 7th

month of gestation. The primary sex ratio has therefore been estimated by various writers, by extrapolation from such figures, at from 125 up to 170. No sound reasons for any such excess of male conceptions, or for such high mortality of male infants in utero, have been put forward.

Dr. Christopher Tietze, a physician and statistician associated with the School of Hygiene, Johns Hopkins University, has reinvestigated the sex ratio of abortions by analyzing the records of the Department of Embryology, which provide the only large series of sex determinations of human fetuses made entirely by trained observers under uniform conditions. A similar analysis published in 1921 by Dr. A. H. Schultz, then a member of the Department, gave an unweighted mean sex ratio of 109 for the 3d to the 7th month of pregnancy inclusive. The Carnegie Collection, having grown greatly, now affords more material for statistical study. Dr. Tietze abstracted from the main catalogue of the Collection records of 5667 fetuses from the 4th to the 7th month. Their sex had been determined by members of the research staff or by trained technicians. This series was supplemented by 120 specimens of the 3d month, the sex of which was determined by microscopic examination of the gonads, largely by the late Dr. George L. Streeter. The help given Dr. Tietze by Dr. Streeter was the last of the latter's many co-operative services to fellow scientists. These 120 specimens of the 3d month had a sex ratio of 93.5 males to 100 females. The fetuses of the 4th, 5th, 6th, and 7th months had ratios of 107.7, 115.6, 109.7, and 100.6 respectively. The mean ratio of the 5787 cases was 107.9. Dr. Tietze's analysis therefore reveals no evidence for a primary sex ratio materially different from that found at the end of pregnancy among the combined live and stillbirths. He tentatively ascribes the

widely different reports based on other material to errors in determining sex made by medical men without special training in embryology, or by midwives. Such errors are the more likely to occur, the earlier the fetuses and embryos studied.

NUMBER OF YOUNG AT BIRTH; NUMBER OF NIPPLES

Dr. Adolph Schultz, of the Department of Anatomy, Johns Hopkins Medical School, a former member of the Department of Embryology, from time to time makes use of the Carnegie monkey colony records and specimens in his biometric studies of primates, as for example in an interesting recent report on the number of young at a birth and the number of nipples in primates. The Carnegie colony of rhesus monkeys has had one twin birth in 188 pregnancies. Schultz has assembled records on twinning in other monkeys, which suggest that twinning occurs in most catarrhine monkeys and in apes with a frequency not far different from that in the human species. A frequently cited theory that twinning in man is a newly acquired character, from the evolutionary standpoint, is not supported by this study.

In the entire suborder of simian primates, 2 pectoral nipples represent the normal condition. Supernumerary nipples, however, have been found in 40 monkeys and apes from 10 species representing all the major groups except the Semnopithecinae. They are not uncommon in human beings. Dr. Harold Speert's Carnegie cases in the rhesus monkey, referred to in Year Book No. 41, and those now reported by Schultz, give a rate of frequency of 14 and 1.1 per cent respectively, which is close to that in man. The general percentage frequency in monkeys, apes, and man is very similar and lies not far above 1 per cent. The primitive character of 2 or 3 pairs of

nipples is more common in the lower primates (prosimians), but the number of nipples varies from species to species and often even within the species.

PALATINE RIDGES OF PRIMATES

In the majority of mammals the lining of the hard palate bears ridges, more or less transversely directed, which differ widely in number and relative size. In the human species the palatine ridges are reduced in number and are usually limited to the area in front of the first molar teeth, but any reader of this report will find, by passing his thumb over the front part of the roof of his mouth, that he possesses 2 to 8 ridges, the average in man being 4.2. Many conclusions have been drawn from the palatine ridges about the evolutionary relationships of mammals, including the relation of man to the other primates. Two great comparative anatomists, Retzius and Gegenbaur, have stated that the human ridges are more numerous in the fetus than in the adult, thus implying that the reduction in number seen in humans occurs ontogenetically, i.e. in the individual, as well as phylogenetically. Volume 33 of the Contributions to Embryology contains a thorough quantitative study by Dr. Adolph H. Schultz of the comparative anatomy of the palatine ridges in primates generally and of their ontogenesis in man. Thirty-two human fetal palates studied by Dr. Schultz were obtained from the collection of the Department of Embryology.

Dr. Schultz concludes that the primitive common ancestor of the primates had numerous large, regular palatine ridges, reaching back to the last molar teeth. This is the usual condition in a great variety of lower mammals. The ridges have become significantly changed in many

groups of primates, and the alterations reveal phylogenetic trends. Dr. Schultz's article must be read to appreciate the variability. He finds that the ridges can be recognized in a human embryo of 28 mm., making their appearance prior to the normal embryonic fusion of the two lateral palatine processes. Thus failure of the latter to fuse, resulting in cleft palate, does not prevent the formation of ridges. The average number of ridges is the same in

fetuses as in adults; this means that there is no reduction in their number during growth of a human individual. The palatine ridges of single-ovum twins show only a limited degree of resemblance, and it appears therefore that the details of the ridge pattern are not closely determined by heredity, although the general degree of regularity and the tendency toward unification and discontinuity of the ridges must have a genetic basis.

DIFFUSION AND POPULARIZATION OF RESULTS

In September and October 1948, Dr. Corner presented lectures, based in large part on work done by himself and other members of the Department of Embryology, before the Linn County Medical Society, Cedar Rapids, Iowa; the Hollywood (California) Academy of Medicine, the San Diego Academy of Medicine, the Southwestern Pediatric Society (Los Angeles), the Los Angeles Obstetrical and Gynecological Society, and the New Mexico Clinical Society. Dr. Burns addressed the Regional Post-Graduate Seminar of the American Urological Association at Buffalo in January 1949, on "The hormones and the differentiation of sex in the mammalian embryo," and also spoke at the University of Rochester on the same subject. Dr. Reynolds gave lectures and talks on problems of uterine physiology

during the year at the University of Maryland, the New York Obstetrical Society, and the Obstetrical Society of Boston. Drs. Corner, Burns, Flexner, Reynolds, and Tyler each gave one or more lectures by invitation before classes in anatomy, physiology, and obstetrics of the Johns Hopkins University, School of Medicine.

Mr. Chester F. Reather, departmental photographer, was given the first award for gross-specimen photography of the Biological Photographic Association for a picture shown at the Association's 1948 annual convention at the University of Pennsylvania. The photograph, a superb picture of a human embryo in its membranes (Carnegie Collection, no. 8537A), has been reproduced by half-tone in *Medical Radiography and Photography* (see bibliography below).

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DEPARTMENT OF GENETICS

Cold Spring Harbor, Long Island, New York

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Members of the Department continued their studies of the nature of the hereditary materials, genes and chromosomes, using a variety of approaches and several different organisms in their experiments. McClintock's cytogenetic studies on maize have given further evidence of the biological complexity of a gene locus, and are revealing one of the possible mechanisms through which genetic instability may be attained and gene differentiation increased. Corroborating evidence of the complexity of a gene locus has been obtained in the work of Demerec and his collaborators with a strain of the bacterium *Escherichia coli*. The biochemical work carried on by Kaufmann, McDonald, and their group with chromosomes of *Drosophila* and several plants is beginning to throw light on the chemical organization of these structures. Studies by Caspari and Dalton, dealing with pigment development in the meal moth and the axolotl, have analyzed the mechanism of the gene action controlling this process. The work of MacDowell and his collaborators with mice has gone farther in demonstrating the importance of factors such as virus infection and mother's and nurse's age in the development of leukemia. Efforts to discover chemicals capable of inducing mutations in *E. coli* have been continued by Demerec, Bertani, and Flint, using a newly developed method involving reversions in this material from the streptomycin-dependent state. Witkin, also working with *E. coli*, has found that sodium nucleate has a striking effect on the time of expression of certain mutants. Doermann investigated a strain of bacterial virus that shows

a high rate of mutations; and Delaporte made cytological observations of the strains of bacteria being used in our various studies. Dobzhansky spent fifteen months in Brazil, studying the dynamics of *Drosophila* populations in tropical and subtropical environments.

The study of mutable loci in maize was continued by McClintock in an effort to determine the mode of origin of mutable loci from normal loci and the nature of events occurring at such a locus to bring about detectable changes in its phenotypic expression. Investigations this year were confined to the *Ac* (activator) locus and to the group of Δ -controlled mutable loci, which require the presence of this second locus for expression of their mutability. It was concluded that the origin of all mutable loci in this class is associated with the transposition of a single locus, *Ds*, from one position in the chromosomal complement to another. The insertion of *Ds* at or near a normal locus may inhibit the action of the normal locus, and thus change its phenotypic expression. Continued changes in the phenotypic expression of the affected locus are related to subsequent events that partially or completely remove this inhibitory action by removal of the *Ds* locus, or that change the constitution or position of *Ds* with reference to the affected locus. The events occurring at *Ds* to bring about such changes result from compound chromatid breaks at this locus. The change in expression of the affected locus depends on the type of fusion of broken ends that follows such breakage events. In several cases, the insertion of *Ds* into or adjacent to a normal locus has

made it possible to analyze more fully the composition and action of the normal locus. This analysis has revealed that one of the normal loci studied is compound, in that it is responsible for at least two reactions associated with the appearance of a single end product. *Ac* itself is a mutable locus. It also undergoes transpositions from one position in the chromosomal complement to another. The mechanism responsible for the transposition of *Ac* and the production of various alleles of this locus is probably the same as or similar to that associated with transpositions and changes of the *Ds* locus.

The patterns of mutation in *E. coli* to streptomycin resistance and dependence, and of reversion from dependence to non-dependence, were studied by Demerec through analysis of the various properties of about 280 separate mutants. Most of these mutants were found to differ from one another, indicating that a reversion from dependence to nondependence probably comes about not through a reversal of the chemical reaction that originally produced the dependent mutant, but through some other change in the *gtnt* complex. Tests made with the K-12 strain of *coli* by Lederberg's method indicate that either a single locus or adjacent loci are responsible for these various mutants. As a rule, each mutant shows the effect in more than one characteristic.

Bertani, Demerec, and Flint investigated mutagenic activity in several chemicals by determining their effect on the frequency of reversions in *coli* from streptomycin dependence to nondependence*. Induction of mutations was observed with the following chemicals: formaldehyde, acriflavine, phenol, caffeine, ethyl carbamate, ammonia, and ferrous chloride. In comparison with radiations and mustard compounds, all these except ferrous chloride

have a low order of mutagenicity. Ferrous chloride in experiments with *coli* is as strong a mutagen as X-rays, ultraviolet rays, or nitrogen mustard.

Demerec also studied in *coli* the patterns of resistance to aureomycin, chloromycetin, and neomycin, and found that there is a stepwise increase in resistance to each of these three antibiotics.

The effect of sodium nucleate on the phenotypic expression of delayed mutations to phage resistance in *E. coli* was investigated by Witkin and Flint. Exogenous nucleic acid can apparently take the place of division in causing delayed mutations to become phenotypically effective.

A new technique for the study of the mutagenic action of certain chemicals was developed by Witkin. The method involves the treatment of bacteria with an inhibitory or toxic compound, the action of which can be specifically reversed by the addition of another compound. This inhibition-reversal system provides a sensitive method for the detection of mutations induced by the inhibitory agent.

Doermann continued investigation of the intracellular growth of bacteriophage along several lines, with emphasis on the genetic approach to this problem. He showed that genetic recombinants are present among the earliest-formed phage particles in bacteria infected with two genetically different phages. This result, taken in conjunction with other genetic information, shows conclusively that phage multiplication is not accomplished by simple fission of virus particles, but that some other mechanism is responsible. The previously described cyanide-lysis method for studying the intracellular bacteriophage population has been modified in such a way that the intracellular phage of individual bacterial cells can be analyzed qualitatively and quantitatively. With this modified method a

study was made to see whether reciprocal exchange is the mechanism of producing genetic recombination among bacteriophages. Although reciprocal exchange was not ruled out completely, its occurrence was shown to be highly improbable, at least during the later stages of virus development.

Doermann and Dissosway made preliminary studies on a genetically unstable series of bacteriophages. The hereditary changes observed appear to be related to alterations in the requirements of the various types for adsorption cofactors. The fact that four new types invariably appear in one plaque arising from a single phage particle implies a fairly high rate of mutation. The quantitative expression of this rate is very much exaggerated, however, by selective factors acting during the growth of the plaque.

Cytochemical studies were continued by Kaufmann, using purified enzymes. A technique of dissecting the chromosome by successive treatments with nucleases and proteases has indicated that this structure represents an integrated fabric, in which no single protein or nucleic acid may be regarded as the primary structural component. In the course of this analysis it was found that the cellular dissolution usually attributed to the specific action of trypsin is due to the removal by water of degradation products of the action of trypsin in combination with electrolytes.

Kaufmann, in extending his studies of the action of near infrared radiation on living cells, did not detect any modification by this portion of the spectrum in the frequency of mutations induced by X-rays in the bacterium *E. coli*.

McDonald found that when dilute solutions of crystalline trypsin in 0.005 N hydrochloric acid are exposed to X-radiation, the ability of the enzyme to hydrolyze

denatured hemoglobin is partially destroyed. For any one enzyme concentration, the residual activity is an exponential function of the dose. In the range of concentrations thus far studied (3×10^4 to 9×10^{16} molar) the ionic yields are not constant, but increase with increasing concentrations of trypsin.

Further studies by MacDowell with transplanted leukemia of line I, freed from a long-unrecognized virus infection, prove that the virus and not the increasing potency of the leukemic cells has been responsible for the death of hosts of a certain foreign strain that had previously been naturally resistant. Hosts of this strain are now as resistant as ever to these virus-free leukemic cells. The virus is lethal to the foreign strain but nonlethal to the strain of hosts that carry the leukemic cells. The same interpretation holds for the breakdown of a 1:1 ratio of susceptible backcross hosts. With virus-free leukemic cells of this line, this ratio has been re-established. On the other hand, the removal of a virus from another line of leukemic cells (line L) has demonstrated that intrinsic changes within the leukemic cells, and not the infecting virus, were responsible in this case for the previously observed increased susceptibility of a strain of foreign hosts.

MacDowell observed also that the age of the nurse has as certain an influence after birth, upon the incidence of spontaneous leukemia and upon the length of life, as the age of the mother has before birth, according to preliminary but unquestionable results of an experiment testing these variables. In F₁ hybrids from leukemic-strain fathers, leukemia is less frequent and life longer when the nonleukemic mothers and nurses are old. But with young mothers, old nurses transmit as much resistance to the appearance of spontaneous

leukemia and as great lengthening of life of both leukemics and nonleukemics as do old mothers with young nurses.

The mechanism of gene action in controlling development of pigment-pattern differences between the white and black strains of the Mexican axolotl was investigated by Dalton, using methods of tissue culture and embryonic transplantation. Study of chromatophores of both strains *in vitro* showed that the genes *D* and *d* do not affect the chromatophores themselves in such a way that intrinsic differences in capacity for proliferation, migration, or pigment synthesis are demonstrable in tissue cultures. Embryonic transplantation experiments indicate that the genetic differences in pigmentation of the two strains are mediated through differences in tissue environment and not in the chromatophores themselves, the white pattern resulting from an inhibitory effect concerned with the migration of pro-pigment cells. The results fail to support the view, suggested in the literature, that the mechanism of gene action in this case involves a diffusible substance necessary for melanin synthesis, contributed to pro-pigment cells by epidermis of the black strain but lacking in the white strain. Furthermore, the assumption, on which this was based, that pro-pigment cells in the white axolotl migrate as extensively as in the black, but do not produce pigment, is rendered questionable by results of Dalton's investigation. Inhibition of chromatophore migration by surrounding tissues of a particular genotype has not previously been described as a mechanism of pigment-pattern formation in amphibians. Demonstration of such inhibition in the white axolotl raises the question whether the pigmentation effects observed in other species combinations after reciprocal transplantations of epidermis may *not* in part depend on simi-

lar relations between epidermis and pigment cells.

The action of the genes *a* (red eyes) in *Ephestia* and *T* (Brachyury, short tail) in the mouse was investigated by Caspari, using serological methods. In *Ephestia*, serological differences between *aa* and *a*a*⁺ genotypes could be established by titration and absorption experiments. An antigen differentiating the two strains was demonstrated in the euglobulin fraction. In the mouse, antigenic differences between *T*/*+* and *+/+* organs were found both by the precipitation method and by the complement-fixation method. With the latter method, the *gent Ki*, which is closely linked and phenotypically similar to *T*, turned out to be antigenically allied to but not identical with *T*.

Caspari, breeding normal animals obtained from crosses involving *Fu* (Fused), confirmed the hypothesis that *Fu*/*+* mothers adversely affect the penetrance of the gene *Fu*. In the offspring of normal animals from crosses of female normal by male Fused, there were occasional phenotypically Fused animals which bred as normals.

An enzyme system catalyzing the oxidation of tryptophane to kynurenin was studied by Caspari in homogenates from mouse liver. The enzyme was partly purified by fractionated precipitation with ammonium sulfate. The properties of the enzyme system and of the reactions catalyzed by it were investigated.

Delaporte studied the cytology of bacteria exposed to various influences such as changes in culture medium, irradiation with ultraviolet, infection by bacteriophage, culture on streptomycin, and inactivation by ultraviolet rays and subsequent recovery by light treatment. She found that all these conditions produce striking effects in the nuclear element of bacterial cells.

A project for study of population genetics of tropical *Drosophila* flies was developed by Dobzhansky, of Columbia University, Research Associate of the Institution, in co-operation with a group of investigators working, during the year 1948-1949, at the University of São Paulo and at the Instituto Agronomico do Norte, Belem do Pará, in Brazil. Samples of wild populations of *Drosophila* were collected in twenty localities, chosen to represent the various bioclimatic regions of Brazil. The collecting work required extensive travel, the distance covered within Brazil by airplane alone amounting to about 28,000 kilometers. More than 100,000 flies were collected and classified. Two species, *ZX willistoni* and *D. pro salterns*, were subjected to cytogenetical analysis, the results of which will be reported later. For the time being, it can be stated that a great amount of genetic variability, both in mutant genes and in chromosomal aberrations, has been discovered in the natural populations of the two species, and furthermore that the quantity of genetic variants present in a population shows a significant relation to the environment in which the population lives.

For the past two years Dr. Ernest W. Caspari has been with the Department as a research associate, while on leave of absence from Wesleyan University, Middletown, Connecticut. He is returning to his former position in September. While here, Caspari carried out extensive research on the action of genes, investigating the chemistry of gene-controlled development of pigment in *Ephesia*, and studying the behavior of several genes in the mouse. He took part in many of the general activities of the Department, serving as chairman of the Seminar Committee and of the Library Committee. His profound knowledge of the literature made him a welcome

participant in discussions and a helpful adviser.

The fellowship program of the Institution has brought to the Department several young members who have been a great asset to the research program. Dr. A. H. Doermann was here for two years, working on reproduction and mutation in bacterial viruses. He left in the summer of 1949 to take a research position at the Oak Ridge National Laboratory of the Atomic Energy Commission. Dr. H. C. Dalton is remaining for another year to continue his research on pigment development in the Mexican axolotl. Dr. Berthe Delaporte, of the Centre National de la Recherche Scientifique in Paris, stayed at the Department for fifteen months, working on various cytological problems with bacteria. Dr. G. Bertani is now studying mutations in bacteria occurring spontaneously and induced by various chemicals.

The research program has been expanded through a grant received from the U. S. Public Health Service in support of Kaufmann's work, and through a grant of the American Cancer Society, recommended by the Committee on Growth of the National Research Council, supporting work of Demerec. Collaboration with the Biological Laboratory adds four research members to the group. Dr. V. Bryson, biologist at the Laboratory, is studying the biological aspects of the origin of bacterial resistance to various chemicals. Associated with him is Dr. B. Prytz, chemist, who is investigating the chemical aspects of the same problem. Dr. A. Kelnar, bacteriologist, who left this summer, was successful last year in discovering the photoreactivation process, whereby microorganisms inactivated by ultraviolet irradiation partially recover after subsequent exposure to visible light. Dr. Bruce Wallace, geneticist, is studying the effect of continuous ex-

posure to radium on the genetic constitution of *Drosophila* populations.

The Cold Spring Harbor Symposium on Quantitative Biology, organized by the Biological Laboratory, brought more than 180 scientists to the Laboratory this June for a ten-day conference on the subject "Amino acids and proteins." Among the speakers at the conference were Kaufmann and McDonald, of the Department's staff. Seven of the speakers came from Europe,

and there were several other participants from foreign countries. During the rest of the summer, more than fifty scientists and their assistants from various institutions stayed at the Laboratory.

During the year the *Drosophila* stock center sent out 318 cultures to research workers, 53 of them to countries in Europe, Asia, and South America. The number of cultures sent to teaching laboratories in high schools and colleges was 666.

MUTABLE LOCI IN MAIZE

BARBARA MCCLINTOCK

During the past year the study of mutable loci in maize has been continued, in an effort to determine the mode of origin of mutable loci from normal loci and to ascertain the events occurring at a mutable locus that result in detectable changes in phenotypic expression. Progress has been made with respect to both these objectives.

As stated in previous reports, two main classes of mutable loci have appeared and are continuing to appear in the maize cultures. One class includes a number of mutable loci that undergo changes in action only when a second locus, the activator (*Ac*), is likewise present. Mutable loci of the second class do not require such an activator locus. During the past year, study has been continued only on the Δ -controlled mutable loci. The decision to confine efforts to these mutable loci was made because all of them respond to the same *Ac* locus, regardless of the diversities of phenotypic expression they represent. On the basis of this common response to the presence of *Ac*, it could be suspected that the events leading to a change in phenotypic expression are of the same nature in all the *Ac*-controlled mutable loci. What are these events? Also, why do normal,

"wild-type" loci suddenly become unstable in these cultures?

Previous reports have discussed in detail the Δ -controlled mutable *Ds* (dissociation) locus. It was shown that *Ac* may induce chromatid breaks at the *Ds* locus that are followed by fusions of broken ends, and that these fusions may result in the formation of a dicentric chromatid and a U-shaped acentric fragment. It was also pointed out that each such event is comparable, with respect to time and frequency of occurrence, to mutations of other loci that produce recognizable phenotypic changes in gene action. It was concluded that *Ac* must give rise to a specific condition in certain cells of the plant that brings about an alteration in the mode of reproduction of the *Ds* locus in these cells during the mitotic cycle. This alteration eventuates in the production of breaks in the sister chromatids at the *Ds* position, as previously described. By genetical and cytological test methods, it was possible to place this *Ds* locus at a position demarcating the proximal third of the short arm of chromosome 9. Continued study, however, has revealed a type of event involving the *Ds* locus that appears to be responsible

for the origin and subsequent behavior of all Δ -controlled mutable loci. This event brings about a transposition of the *Ds* locus from one location in the chromosome complement to another. In its new position, *Ds* responds to *Ac* just as it did in its previous position. (The position of *Ds* in the short arm of chromosome 9, where it was first detected, has been designated the "standard position.") These transpositions of *Ds* are not infrequent. In the sporophytic tissues, they usually occur late in development and in individual cells of the plant. For transposition to occur, *Ac* must likewise be present. When *Ds* is transposed from its standard position to another position in the short arm of chromosome 9, the new location may be readily determined.

THE MECHANISM OF TRANSPOSITION OF THE *Ds* Locus

A number of cases of transposition of *Ds* are now under investigation. In some of these, a gross chromosomal alteration has accompanied the transposition of *Ds*. By cytological and genetical analyses of the cases involving gross chromosomal aberrations, it has been possible to reconstruct in considerable detail the events that must have occurred to bring about a transposition of the *Ds* locus. These events are similar in all analyzed cases, and can be summarized as follows: During a mitotic cycle a condition may be produced at the *Ds* locus that results in the removal from one or both chromatids of a submicroscopic fragment of chromatin containing the *Ds* locus. Both ends of this fragment are unsaturated; and the mechanism of removal of the fragment may be a tearing process, since unsaturated ends, capable of fusion, are produced in each of the chromatids of chromosome 9 at the position where the fragment was situated. If, dur-

ing the same mitotic cycle, a spontaneous break occurs elsewhere in the chromosome complement, four additional broken ends may be present in the nucleus. Since any unsaturated broken end is capable of fusion with any other unsaturated broken end, a number of different consequences of fusion among the twelve broken ends can arise. If the spontaneous break occurs in the short arm of chromosome 9 at a position other than the *Ds* locus, several types of altered chromosomes 9 can be formed. These may have a deficiency, a duplication of a segment of the short arm—either in a normal or in an inverted order—or an inversion. On the other hand, fusions of broken ends can bring about a transposition of the *Ds* locus without an accompanying gross chromosomal rearrangement. If the spontaneous break occurs in one of the other chromosomes of the complement, a translocation between the short arm of chromosome 9, at the position of the *Ds* locus, and this other chromosome can be produced. A transposition of *Ds* may likewise accompany such an event. Examples of these various kinds of translocation and transposition have been found. Those involving transpositions of *Ds* within the short arm of chromosome 9, either accompanied or unaccompanied by gross chromosomal rearrangements, have been selected for continued investigation.

In the analyzed cases of transposition of *Ds*, the inserted segment of chromatin containing the *Ds* locus is not visible in its new position with the light microscope. It is also too small to affect detectably the percentage of crossing over in adjacent regions in plants heterozygous for the transposed *Ds* locus. Its detection in the new position is easy, nevertheless, because it behaves as it did in its former position; dicentric chromatids and acentric fragments may be produced by subsequent

breaks and fusions that now occur at this new position. Because it behaves in its new position as it did in its former position, transposition from this new position to still another position may occur subsequently.

The discovery of the transposition of the *Ds* locus, and the knowledge gained in determining the principal events responsible for it, have supplied the information needed for understanding the origin of other \wedge -controlled mutable loci. It has also become possible to formulate a more direct approach for investigation of the primary effect of *Ac* on the *Ds* locus, wherever it may be, and to determine more fully the various changes that are known to occur at the *Ds* locus itself.

THE ORIGIN OF \wedge -CONTROLLED MUTABLE LOCI

In Year Book No. 47 (1947-1948), the sudden appearance of an *yic*-controlled mutable *c* locus was described. It was found in a single one of the tested male gametes produced by a plant having one *Ac* locus. This plant was also homozygous for a normal *C* locus and for *Ds* in its standard position. In this gamete, the action of the *C* locus had changed. It behaved thereafter like the known recessive (*c*) but, unlike this recessive, was capable of mutating back to a normal *C* action when *Ac* was present.

Study of the $c^{TM'}$ locus has been of particular importance in revealing the factors associated with the origin and subsequent behavior of \wedge -controlled mutable loci. It is now apparent that the mutable *c* locus arose when the *Ds* locus was transposed from its standard position to a position within or close to the normal *C* locus. This event occurred late in the development of the parent plant, and probably only in a single cell of this plant. No gross

chromosomal rearrangements accompanied the transposition. The chromosome 9 carrying this transposed *Ds* locus is morphologically normal. The transposition of *Ds* was recognized by the altered position of the chromatid breaks associated with *Ds* behavior and the concomitant disappearance of such events at the standard location. Both cytological and genetical test methods, used to determine the location of these breaks, were in agreement in placing the D^{\wedge} -type activity at the known position of the normal *C* locus in the short arm of chromosome 9. In its new position, the *Ds* locus presumably inhibits the normal action of the *C* locus. The *C* locus, although present, does not appear to function, and as a consequence no aleurone color is produced. With respect to pigment formation, the tissue response is the same as that given by the known recessive allele, *c*, or by a deficiency of the *C* locus. This inhibited *C* locus, however, can mutate to a state that re-establishes its former action. This occurs only when *Ac* is also present in the nucleus. The restoration may be permanent. The restored *C* locus no longer shows unstable behavior in the presence of *Ac*, and it cannot thereafter be distinguished from a normal *C* locus. What occurs, then, at the inhibited *C* locus to restore its normal action?

As stated previously, the studies of a number of different transpositions of the *Ds* locus have shown that *Ds* may be removed from a chromatid and that the mechanism of removal involves compound chromatid breakage at this locus. The removed fragment containing the *Ds* locus has unsaturated broken ends, and the ends formed in the chromatid by its removal are also unsaturated and capable of fusion. It is known that *Ds* activity usually disappears completely at the $c^{TM'}$ locus when a mutation from *c* to *C* occurs. The known mechanism of removal of *Ds* from a

chromatid, gained from a study of transpositions of *Ds*, suggests an explanation of these mutations. An event leading to removal of the inserted *Ds* segment from the *C* locus would give rise to two broken ends in the chromatid. Fusion of these broken ends would re-establish the former normal gene order, and remove the inhibitory action on the *C* locus induced by the inserted segment; and as a consequence a mutation from *c* to *C* would be evident. No further changes at this locus would occur, for no *Ds* locus would be present to produce them. The *C* locus would be completely normal again. If this primary event is responsible for the *c* to *C* mutations, it also explains why a few of these mutations are accompanied by detectable transpositions of *Ds*. Transpositions could take place if a spontaneous chromosome break, elsewhere in the chromosome complement, occurred in the same mitosis that removed *Ds* from the *C* locus.

The analysis of the events occurring when *Ds* is inserted into or close to the normal *C* locus has made it possible to interpret a previously puzzling aspect of *Ds* behavior at its standard location. At this position, two contrasting "states" of the *Ds* locus have long been recognized. When one of these states (state I) is in effect, the majority of mutational events occurring at the *Ds* locus result in the formation of a dicentric chromatid and a U-shaped acentric fragment. In the contrasting state (state II), there is a markedly lower frequency at this locus of breaks and fusions resulting in the formation of dicentric chromatids or other gross chromosomal rearrangements.

The above two contrasting states of *Ds* may be recognized when it is at the *C* locus (c^{*1}). In the original isolate of r^{*1} a state I *Ds* locus was present. This was the same state of *Ds* that had been present in the chromosome before its trans-

position to the *C* locus. In kernels having this state of *Ds*, only a few mutations giving a *C* phenotype appear. This state of the *Ds* locus at c^{*x} changes rather frequently, and by a single event, to one that is comparable to state II of the *Ds* locus at its standard position. The event is made evident by a greatly lowered frequency of dicentric chromatid formation. The rate of *c* to *C* mutations rises to a frequency that is comparable to the previous rate of dicentric chromatid formation. It has been determined that the *c* to *C* mutations are associated with a simultaneous loss of *Ds* activity. This relationship indicates that the change from a *c* to a *C* phenotype is associated with an event involving the *Ds* locus itself. A normal chromosome 9 having a fully active *C* locus but no *Ds* locus is the usual consequence. An interpretation of the event leading to a *C* phenotype has been given above. On this interpretation, the two contrasting states of the *Ds* locus reflect the relative frequencies of alternate consequences of the breakage events occurring at this locus. Both types of consequence are recognized when *Ds* is at the c^{*1} locus but only those giving dicentric chromatids or other gross chromosomal abnormalities are detectable when *Ds* is at its standard position. At this latter position, *Ds* may inhibit the action of the adjacent loci, but the inhibition may not be recognized because it results in no obvious change in a readily detectable phenotypic character. In this case, neither the inhibition of gene action brought about by the insertion of the *Ds* locus nor the release from inhibition following its removal would be evident. Detection of the frequency of breakage events at the *Ds* locus would be confined to the fraction that results in the formation of a dicentric chromatid and a U-shaped acentric fragment. This fraction may be high or low, depending on the state of the *Ds* locus.

That the time and frequency of aberrant events occurring at the *Ds* locus may be the same for each of these contrasting states will be indicated in a later section. The important difference is in the consequences of the breakage events, not in the frequencies of the events themselves.

The recognition of different states of the *Ds* locus makes it necessary to consider the factors responsible for the origin of these states and the conditions present in each. Two clearly distinguishable states of *Ds* have been described above. Other states of this locus have been recognized. When *Ds* is at the *C* locus (c^{m+1}), these several states are distinguishable, one from another, by the relative frequencies of the two main consequences of events occurring at *Ds*—that is, dicentric chromatid formation or *c* to *C* mutations. At the standard position, the comparable states are distinguished, one from another, by the relative frequency of only one of these consequences—"dicentric chromatid formation. These states appear to be intermediates between the extreme state I and the extreme state II. It has been well demonstrated that a *Ds* locus giving a high frequency of dicentric chromatid formation may change at a single mitosis to one that gives a low frequency. A *Ds* locus giving a low frequency of dicentric chromatids, on the other hand, does not change to one giving a high frequency at a single mitosis. This change from extreme state II to extreme state I requires several stepwise events, reflected in the intermediate states. These observations would suggest that the individual states of the *Ds* locus are indications of the number of active *Ds* units that may be present in a small chromatin segment, and that the change from one state to another involves a change in number and/or distribution of these units within the segment. Such changes might be expected to occur as one of the consequences

of the chromatid-breakage-and-fusion mechanism associated with the aberrant events occurring at the *Ds* locus. On this interpretation, it could be concluded that the extreme state II *Ds* locus has few *Ds* units and that the extreme state I *Ds* locus has many such units; for the mechanism could readily reduce the number of units through losses at a single aberrant mitosis, but would require a series of such mitoses to build up a large number of units.

The analysis of the origin and subsequent behavior of *Ds* at the *C* locus has served to clarify some other aspects of this study of mutable loci. Why did new *Ac*-controlled mutable loci arise in these stocks? Why did a normal "wild-type" locus suddenly behave as a mutable locus? What event occurred at the locus to bring about a mutation, that is, a change in phenotypic expression? The analysis of the origin and behavior of c^{771m} has made it possible to approach these questions and to formulate a concise interpretation of the origin and behavior of the other *Ac*-controlled mutable loci. Inhibition of a locus, either qualitatively or quantitatively, by insertion of a foreign bit of chromatin can be followed by release of this inhibition if the foreign chromatin is removed, transposed, or in some manner altered in position with respect to the inhibited locus. The primary mechanism that allows for such changes at a locus is associated with compound chromatid breaks at the locus and subsequent fusions of the broken ends. In its initial aspects, it is only necessary to consider a single locus having the peculiar faculty of undergoing such breakage events, at whatever position it may be located, to account for the origin and behavior of many different mutable loci.

TRANSPOSITION OF THE *AC* LOCUS

During the past year, an extensive study of the inheritance behavior of the *Ac* locus

was undertaken. This study has established that *Ac* is inherited as a single unit. It shows typical Mendelian inheritance, with one important exception. This exceptional type of inheritance behavior is the same as that shown by *Ds*: transposition of the locus from one position in the chromosomal complement to another. Two or three per cent of the gametes of an *Ac Ac* plant may be derived from cells in which a transposition of *Ac* has taken place. These transpositions usually occur relatively late in the development of the plant. Plants derived from zygotes that have *Ac* loci in allelic positions in each of two homologous chromosomes may give rise to a few gametes with either (1) two *Ac* loci showing no linkage with one another, (2) two *Ac* loci completely linked or very closely linked, or (3) no *Ac* locus at all. When an *Ac* locus is transposed to a new position, it shows typical Mendelian inheritance at this new position. Linkage with known genie markers can be established. Here, again, exceptions may arise as the consequence of a few transpositions from this new position to still another position. The frequency of these transpositions is not high enough, however, to distort seriously the statistical data of linkage studies. It is likely that the mechanism producing transpositions of *Ac* is the same as or quite similar to that producing transpositions of *Ds*.

Ac itself is a mutable locus. It can be identified only by its action on *Ds*. Its mutations are made evident by changes in the time and frequency of *Ds* mutations. (The events at the *Ds* locus that result in either dicentric chromatid formation or a change in phenotypic expression of a *Ds*-inhibited locus will be termed "*Ds* mutations" in this account.) It is known that the number of *Ac* loci in the nucleus controls the time and frequency of *Ds* mutations. Increased doses of *Ac* loci (from 1

to 3 in the triploid endosperm) result in an increasingly delayed time of occurrence of *Ds* mutations. Similar changes in the mutational response of *Ds* will be registered after a somatic mutation in a single *Ac* locus. These responses indicate that some quantitative change may take place at the *Ac* locus when it mutates—probably an increase or decrease in the number of subunits at this locus. Thus, superimposed on those quantitative changes that can be produced by additions of whole *Ac* loci through controlled chromosome combinations in diploid tissues of the plant or in triploid tissues of the endosperm are those that can occur at a single *Ac* locus.

There is a ready method of identifying those kernels on the ears of *Ac Ac* plants that are likely to have a transposed *Ac* locus. This involves crossing plants having no *Ac* locus to plants having a single *Ac* locus in which the *Ac* state is known (determined by its effects on *Ds* in 1, 2, and 3 doses). The *F₁* plants are selfed and the *F₂* progeny grown. The *F₂* plants are then crossed by plants having no *Ac* locus but carrying *Ds* at its standard location in each chromosome 9. The ears produced by the *Ac Ac F₂* plants are selected, and an examination is made of the *Ds* mutation rates in the kernels. If, in the *Ac Ac F₂* plants, no mutations have occurred at the *Ac* locus and no transpositions have taken place, all the kernels should show the same pattern of *Ds* mutations. In other words, the control of these *Ds* mutations should be the same, since all the kernels should have two *Ac* loci in the endosperm cells and all the *Ac* loci should be alike. The majority of the kernels on such ears do show a remarkable similarity in the pattern of expression of *Ds* mutations. A small percentage of the kernels, however, are markedly different. These exceptional kernels fall into three classes: (1) those showing no *Ds* mutations at all,

(2) those showing a few very late-occurring *Ds* mutations that suggest an increase in *Ac* dosage, and (3) those showing a time and frequency of *Ds* mutations that suggest a lowered dosage of *Ac*. A preliminary test was made in an attempt to determine the reason for the changed responses of *Ds* in the kernels of types (1) and (2). Twenty-five such kernels were selected from these ears, and plants were grown from them. Tests were conducted to determine (1) the presence or absence of *Ds*, (2) the presence or absence of *Ac*, and (3) the action of *Ac*, when present, in one and two doses. Eleven of the plants arising from these selected kernels gave no evidence of *Ac* at all; the *Ac* locus was either absent altogether or completely inactive. Ten other plants had two independent, nonlinked *Ac* loci. In four plants, *Ac* was inherited as a single unit; but this unit, in a single dose, produced the same effect on *Ds* mutations that two units of the original *Ac* locus, from which it was derived, had produced.

One type of event, the transposition of *Ac*, will account for these results. If, in these *Ac Ac* F₂ plants, transposition of one of the *Ac* loci occurred in a meiotic or premeiotic mitosis, two *Ac* loci would still be present in the nucleus, but they would no longer be allelic with respect to position in the chromosomal complement. If the transposed *Ac* locus were inserted into a nonhomologous chromosome, meiotic segregations could give rise to gametes with either (1) one *Ac* locus, in its original position or its new position, (2) two *Ac* loci, one in each of two nonhomologous chromosomes, or (3) no *Ac* locus. Transposition within the same chromosome (or homologue), or insertion of the *Ac* locus of one chromatid adjacent to the *Ac* locus of the sister chromatid, would give comparable meiotic segregations with respect

to the production of gametes with two *Ac* loci or with no *Ac* locus.

In the given cross, the kernels arising from the megaspores having no *Ac* locus would show no *Ds* activity; for no *Ds* mutations occur without *Ac*. Tests for *Ac* in the plants arising from these kernels would be negative, because no *Ac* locus would be present. Kernels developing from megaspores receiving a single *Ac* locus, either in its original position or transposed but unmodified in its action, would show the characteristic effect on *Pi*-mutations produced by the *Ac* locus when two are present in the endosperm. (It should be recalled that the female parent contributes two nuclei to the triploid endosperm tissue, and the male parent one.) Those developing from megaspores with two *Ac* loci, either linked or situated in different chromosomes, would give rise to endosperms with four instead of two *Ac* loci. It is known that increases in the dose of *Ac* will delay the time of appearance of *Ds* mutations, and that this effect is proportional to dosage—the higher the dose, the more effective the delay. With four doses of *Ac* instead of the usual two, the delay may be so effective that either no *Ds* mutations will occur during the development of the tissue or only a few will occur very late in the development of the endosperm. In either case, the kernels having such increased doses of *Ac* will be strikingly different in appearance from the majority of kernels, that is, those with two *Ac* loci in their endosperm cells. It was this striking difference in appearance of a few kernels on these ears that allowed the selection to be made. The analysis of the *Ac* composition of the kernels has led to the conclusion that they develop from ancestor cells in which a transposition of *Ac* has occurred.

For comparison, plants were grown from some of the kernels on these F₂ ears that

showed the characteristic type of *Ds* mutational response known to be associated with the presence in the endosperm of two *Ac* loci. Tests of the *Ac* constitution of these plants gave the expected results. One *Ac* locus was present in each of the tested plants, and its control of the time and frequency of *Ds* mutations, in one or two doses, was similar to that in the parent plant.

These studies have been expanded during the summer of 1949; but the results of the preliminary tests are sufficient to indicate the factors responsible for apparent exceptions to the expected Mendelian inheritance of *Ac*. They have also made possible an interpretation of one of the several kinds of event that occur during the development of the plant or of the endosperm to bring about pronounced changes in the action of *Ac* on *Ds*. These changes are registered by the appearance of precise sectors showing altered *Ds* responses. Tests are now being conducted to distinguish between changes in state of the *Ac* locus—that is, between changes in quantitative action of an *Ac* locus that is inherited as a single unit, and changes that are caused by an increase in numbers of such loci after transposition of *Ac*, as outlined above. The phenotypic effects of these two types of change overlap, but the causative series of events, although related, are nevertheless separable.

The mechanism responsible for transposition of the *Ac* locus has not been analyzed. It is thought likely to be the same as or similar to that producing transpositions of *Ds*. If so, some of the transpositions of *Ac* should be associated with chromosomal rearrangements. A chromosomal translocation was recognized in one of the cases cited, but it has not yet received adequate analysis.

THE ACTION OF *AC* ON THE MUTABLE LOCI IT CONTROLS

It has been emphasized repeatedly that *Ac* controls the occurrence of *Ds* mutations and that its quantitative levels control the time and frequency of these mutations. In this report, it has been shown that the mutable c^{m-x} locus is merely a transposed *Ds* locus situated at or close to the *C* locus. The analysis of this c^{TM-1} locus and of its origin from a transposition of *Ds* has suggested that all \wedge -controlled mutable loci arise from transpositions involving, originally, only one *Ds* locus. According to this interpretation, *Ac* does not control the mutability of many different loci, but only the mutability of a single locus—the *Ds* locus—wherever it may be situated in the chromosomal complement. Mutations of *Ds* in these various positions may result in changes in phenotypic expression that are strikingly different. The change in phenotype, in any one case, depends on the kind of locus that has been inhibited by the insertion of *Ds*. In their normal action, these various *Di*-inhibited loci must control quite different chemical processes. The events at the *Ds* locus that result in a return to partial or complete action of the inhibited locus must therefore involve a different series of changes in chemical processes in each case. Without an integrative understanding of the events that occur at such mutable loci, it would be difficult to understand why *Ac* should control the mutability of loci concerned with such unrelated processes, and why each such locus should respond to a particular *Ac* locus and dosage in an exactly comparable manner. There is no difficulty, on the basis of the given interpretation, in appreciating the apparent nonspecificity of control of mutable loci by *Ac* and the similarity in response of these mutable loci to changes in *Ac* state and dosage.

In order to obtain more specific information about the nature of the action of *Ac* (other than its known effects in producing chromatid breaks at the *Ds* locus and controlling the time and frequency of these breaks), combinations of *Ds* loci at various positions in the short arm of chromosome 9 have been made. These combinations were made in an attempt to answer the following question: Does *Ac* produce a cellular or nuclear condition in a certain cell, at a certain time in development, to which all *Ds* loci will respond? An instructive example for this purpose is a combination of c^{TM^1} (*Ds* at or close to the *C* locus) with *Ds* at its standard location. If a plant carrying c^{TM^1} and *wx* in its chromosomes 9 is crossed by a plant carrying c^s (stable *c*, nonmutable with *Ac*), *Wx*, and *Ds* (standard location, to the right of *Wx*), kernels will be produced that are $c^{TM^1} wx/c^{m \sim x} wx/c^B Wx Ds$. This combination should show whether or not mutations in the several *Ds* loci will occur at the same time in the same cell, and whether this response will be of the same order with one and with more doses of *Ac*. Simultaneous mutations would be revealed in these kernels provided an extreme state II *Ds* locus were present at $c^{mm\%}$ (mutation from *c* to *C* and few if any dicentric chromatid formations), and an extreme state I *Ds* locus were present in the $c^s Wx Ds$ chromosome (high rate of dicentric chromatid formation). If all *Ds* loci respond to some particular developmental change that is brought into being by the presence of *Ac*, then when this changed condition arises in a cell, a mutation of *Ds* at the $c^{m \sim l}$ locus should give a *C* phenotype in the descendent cells. A mutation at the *Ds* locus in the $c^{\circ} Wx Ds$ chromosome should also occur. A *wx* phenotype would then appear in the descendent cells, because a *Ds* mutation in the $c^s Wx Ds$ would produce a dicentric

chromatid and a U-shaped acentric fragment; this acentric fragment would carry the *Wx* locus, and consequently *Wx* would be lost from the nuclei during a mitosis. The effects produced by such simultaneous mutations of the several *Ds* loci should be visible in the mature kernel. Colored areas (the *c* to *C* mutations) should appear, and the underlying starch should be *wx*. Also, the borders of the sectors having both of these altered phenotypes should correspond exactly. In the examined kernels having these given constitutions, a high percentage of the *C* areas had underlying *wx* starch, and the borders of the sectors did exactly correspond. Exceptions were expected, and a number were observed. Some examples were: *C* areas with underlying *Wx* starch, *wx* areas with overlying colorless aleurone, *C* areas with only half of the underlying sector composed of *wx* starch, or *wx* areas with only half of the overlying aleurone layer showing a *C* phenotype. It is hoped that an extended analysis of the various classes of exceptional areas in these kernels will reveal the more unusual consequences of the events that occur at the *Ds* loci in these mutation-producing mitoses, and the resultant organization in the two affected sister chromatids.

Tests have also been constructed to determine the relation between the mutations of *Ac* and those of *Ds*. Although the analyses of these tests are incomplete, it seems apparent that *Ac* tends to mutate in the same cell in which a *Ds* mutation is occurring, or in an immediate ancestor cell. The combined evidence suggests that some condition, under the control of the *Ac* locus and depending on its state and dosage, must develop in specific cells at specific times, to produce a mutational response (chromatid breaks) at *Ds* loci as well as at the *Ac* locus itself. The consequences of such mutation are the observed changes in

genic action, transpositions or losses of *Ds* or *Ac*, and production of gross chromosomal rearrangements with or without accompanying transpositions of *Ds* or *Ac*.

MUTABLE LOCI $c^{m\sim 2}$ AND $wx^{m\alpha}$

The \wedge -controlled mutable loci $c^{m\sim 2}$ and $wx^{m\sim 1}$ were described in Year Book No. 47. A few salient facts and conclusions based on the continued study of these loci are as follows: Both loci express their mutations quantitatively. A series of alleles derived from such mutations, which show gradations of quantitative expression, have been selected for study. When *Ac* is absent, a particular expression of an allele can be held constant, for no somatic mutations of these alleles occur. When *Ac* is present, the alleles may continue to mutate to either higher or lower levels of quantitative expression. For a study of the action of any one allele, therefore, it is important that no *Ac* locus be present.

It has been determined that chromatid breaks may occur at these two mutable loci; in this respect, they are similar to c^{TM^1} . Both $c^{m\sim 2}$ and $wx^{m\alpha}$ were isolated from stocks known to have a *Ds* and an *Ac* locus. Unlike $c^{m\alpha}$ they were not detected at the time of their origin. It is therefore impossible to reconstruct the particular events associated with their origin from a normal *C* locus and a normal *Wx* locus. The presence of *Dy*-type behavior at these mutable loci points to a mechanism similar to the one associated with the origin of $c^{\wedge 1}$.

In the case of $c^{m\sim 2}$, the position of insertion of *Ds* into or adjacent to the *C* locus may differ from its position of insertion in $c^{m\alpha}$; for two qualitatively different types of phenotypic expression of the *C* locus result from mutations of $c^{m\sim 2}$, whereas only one type regularly follows mutations of $c^{m\alpha}$. Both types of qualita-

tively distinguishable mutations at $c^{m\sim 2}$ result in pigment formation in the aleurone layer. Within each of the two qualitative types there occurs a series of mutants showing various degrees of quantitative expression. The color intensities produced by the different mutants of both types range from a faint pink to a deep red (in *pr pr* constitutions). The two series of mutants are distinguished from each other mainly by the fact that a different diffusible substance (or substances) is produced by the members of each. Both substances are concerned with pigment formation. The diffusible substance produced by type 1 mutants may be utilized by a cell having a normal *C* locus, or by a cell having a type 2 mutant, to intensify the color of the cell pigment. The normal *C* locus and the type 2 mutants, on the other hand, both "produce a diffusible substance that can be used by type 1 mutants to intensify pigment color. Thus, the type 2 mutants and the normal *C* locus are much alike; they both produce a diffusible substance that type 1 mutants can use, and they both can use a diffusible substance produced by type 1. This relationship suggests that a normal *C* locus is probably responsible for the production of at least two diffusible substances, both of which are required for pigment formation. It also suggests that the dosage responses noted for the normal *C* locus may be the consequence of a limited production of one of these substances by a single *C* locus: the more *C* loci were present, the more of this substance would be produced and the deeper would be the pigment color. The quantitative grades of expression of the alleles within the two types of mutations arising from $c^{m\sim 2}$ may reflect the relative quantities of the two substances produced by individual members of a type—limitations in the production of one of these substances conditioning the amount of pig-

ment that can be formed, and thus the depth of color that can appear.

The conclusions derived from study of $c^{m\sim 2}$ regarding the action of the normal C locus are noteworthy, in that they consider a double function of a single unit in inheritance. This unit, concerned with pigment production in the aleurone layer of the endosperm, appears to be composed of at least two qualitatively different subunits, both of which determine the production of substances required for pigment formation. It is possible that this C locus behaves as a unit in inheritance not only because all the subunits are needed for the production of pigment, but also because a particular spatial relation of the units at the locus is required to assure a definite sequence of reactions.

Mutations of the $wx^{m\sim x}$ locus have been similarly instructive in considering the action of the normal Wx locus, but for reasons other than those just discussed for $c^{m\sim 2}$. Here, alleles showing various quantitative levels of expression are produced by mutations of $wx^{m\sim L}$. The levels are expressed by the percentage of amylose in the starch component of the endosperm cells. When only the recessive, wx , is present, no recognizable amylose starch is produced. The selected alleles derived from mutations of $wx^{m\sim x}$ form a series in which a single dose (Wx allele, wx , wx) produces quantities of amylose ranging from very little (less than 1 per cent) to as much as the normal Wx locus produces in three doses. Chemical analyses of the percentages of amylose starch produced by several of these alleles have been conducted by Miss Ruth Sager and Dr. Charles O. Beckmann, of Columbia University. These analyses have shown that the type of color reaction produced by staining with iodine is a relatively reliable indication of the approximate percentage of amylose present, interest in this case centers not so much

in the appearance of alleles having lower activity than the normal Wx locus as in those having higher activity than the normal locus. Is the normal Wx locus partially inhibited, or do the Wx alleles showing greater than normal activity arise from duplications of the locus? In Ac -carrying plants, the chromatid-breakage-and-fusion mechanism associated with mutations at the $wx^{m\sim x}$ locus or its intermediate alleles should give rise, in some cases, to duplications or multiplications of units of the Wx locus. It is hoped that a study of the different amounts of amylose produced by sister chromatids after mutation of $wx^{TM'}$, or one of the intermediate alleles, will furnish some information with reference to this question.

CONCLUSIONS

The purpose of the foregoing sections has been to indicate the progress made during the past year in attacking fundamental aspects of the origin and behavior of Ac^* controlled mutable loci. It was concluded that only two loci are involved in all these cases: the Ds locus and the Ac locus. The origin and subsequent behavior of newly arising mutable loci depends on the transposition of a Ds locus and its insertion into (or adjacent to) a normal locus, and on the constitution of this inserted Ds locus. The gene action of a normal locus may be inhibited by such an insertion. Subsequent events at this new position may remove the inserted segment and its inhibitory action altogether; or changes in the constitution or position of the Ds locus may result in changes in the degree of inhibition of the affected locus. It was also concluded that the events occurring at Ds during a mutation-producing mitotic cycle result in compound chromatid breaks at this locus, and that the observed consequences depend on subsequent fusions of the broken ends.

The fusion phenomenon, of utmost importance in these cases, calls for no new interpretations, since the fusion of newly broken (unsaturated) chromosome ends has been well investigated and could be anticipated.

Both *Ac* and *Ds* are mutable loci, for their mode of action changes as the consequence of events occurring at these loci in certain cells of the plant. Like *Ds*, *Ac* also undergoes transposition from one location in the chromosomal complement to another. The mechanism of transposition, although not directly analyzed, is possibly similar to that associated with the transpositions of *Ds*. The evidence also indicates that changes in *Ac* as well as *Ds* are associated with chromatid breakage and fusion. It is necessary to determine, then, the nature of the events occurring at these two mutable loci, during a particular mitotic cycle, that will result in the observed breakage-and-fusion phenomena. Unquestionably, these events are primarily responsible for all the observed changes at these mutable loci. It is suspected that they are associated with some aberration in the mode of reproduction of a particular type of molecule in the chromosome during a mutation-producing mitotic cycle. Both *Ac* and *Ds* are assumed to have such molecules. If the aberration involves a chemical bonding of the newly formed molecule with the original molecule, which holds at least until after the forced separation of sister chromatids during the prophase period, a rupture of the chromatid could occur at the affected locus during this separation period. It is known that the bonds holding the molecules together in a linear order in the chromosome may be ruptured by mechanical pull, and that the broken ends so produced are unsaturated and capable of fusion with other unsaturated broken ends. It is therefore necessary to assume, in this interpretation,

that the bond connecting the newly formed molecule with the original molecule is stronger than the bond holding the molecules together in linear order.

The study of transpositions of the *Ds* locus has shown that a rupturing mechanism of this type, or at least one that leads to similar consequences, must be involved. It has been established that the transposition phenomenon is associated with chromatid breakage; the *Ds* locus is inserted into a position where a spontaneous break has occurred. The transposition phenomenon is readily explained if it is assumed that break-producing events at the *Ds* locus may sometimes result in the tearing-out of a minute fragment containing *Ds* and having two unsaturated broken ends. The insertion of *Ds* into a new position would result merely from fusion of unsaturated ends. If the broken ends arising from the spontaneous break are labeled 1 and 2 and those of the fragment 3 and 4, the fusion of 1 with 3 and 2 with 4 would accomplish the transposition. The above-described process of mechanical rupture of the chromatid at the *Ds* position could result in just such a torn-out fragment. The consequence of any one rupture would depend on the type of fusion of broken ends that followed. Not only could transpositions occur, but the *Ds* locus could be lost altogether, or two *Ds* loci could enter one chromatid, leaving none in the sister chromatid. Such duplications (altered states of *Ds*) could, in turn, initiate a series of new consequences when the aberrant type of event, leading to chromatid rupture, again occurred in a descendant of this chromatid.

The analysis discussed in this report of the factors associated with the origin and behavior of Δ -controlled mutable loci in maize has led to a relatively simple interpretation of the nature of the events responsible for changes in action of the genes

involved. The types of phenotypic change that follow mutations of non- λ -controlled mutable loci are similar to those shown by the λ -controlled mutable loci. It is quite possible that the same or similar events are primarily responsible for these changed phenotypes also.

Mutable loci have been described in a number of organisms. Many of them show changes in phenotypic expression similar to those now being observed in maize. The events responsible for changes in expression of gene action may be simi-

lar in these organisms to those occurring in maize. The investigations described in this report cast doubt on interpretations that postulate a "true gene mutation," that is, a chemical change in a gene molecule, resulting in a changed specificity of its active product. Phenotypic change may well be related to inhibition of the action of a normal gene followed by partial or total release of this inhibition, together with such duplications or deficiencies of the locus as could be produced by the mechanism outlined above.

THE GENE

M. DEMEREC, B. WALLACE, E. M. WITKIN, AND G. BERTANI

During the past year our group has been engaged in studies of a number of problems dealing with *gent* mutations. In *Escherichia coli* an extensive study was made of the genetic mechanisms responsible for changes to streptomycin resistance and dependence, and reversions from dependence to nondependence. This study revealed a complex system of different mutations that can be recognized by their effect on several other properties in addition to streptomycin resistance. Results so far suggest that the majority of these mutations are due to changes either in a single *gene* locus or in adjacent loci. The effect of sodium nucleate on mutations in *coli* was studied; and the resistance patterns for aureomycin, chloromycetin, and neomycin were investigated. Survey studies of the mutagenic action of chemicals were continued, using both *E. coli* and *Drosophila*.

We were assisted in this work by Misses J. Flint, E. Lively, H. Spring, and L. Hahn, Mrs. J. Buchanan, Mr. R. Millemann, and Mr. W. Reiser, and during the summer by Miss Rada Demerec, of Swarthmore College, and Mr. Norton Zander, of the University of Wisconsin. The work was

aided by a grant from the American Cancer Society, recommended by the Committee on Growth of the National Research Council.

GENETICS OF STREPTOMYCIN RESISTANCE IN *ESCHERICHIA COLI*

Our earlier work (Year Book No. 45, 1945-1946, pp. 152-153) showed that bacteria resistant to streptomycin originate through mutations, and also that completely resistant mutants may be obtained in one step from sensitive bacteria. Even though the rate of mutation to complete resistance is very low, it is not difficult to measure, since several billion bacteria may be plated on a Petri dish with medium containing streptomycin and only the resistant will grow to form colonies. As in the earlier studies of mutation to bacteriophage resistance, extremely large numbers of individuals can be used in these experiments, and thus very exact determinations of spontaneous and induced mutability can be made. Therefore, investigations of streptomycin-resistance mutability were continued, with the purpose of developing another method in mutation studies to

complement the phage-resistance method, which we have been using with good success since 1942.

Rate of spontaneous mutation to resistance. Determinations were made by M. Demerec and E. Lively of the rate of spontaneous mutation to streptomycin resistance, using the method of independent cultures developed by Luria and Delbrück. In each experiment about one hundred broth cultures of 1 ml. each were grown to saturation, and the proportion of cultures without mutants was determined by plating the whole contents of each tube into agar-broth medium containing 25 µg. per milliliter of streptomycin. In addition, 5 cultures in each experiment were assayed to determine the average number of bacteria present. Using Delbrück's formula, mutation rate was calculated in four experiments as follows: with strain B/r, 0.73 and 1 per 10^9 ; with strain B/i, 1.3 per 10^9 ; and with strain B/6, 2.6 per 10^9 . From these data it may be concluded that for the B strain the rate of mutation to streptomycin resistance, using concentrations of 25 µg. per ml, is about 1×10^{-9} .

It has been found that a portion of streptomycin-resistant mutants are dependent on streptomycin; that is, they require it in order to divide. About 60 per cent of mutants in the B/r strain are of this dependent type.

Spontaneous reversions from dependent strains. Dependent mutants are able to pass through only a few divisions on medium without streptomycin. The number of divisions depends on the mutant itself, on the concentration of streptomycin on which it grew, and on the degree of crowding of the cells on the culture. Therefore when dependent bacteria are plated on medium not containing streptomycin, they will soon stop growing; but any mutant to nondependence (a reversion) that occurs during these divisions

will grow and form a colony. Thus when information is available about the number of dependent bacteria plated, the number of divisions passed through on medium without streptomycin, and the number of nondependent colonies appearing, it is possible to calculate the mutation rate from dependence to nondependence. Since large numbers of bacteria can be used, this method is suitable for studies of low rates of spontaneous and induced mutations.

The experimental studies of spontaneous reversion rates were carried on by M. Demerec, G. Bertani, and E. Lively.

The behavior of strain Sd-4 (B/r/Sd-4) has been analyzed in detail. The number of residual divisions on plain mutant agar for Sd-4 bacteria grown on 25 µg. of streptomycin per ml. was determined by washing the plate with 10 ml. of broth and assaying the suspension. The effect of number of bacteria plated on the number of residual divisions has also been studied. When 10^7 to 5×10^7 bacteria are plated, the number of divisions they pass through is approximately 3.5. These divisions are completed in about 12 hours at 37° C. After this time the bacteria stop dividing, but continue to grow in length, forming filamentous polynucleate cells ("snakes"). A high percentage of these long cells are still "alive" (i.e., able to recover and grow normally if streptomycin is added) after 1 to 2 days. Two facts seem to indicate that mutations can take place in the filamentous stage: (a) new mutant colonies are still appearing after as much as 5 days; (b) when cells are irradiated after completion of residual divisions, the number of mutant colonies increases. This means that an estimate of rate of mutations from dependence to nondependence calculated from the number of living bacteria present on the plate will be too high. Under the conditions stated above for streptomycin concentration and crowding, the average

number of reversions, scored after 6 to 7 days of incubation at 37° C, is 36.8 ± 2.8 (average from 17 experiments; 7 to 20 plates per experiment) per 10^8 plated bacteria. Taking into account the residual divisions the bacteria pass through, an estimate of the rate of mutation from streptomycin dependence to nondependence per bacterium per division can be calculated as 37×10^{-5} . This does not take into account the nuclear divisions occurring during formation of filaments. Among the mutants obtained, some (about 25 to 30 per cent for this particular strain) are resistant, some are sensitive. Sensitive mutants are obviously selected against as long as the dependent strain is cultured in the presence of streptomycin. Resistant mutants might theoretically form a "background" in Sd cultures (as happens with mutants resistant to phages); but timing of the appearance of the mutant colonies does not yield any evidence of such "background." In the presence of dependent bacteria and streptomycin, resistant mutants also are probably selected against.

Patterns of residual growth, rate of mutation, and ratio of resistants to sensitives among reversions are different for different dependent strains.

Mutations induced by irradiation. An extensive series of experiments, using ultraviolet rays of wave length 2537 Å and X-rays, was carried on by M. Demerec and E. Lively to determine the rate of induction of mutations from B to resistance and of reverse mutations from dependence to nondependence. Previous work with radiations (see Year Book No. 44, 1944-1945, pp. 115-116) had shown that induced mutations to phage resistance are of two types: the so-called "zero-point" mutations, which are expressed before the irradiated bacteria have passed through a cell division; and "end-point" mutations, which are not expressed until after at least one

cell division has taken place. It had also been found (see Year Book No. 45, pp. 143-144) that with X-rays the mutation rate is directly proportional to the dose, giving a single-hit curve, whereas with ultraviolet rays there is a disproportionately rapid increase with increased dose, indicated by a multiple-hit curve.

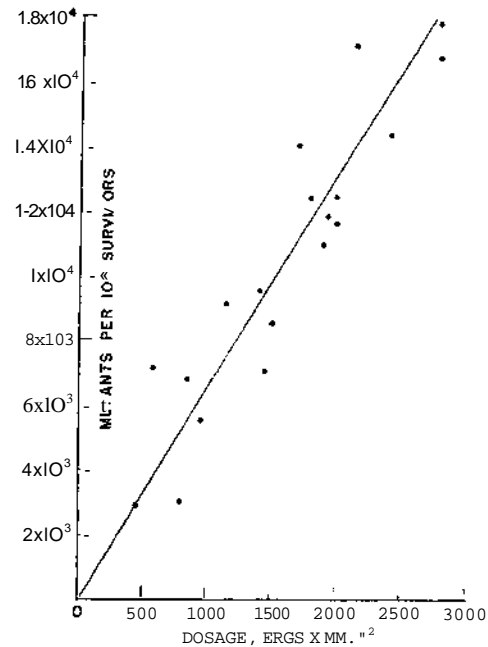


FIG. 1. Reverse mutants induced in a streptomycin-dependent strain (B/r/Sd-4) of *Escherichia coli* by various doses of ultraviolet rays.

This year's experiments showed that in the streptomycin-resistance system zero-point mutants do not appear, either among the resistant mutants or among the reversions. Studies of the relation between dosage and mutation rate, made with reversions in the streptomycin-dependent strain, demonstrated that increase in mutation rate is proportional to increase in dosage in material treated either with ultraviolet rays or with X-rays (figs. 1,2).

Studies of induced mutation from sensitivity to resistance indicated that not all

the induced mutants show up by the time surviving bacteria have doubled in number. This may be due to the same cause as the delay observed in the expression of end-point mutations to phage resistance (see Year Book No. 44, pp. 115-116).

Mutations induced by chemicals. It was found convenient to use induction of reversions from streptomycin dependence to nondependence as an index in studying the mutagenic properties of chemicals.

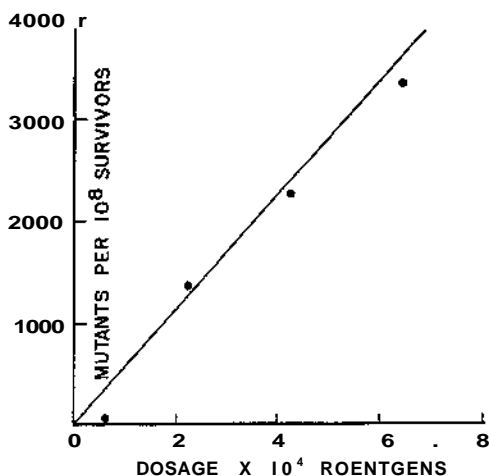


FIG. 2. Reverse mutants induced in a streptomycin-dependent strain (B/r/Sd-4) of *E. coli* by various doses of X-rays.

Resting bacteria of the dependent strain Sd-4 were treated with a certain chemical in aqueous solution, plated on broth agar, and incubated for 7 days; then the number of mutants on each plate was determined. Occasionally the treatment caused the bacteria to clump; and to avoid the variable that might thereby be introduced, the treated bacteria were examined under the microscope and used only if clumping was not evident. Untreated controls were included in each experiment.

This test—because of the difficulty of checking the amount of residual growth—is not the most favorable for quantitative

studies of relations between mutation rate, concentration of chemical, and length of treatment. It is probably the most expedient, however, when the main problem is whether or not a substance is mutagenic.

These experiments were done by G. Bertani, M. Demerec, Jessie Flint, and Eileen Yongen, who is working under a grant from the Jane Coffin Childs Memorial Fund, administered by the Biological Laboratory.

Chemical substances to be tested for mutagenic potency were chosen according to two main criteria: either because they had shown definite or probable mutagenicity in earlier genetical tests (induction of lethals in *Drosophila*, induction of resistance to phage Ti in *E. coli*, etc.), or because they represented particular chemically defined groups of substances. The data collected for various chemicals are summarized below.

Formaldehyde: Previously shown to be mutagenic in *Drosophila* feeding-technique experiments. Streptomycin test showed definite mutagenicity, even at survival levels as high as 60 per cent. Tenfold increases in number of mutations, as compared with controls, were detected in some experiments. Preliminary experiments showed that treatment with formaldehyde has no considerable effect on residual growth.

Acridine: Previously shown to be mutagenic in phage-resistance tests. In the present test, mutagenic effect could be detected at a survival level of less than 1 per cent.

Phenol group: Phenol is known to be mutagenic in *Drosophila*, when the treatment is applied by means of special transplantation techniques. With the streptomycin test it was also positively mutagenic, in a survival range of 0.5 per cent to 34 per cent, with increases up to tenfold in number of mutations as compared with controls. Two other substances, chemically closely related to phenol, were tested, namely, picric acid and alpha-dinitrophenol. Both showed mutagenicity of the

same order and in the same survival range as phenol.

Caffeine: Known to be mutagenic for molds. Preliminary experiments showed positive results in the streptomycin test.

Sodium desoxycholate: Previous phage-resistance tests showed a very low degree of mutagenic potency. In the present test, no induction of mutations could be detected. This may indicate that the streptomycin method of testing is less sensitive than the phage-resistance method.

Ethyl carbamate (urethane): Known to be mutagenic for *Drosophila*. In the streptomycin test, definitely mutagenic when survival is less than 10 per cent, weakly mutagenic when survival is as high as 60 per cent.

Ammonia: Detectably mutagenic only when survival is lower than 2 per cent. The same effect seems to be typical of ammonium chloride.

Inorganic acids: Phosphoric, hydrochloric, nitric, and sulfuric acids were tested. None showed niutagenic activity.

Organic acids: Acetic, formic, and lactic acids were tested. Results with this group of substances were very irregular and are still in progress. Formic acid is probably weakly mutagenic.

Alkalis: Sodium hydroxide and potassium hydroxide were tested. Neither is mutagenic.

Heavy metal salts: Copper sulfate, mercuric chloride, and silver nitrate were tested. At high survival levels (more than 10 per cent) none of them is mutagenic. Experiments with low survival ranges (which are difficult to obtain with short periods of treatment, such as have commonly been used in these experiments) are still going on.

Ferrous chloride: This compound gave a very high number of induced mutations at high survival levels. Mutagenic potency compares well with that of nitrogen mustard and radiations,

Mutation pattern of the streptomycin re si stance wstcm.* Mutants of the B strain of *coli* that are resistant to streptomycin (B^rS) or dependent on streptomycin (B/Srf) may be isolated by plating large num-

bers of the bacteria in medium containing streptomycin. Reverse mutants (R) from the dependent strain may be isolated by growing B/Sd on medium lacking streptomycin. By the same process of selection, resistant and dependent mutants can be picked up from the reversions, and from these second-order dependents another set of reversions may be obtained. By continuing the procedure, resistant mutants and reversions of higher orders may be produced. M. Demerec, Ethelyn Lively, and Helen Spring studied such a chain of mutational changes through four orders, and made an extensive analysis of 163 dependent mutants and 120 reversions isolated in this series of experiments. The following properties were studied: (1) mutation rate, (2) mutation pattern (i.e., proportions of B/S and B/Sd mutants and R and S reversions given by the different R's and B/Sd's), (3) growth rate, (4) biochemical deficiencies, which frequently are connected with these mutational changes, (5) sensitivity to ultraviolet radiation, and (6) dependence on certain degradation products of the streptomycin molecule.

From the results of all these tests together, it was evident that hardly any two of the mutants studied were alike; and we used only six out of many possible criteria to detect differences between them. This means that a reversion from a B/Sd to an R does not come about by a reversal of the chemical reaction that originally produced the B/Sd (B \rightleftharpoons B/Sd), but must originate through a change independent of the original one. Therefore there must be a considerable number of independent reactions that give rise to either streptomycin-dependent, streptomycin-resistant, or streptomycin-sensitive mutants.

Genetic mechanisms of the resistance pattern. An effort was made to find out whether one or several gene loci are involved in the control of reactions in the

streptomycin-resistance system. The most direct way of solving such a problem is to determine genetic relationship by intercrossing. Unfortunately, this cannot be done with bacteria, at least not with the B strain of *E. coli*. Lederberg, however, working with the K-12 strain of *coli*, has shown that if two lines, each carrying several heritable characteristics, are plated together, there occur interchanges between the two sets of characters comparable to the crossing over observed in higher organisms. He has worked out a technique whereby the amount of crossing over can be determined and the distances between loci calculated. If no recombination is obtained between two characters, this indicates either that their genes are in the same locus (allelic) or that they are located in closely adjacent loci.

We have partially repeated with the K-12 strain the experiments made with our B strain, and have found that they have a similar pattern of resistance to streptomycin. Intercrosses were made between six mutants of K-12/S and four mutants of K-12/Sd, all of different origin and some having special properties. The results indicate that these mutants are either alleles or located close together. The crosses were carried out by Mr. Norton Zinder, a graduate student of Dr. Lederberg, who spent six weeks with us during the summer. The work is now being extended to additional mutants of higher orders.

Correlation between spontaneous and induced mutation rate. It has already been mentioned that B/Sd mutants differ in their rates of reversion. For example, reverse mutations were observed to occur in Sd-4 with a frequency of about 3.7 per 10^7 plated bacteria, whereas in Sd-12 only one mutant was found among 4.72×10^{10} bacteria. Experiments by Demerec and Lively showed that ultraviolet treatment that in-

duced 6600 mutants in Sd-4 did not induce any in Sd-12.

Streptomycin dependence. In our studies of the streptomycin-resistance system we have accumulated a large collection of streptomycin-dependent strains of separate origin. Of these, 124 were tested by Demerec and William Belser for ability to grow on two streptomycins (dihydrostreptomycin and mannosidostreptomycin) and on five degradation products of the streptomycin molecule: (1) tetrahydroanhydrostreptobiosamine hydrochloride, (2) streptobiosamine hydrochloride, (3) streptidine dihydrochloride, (4) streptidine sulfate, and (5) streptamine dihydrochloride. The two streptomycins, and degradation products (1), (2), (3), and (5), were received from Dr. Josef Fried, of E. R. Squibb & Sons; and degradation product (4) was received from Dr. Amel Menotti, of Bristol Laboratories, and Dr. Karl Folkers, of Merck & Company.

Both the streptomycins were adequate to supply the deficiency in each of the 124 dependent strains. Degradation products (3), (4), and (5) were not adequate to supply the deficiency in any of our strains; whereas 44 strains were able to grow on (1) but not on (2), 9 strains on either (1) or (2), and 1 strain on (2) but not on (1). Considerable differences were observed among the various Sd forms with respect to rate of growth on these compounds, as well as on streptomycin.

General considerations. The analysis of the streptomycin-resistance system indicates that here a series of reactions is controlled by what appears to be a single gene locus. One change in this locus produces a mutant resistant to streptomycin; another change in the same locus produces a mutant dependent on streptomycin; another, a resistant slow grower; still another, a dependent biochemically deficient mutant; and so on. It appears that this par-

ticular locus controls a series of interrelated reactions—a chain of reactions—the end result being determined by the point at which the chain is interrupted or broken by the gene change.

This chain of reactions may be lineal; or it may be branched—that is, some of its links may serve as the starting points of new reaction chains. Our results indicate that, after this chain has been interrupted by a mutation, continuity is re-established (i.e., reversion occurs) not through a repair in the interrupted link, but rather through a change at some other point, which either re-establishes the original reaction or induces the development of a product to replace the one suppressed by the mutation.

According to information now available, this interrelated system of reactions controls the following properties: resistance to streptomycin; dependence on either streptomycin or some part of the streptomycin molecule; resistance to radiations, mustard compounds, and penicillin; some not yet identified biochemical deficiency; mutation rate; and mutation pattern. It is not associated with resistance to the seven phages of the T series. We have tested about 500 streptomycin-resistant and -dependent mutants with phages of the T series and have detected no instance of a change involving both resistance to streptomycin and resistance to phages. This suggests either that bacterial cells possess several completely independent systems of interrelated reactions, or that there is only rarely a connection between the reactions involved in different systems.

BACTERIAL RESISTANCE TO AUREOMYCIN,
CHLOROMYCETIN, AND NEOMYCIN

Our earlier work (see Year Books No. 43> *mrm4*> PP- 109-110; No. 45, 1945-1946, pp. 152-153) has shown that bacteria

resistant to penicillin and to streptomycin originate as genetic mutants. We have also worked out the patterns followed in the development of high-degree resistance to these two antibiotics. This year we studied the resistance patterns of aureomycin, chloromycetin, and neomycin in *E. coli*. The work on aureomycin was done by M. Demerec and Ethelyn Lively, and the rest by M. Demerec and Rada Demerec, who spent the summer of 1949 at this laboratory under a grant from the National Tuberculosis Association, administered by the Biological Laboratory.

Aureomycin. Results of the experiments indicate that high resistance cannot be obtained in one step, but may be built up in several steps. Because of its action on bacteria, this antibiotic was found unsuitable for detailed analysis of the resistance pattern. In addition to killing some of the bacteria, it suppresses division in the survivors. Since it loses some of its potency during an experiment, these suppressed bacteria begin to divide again when the concentration of aureomycin has decreased to a certain point. This behavior hinders the quantitative analysis of resistance.

Chloromycetin. The material used in experiments was obtained from Dr. John Ehrlich, of Parke, Davis & Company. This antibiotic becomes effective—that is, a detectable reduction in number of survivors is observed—at a concentration on agar plates of between 4 and 5 pg. per rnl. With further increase in concentration, the number of survivors drops rapidly, and at 13 pg. per ml. no survivors are found. Strains isolated from survivors of the highest concentrations are more resistant than the original strain (first-step resistance). They begin to be affected at concentrations of between 10 and 13 pg., and require up to 35 pg. for complete elimination. Second-step resistant strains are very resistant. For them the effective concentration is between

30 and 50 jg., and the lethal concentration about 100 ng.

Neomycin. The material for the experiments was obtained from Dr. Gladys L. Hobby, of Chas. Pfizer & Company. The bacteria are affected by concentrations of between 1 and 15 units per milliliter, and about 25 units are required to eliminate all of them. First-step resistant strains show a considerable degree of variability with respect to the concentrations needed to affect them. The observed range among 7 strains tested was from 4 to 15 units. The lethal concentration is between 25 and 35 units; that is, only slightly higher than the lethal concentration for the original strain. Second-step resistant strains, likewise, are only slightly more resistant than the first-step resistant strains.

Cross-resistance tests. Tests were made to determine whether strains resistant to streptomycin are also resistant to chloromycetin and neomycin. All together, 42 strains were tested with each antibiotic. All tests were negative; that is, resistance to streptomycin did not affect sensitivity to the other two antibiotics.

General considerations. Since the development of resistant strains is an important factor affecting the clinical usefulness of antibiotics, information about resistance patterns is essential for effective use of these substances in medical treatment. Our earlier work showed that first-step strains resistant to penicillin are all very uniform and are only slightly more resistant than the original strain. Highly resistant strains can be obtained only in several steps, by always selecting the most resistant survivors for further propagation. Therefore it is relatively simple to prevent the development of highly resistant strains if the concentrations of penicillin used in treatment are sufficiently high to eliminate all the bacteria present.

The situation is quite different with

streptomycin. Here the variability among first-step resistant strains is very great, and some of them are completely resistant. Since high resistance may be attained in one step, the development of highly resistant strains cannot well be avoided.

The patterns of resistance to aureomycin and chloromycetin resemble that for penicillin. Therefore it may be expected that the clinical use of these two antibiotics will not be complicated by the development of resistant strains.

Neomycin, whose antibiotic activity closely resembles that of streptomycin, evokes a resistance pattern that is intermediate between those for penicillin and streptomycin. First-step resistant strains show a considerable degree of variability, and some of them have fairly high resistance. The development of highly resistant strains can be avoided only if a sufficiently high concentration can be used in treatment to eliminate all first-step mutants. For our strain of *coli*, this concentration is not lower than 25 units per milliliter. Since strains resistant to streptomycin are sensitive to neomycin, the use of a mixture of these two antibiotics would greatly lower the required concentration of each, provided chemical action of the compounds is not neutralized in mixtures.

PHENOTYPIC EXPRESSION OF DELAYED MUTANTS

During the past year an interesting genetic effect of yeast ribose nucleic acid was observed and investigated in *Escherichia coli* by Witkin and Jessie Flint. Cultures of strain B/r grown in broth containing 0.5 per cent sodium nucleate (Schwartz) were found to yield, on the average, significantly higher numbers of mutants resistant to bacteriophage T_i than a series of control broth cultures. Tests made during the logarithmic growth phase showed

that the increase was not due to a heightened rate of mutation during the growth of the nucleate cultures. No difference in number of mutants between cultures grown with and without nucleate was observed until the "stationary" phase of the culture cycle was well under way. The average number of mutants rose continuously, in both broth controls and cultures containing nucleate, for about 72 hours, with the nucleate cultures maintaining a significantly higher average than the controls after the end of the logarithmic growth phase. A careful investigation of the effect of sodium nucleate on the growth rates of sensitive and resistant bacteria ruled out the possibility of differential stimulation of division in resistant mutants, or more active total division in the nucleate cultures.

Observation of individual nucleate cultures led to the formulation of the following hypothesis: that sodium nucleate increases the frequency of Tr-resistant mutants by hastening the phenotypic expression of spontaneous "delayed" mutants, which would ordinarily remain phenotypically sensitive until the occurrence of one or more divisions. This hypothesis was suggested primarily by the observation that large numbers of small-colony Ti-resistant variants occasionally appeared after 48 hours in a nucleate culture known to contain only the more common large-colony type of mutant at 24 hours. Since the small-colony Ti-resistant variant is relatively rare, it is very unlikely that any one culture will contain more than one clone of this type. The amount of division occurring between 24 and 48 hours of incubation is not sufficient to explain the new appearance of a large clone. Therefore, it seems likely that the clone of small-colony Ti-resistant variants must have been present at 24 hours in a phenotypically sensitive state.

A critical test of the hypothesis that sodium nucleate develops the phenotypic expression of delayed mutants is now under way. Ultraviolet-irradiated cultures, which are known to contain extremely large numbers of induced delayed Ti-resistant mutants, are being used in this investigation. Preliminary results indicate that sodium nucleate converts delayed mutants into "zero-point" mutants; in other words, that the ultraviolet-induced delayed mutants can be detected after treatment with sodium nucleate under conditions precluding division. In the absence of nucleate, division is required before the delayed mutants become phenotypically resistant.

Similar results have been obtained with high concentrations of casein hydrolysate. High concentrations of glucose and certain amino acid mixtures fail to duplicate the nucleate effect.

INHIBITION-REVERSAL TECHNIQUE

Sodium nucleate was used also by Witkin in the development of a new technique for investigating chemically induced bacterial mutations. The inhibitory action of a number of antibacterial compounds can be reversed by the addition of sodium nucleate. Among these are acriflavine and caffeine, which have been shown to be mutagenic (see Year Books Nos. 46 and 47), and various analogues of the naturally occurring purines and pyrimidines, which have not yet been tested for mutagenic activity. The method can be illustrated by describing its application to the study of mutations induced by acriflavine. Twenty-five tubes containing 5 ml. of 0.01 per cent acriflavine dissolved in nutrient broth are inoculated with io^T bacteria from a fresh broth culture of strain B/r. The tubes are incubated for 4 hours at 37° C, at which time the survival in each tube is about

10^{-3} . Sodium nucleate is added to each tube to give a final concentration of 0.5 per cent, and the tubes are incubated 18 to 24 hours. The bacteria not yet killed by the acriflavine at the time of addition of nucleate are able to divide at the normal rate to give cultures of full titer after incubation. Each culture is assayed to determine the number of bacteria per milliliter and the number of Ti-resistant mutants per 10^8 bacteria. The frequency distribution of mutants in the nucleate-reversed series of cultures is plotted and compared with the frequency distribution of mutants in a similar series of control cultures. The control cultures are inoculated with the same number of bacteria found to be viable in the acriflavine series at the time of addition of nucleate, and are grown in the presence of 0.5 per cent sodium nucleate without exposure to acriflavine. The distribution of mutants in the control series is extremely constant, with most of the cultures in the range from 10 to 50 mutants per 10^8 bacteria. In the acriflavine-nucleate series, most of the cultures have over 200 mutants per 10^8 bacteria at the end of growth. Experiments with artificial mixtures of sensitive and resistant bacteria have indicated that selection is not responsible for the effect.

Both zero-point and delayed mutations are detected by this technique, as both types of induced mutations contribute to the final crop of mutants in the experimental cultures. The method has other advantages over those used previously: (1) The nucleate reversal effectively removes the acriflavine from further activity, more completely than several centrifugations and washings. (2) Selection due to differential survival of resistant mutants initially present in the treated population can be ruled out directly, by using inocula small enough to contain no "background" mutants. (3) Statistical error in the estimation of muta-

genic potency is considerably less than with methods in which the observation of small numbers of mutant colonies is the basis of calculation.

The inhibition-reversal method, as illustrated by the acriflavine-nucleate system, is applicable to a great many other similarly reversible systems, some of which are already under investigation.

EXPERIMENTS WITH DROSOPHILA

During the past year a great deal of work has been done in continuation of the study of mutagenic action of chemicals on the sperm of *Drosophila*. This research has been carried on by Wallace, assisted by Leona Harf, Helen Spring, Mrs. Jennie Buchanan, and Raymond Millemann. If one examines the data gathered throughout the years this program has been pursued, one finds that they indicate several fairly distinct periods: (1) During the initial period the aerosol technique was being tested with miscellaneous dyes and organic salts. The results of these experiments were uniformly negative' (Year Book No. 45, pp. 156-157). (2) A second period started with the first test of the carcinogenic substance dibenzanthracene. During this period the results indicated that certain chemicals were mutagenic; chromosomal aberrations were induced by these chemicals, and the results of different experiments using the same chemical were fairly consistent (Year Book No. 46, pp. 127-131)* (3) The third period was a short, not too distinct, time during which the variability from experiment to experiment became alarming and only occasionally was it possible to obtain confirmation of previous experiments (Year Book No. 47, p. 170). (4) The fourth period, encompassing all of the past year, has been characterized by uniformly negative results except in those experiments us-

ing nitrogen mustard, methyl- ϵ -V(beta-chloroethyl)amine hydrochloride. A summary of the experiments made during this period is presented here (table i), but a fuller analysis will be published elsewhere.

Testing the data given in table 1 for homogeneity by the Brandt and Snedecor method of computing chi square gives the

ments, we obtain a chi square of 1307 with 19 degrees of freedom; the probability of getting such a chi square from a homogeneous population is negligible, and it can be concluded that nitrogen mustard does induce mutations.

The study of lethals included analysis not only of their frequency, but also of

TABLE 1

SUMMARY OF THE FREQUENCY OF LETHALS OBTAINED DURING 1948-1949 BY TREATMENT OF DROSOPHILA MALES WITH VARIOUS CHEMICALS

Treatment	No. sperm tested	No. lethals	% lethals
Control	39,928	122	0.306
1,2,5,6-dibenzanthracene	60,758	186	0.306
\wedge -hydroxyazobenzene	7,178	19	0.265
beta-naphthylamine	1,980	5	0.253
azoxybenzene	773	1	0.129
azobenzene	1,907	2	0.105
\wedge -dimethylaminoazobenzene	2,674	7	0.262
3,4-benzpyrene	1,499	4	0.267
1,2-benzanthracene	1,185	1	0.084
methylcholanthrene	3,003	9	0.300
acetylaminofluorene	1,172	2	0.171
pyrene	1,146	2	0.175
anthracene	920	4	0.435
alpha-naphthylamine	1,296	6	0.463
pEenanthrene	1,044	7	0.670
\wedge -aminoazobenzene	741	2	0.270
\wedge -diethylaminoazobenzene	859	2	0.233
acriavine	873	2	0.229
sodium desoxycholate	1,713	5	0.292
Subtotal	130,649	388	0.297
nitrogen mustard	3,757	153	4.07
Total	134,406	541	0.403

following results: If nitrogen mustard results are omitted, the chi square for 18 degrees of freedom is 14.06; the probability of obtaining a chi square as large as this from a homogeneous population is 0.74. There is no reason, then, to conclude from these experiments that any one of these chemicals differs from any other, or from the control, in its mutagenic properties. If we include the nitrogen mustard experi-

their location. Of the 388 lethal gene mutations obtained in experiments other than the nitrogen mustard experiments, 372 were located in one or another of the five regions of the X chromosome formed by the mutant genes *ec*, *ct*, *v*, and *g*. No evidence was found that any of these 372 lethals was involved in a chromosomal rearrangement. It should be emphasized that 114 lethals arising spontaneously in

the control experiments were among those tested for position and for the presence of concurrent chromosomal rearrangements. The confidence interval, then, for the frequency of chromosomal rearrangements occurring with spontaneous lethal gene mutations is 0 to 0.03. (This interval is calculated by use of the equation $i-p = \text{antilog}\left(\frac{\log a}{n}\right)$, where p is the upper limit of the confidence interval, a is the level of significance, 0.05, and n is the number of observations, 114.) If we turn now, for example, to experiment 185 (one of the experiments using p-hydroxyazobenzene made during 1947), in which 3 aberrations were found among 35 lethal gene mutations, we can calculate the probability that these were spontaneous gene mutations occurring concomitantly with aberrations. Using the 0.03 upper limit and fitting the normal curve to the binomial with Yates' correction for small figures, it is found that the probability of getting 3 aberrations with 35 lethal mutations is 0.0375. Since this figure is obtained by use of the upper limit, we conclude that some factor other than chance was operating in experiment 185 and that the aberrations observed were not of spontaneous origin.

In conclusion, it may be mentioned that the following factors have been considered in an effort to locate the source of variability in our experiments: genetic constitution of the treated flies, solvents, temperature, nebulizers, length of treatment, killing of treated flies, size of treated flies, condition of chitin, rate of respiration (as controlled by CO₂), and condition of the M-5 tester chromosome as determined by cytological and genetical analysis.

An analysis of the relation between dominant and recessive lethals induced by nitrogen mustard has been undertaken during the past year. The purpose of this

analysis was to obtain data bearing on the theory that dominant lethals are the result of inviable chromosomal rearrangements. The arguments presented by Muller and by Lea (D. E. Lea, *Actions of radiations on living cells*, 1947) are convincing but are based on differential genetic effects of varying X-ray treatment. Since nitrogen mustard induces both gene mutations and chromosomal rearrangements, but in a ratio different from that induced by X-rays (Auerbach and Robson, *Proc. Roy. Soc. Edinburgh* (B), vol. 62, pp. 271-283, 1947), it seemed that dominant-lethal data from nitrogen mustard experiments would be enlightening.

In contrast with X-rays, whose dosage can be measured and controlled accurately, nitrogen mustard, as applied to flies by the aerosol technique, gives variable results. Because of this, it was decided that the ratio of dominant to recessive lethals in a number of experiments should be obtained and compared with similar data obtained for various doses of X-rays.

The dominant-recessive relation for nitrogen mustard (25 separate experiments) was found to be nearly identical with that expected for X-rays. Since viable rearrangements are less frequent with nitrogen mustard than with X-rays, but dominant lethals for the two are equal, it seems profitable to investigate this problem further. The observed difference may be caused either by a greater induction of primary breaks by X-rays or by the greater opportunity for restitution in the nitrogen mustard experiments.

A third series of experiments, involving X-radiation, was initiated by our group and is now being pursued at the Biological Laboratory of the Long Island Biological Association. It was necessary to know whether eggs and larvae can survive a chronic treatment of 133 r units per day.

and whether, if they do survive, an appreciable number of lethal gene mutations can be found in the adult flies.

The eggs of a large number of Oregon-R parents were collected daily for 5 days. Those collected on the first day were irradiated daily for 5 days from the day of collection, those collected on the second day were irradiated for 10 days from the day of collection, those of the third day for 15 days, the fourth for 20 days, and the fifth for 25 days. These treatments covered, respectively, the periods egg to larva, egg to pupa, egg to 5-day-old adult, egg to 10-day-old adult, and egg to 15-day-

old adult. The data obtained are given in table 2, combined with tentative data of a confirmatory experiment carried out at the Biological Laboratory.

The proportion of lethals recovered in the sperm of adult males increases linearly with time until the fifteenth or twentieth day, when it becomes stabilized. This agrees with the work of others (see Demerec and Kaufmann, *Amer. Naturalist*, vol. 75, pp. 366-379, 1941), who have found that sex-linked lethal gene mutations and dominant lethals are eliminated from sperm about 14 to 17 days after treatment of male *Drosophila* with X-rays.

TABLE 2
FREQUENCY OF SEX-LINKED LETHALS OBTAINED BY IRRADIATION OF GROUPS OF FLIES FOR VARYING NUMBERS OF DAYS

Treatment Days	r units	No. sperm tested	% lethals ± S.E.	Expected*
0	0	2107	0.190 ± 0.095	...
5	539	2564	1.21 0.216	1.44
10	1093	1102	3.99 0.590	3.26
15	1617	2454	4.60 0.423	4.32
20	2156	1336	6.14 0.657	5.76
25	2695	327	5.20 1.23	...

* Expected on the basis of a linear increase for 20 days.

CYTOLOGY OF BACTERIA

BERTHE DELAPORTE

To ascertain, if possible, whether the nuclear element seen in all bacterial cells is haploid, diploid, or polyploid—that is, whether it is a single nuclear unit or is composed, at least at certain times during the growth of a bacterial culture, of several fused nuclear units—studies have been made of the bacterial nuclear element under many different conditions of observation, and also in cells treated with radiations or chemical mutagens, during lysis by bacteriophage, and in different strains grown under various conditions.

The technique used most often was staining with Giemsa after fixation with osmium tetroxide vapor and hydrolysis with *N* HCl at 60° C. for 10 minutes. Post-fixation with alcoholic sublimate after the osmium tetroxide treatment gave identical results. Fixation of cells on the agar block before making an impression of the culture gave the same results as fixation after impression, provided the bacteria were not allowed to dry before fixation.

Permanent mounting medium. Since no good technique of mounting was known

that would allow preservation of Giemsa-stained preparations for a few months, several procedures were tried, and Abopon, a water-soluble resin used by E. Lieb to mount amyloid-stained preparations, was found to be convenient and satisfactory. Eight months after mounting, the preparations are the same as on the first day. Staining with Giemsa and with Feulgen gives the same results, but with Feulgen the nuclear element is smaller than with Giemsa.

INFLUENCE OF CULTURE CONDITIONS ON STRUCTURE

Bacteria of the same strain of *Escherichia coli* (B/r/i, for example), grown in different aerated liquid media (broth or the GR medium of Doermann) and subcultured in one or the other medium, show, at the same age, differences in length of cells and in shape of the nuclear element. Culture for 24 hours in broth followed by 105-minute subculture in broth results in very short cells, sometimes almost spherical, with large round or ovoid nuclear elements. Culture for 24 hours in GR medium followed by 105-minute subculture in broth produces medium-length cells with globular nuclear elements. Culture for 24 hours in GR and 105-minute subculture in GR produces long cells in which the nuclear element often appears as an axial rod.

When *E. coli* (B/r) is grown in non-aerated broth, it produces, in a 4-hour culture, medium or long rods with 4 to 8 nuclear elements, isolated but close together, so that they appear as transverse rods (perpendicular to the long axis of the cell), which divide lengthwise and therefore often are seen as V or U shapes.

INFLUENCE OF ULTRAVIOLET RADIATION

When cells of *E. coli* are irradiated, they form "snakes." B-strain cells, irradiated

during the exponential phase of growth, produce snakes in which the nuclear element is seen, most frequently, as a line of adjacent granules along the axis of the filament, or else as an axial line showing no distinct granules. Sometimes the granules are separated, isolated or two by two. The ends of snakes contain no nuclear substance.

ACTION OF BACTERIOPHAGE

When a suspension of bacteriophage is added to a sensitive strain in exponential growth in GR medium (T4 to strain B/r/i, for example), no change is evident for the first 10 minutes, except that in some cells a few granules are visible at the periphery, but after 20 minutes most of the cells are completely and uniformly filled with chromatic substance. (A few cells, resistant to infection, will maintain a normal aspect throughout the process.) One to four small chromatic granules are seen at the surface of some cells. After 25 minutes the membrane has lost its rigidity and sharpness, and small knobs are visible on the cells. After 30 minutes this effect is accentuated. After 38 minutes lysis begins; the cell expands considerably, taking the shape of a racket with a short handle or of a complete sphere in which the whole interior is uniformly chromatic, and then bursts, losing all its chromatic substance. Around newly lysed cells it is possible to see a large number of small points, stained with Giemsa. It is difficult to determine whether or not these are phages, or aggregates of phages, just leaving the cell. These phenomena have also been observed with the phase-contrast microscope. The infected cells remain dark during the entire time of infection and internal multiplication of the phage, and then are seen to swell rapidly into round bodies, which burst a few seconds later and immediately become transparent,

with a remnant of membrane visible as a ghost.

When phage is added to a culture of *E. coli* containing snakes induced by ultraviolet radiation, large, round swellings are observed in the snakes—one or two in short snakes, several in long ones. With the phase-contrast microscope, a few minutes after the beginning of the experiment, one *sets* transparent spaces, approximately square and occupying the whole width of the snake, like complete interruptions of its living substance. A few minutes later, these spaces swell rapidly into round, transparent bodies, sometimes 15 to 20 microns in diameter, sometimes larger. Occasionally smaller round bodies appear on the sides of snakes, but these are filled with protoplasm like the interior. Sometimes the large bodies are in the form of spindles. Staining shows granules of nuclear substance at the periphery of the large bodies. In the remainder of the snakes, the chromatin is in shapeless masses. Some snakes look like a string of beads, which are sometimes closely joined together in an irregular line, but more often separated by short threads of chromatic substance. About 35 minutes after infection, many snakes consist of lengthened masses of chromatic substance, two or three times the normal diameter of cells, separated by thinner, threadlike sections, which are sometimes very long. In living preparations, sometimes one of the large bodies bursts, leaving only debris in its place. In both stained and living preparations, some of the snakes are ghostlike, of normal or smaller diameter, with a few chromatic granules at the periphery.

Lysis by bacteriophage was also observed on a strain of *Bacillus cereus*. Ten minutes after addition of the phage, the nuclear elements of the cell fuse and 10 minutes later the whole cell interior is filled with a chromatic, homogeneous substance. At 60

minutes the cell is considerably larger in volume, the contents always homogeneous and the membrane neatly outlined. At lysis, the chromatic substance recedes from the periphery, in deeper and deeper scallops, until first only an axial rod is left, then only a few granules, which finally disappear, leaving the cell with no chromatic substance. The bacterial membrane keeps its cylindrical shape throughout the process; but the cells, which at first are in long chains, later become isolated. The above-described process occurs simultaneously in all cells of a culture.

ACTION OF STREPTOMYCIN

Antibiotics, when added to a culture, bring about changes in cell form. Penicillin, for example, induces the formation of round bodies on the cell. In certain mutant strains, the cell shape is modified only when cultures are grown on normal medium containing no antibiotic. For example, the streptomycin-dependent strain B/r/Sd, which has normal cell structure when cultured on streptomycin-agar, grows very slowly and forms "snakes" on medium lacking streptomycin. The cells first become elongated, with the nuclear substance in the shape of an axial rod, then gradually develop into long snakes. These are filled with nuclear substance, continuously or in fragments, with occasional swellings or enlargements in which the nuclear substance appears as either contiguous granules, an axial thread, or a loose net of granular filaments.

INACTIVATION BY ULTRAVIOLET AND RECOVERY BY LIGHT

Exposure of a culture of *E. coli* to an appropriate dose of ultraviolet rays causes death of 99 per cent of the cells. These cells do not at once show changes in their structure, except for the appearance of a

few small granules of chromatic substance—perhaps detached from the central mass of nuclear substance—at the periphery of some cells, most often near a pole. Observations were made on strains B and B/r. After about an hour of culture in the dark in aerated broth or agar, the dead cells appear to be smaller, and their chromatin is diffused uniformly throughout (or almost throughout) the cell so that it is more or less completely mixed with the cytoplasm. This effect persists for a number of hours before destruction of the cell is complete. The few cells that do not die maintain their normal structure and begin to multiply normally after a longer than usual lag phase. Later, in B/r (in aerated broth or M-9), some snakes are formed; these have isolated nuclear granules either in an axial line, scattered, or grouped near the middle of the snake. The cells that remain short have only one central granule, or two when they are dividing. In strain B, on agar, almost all the surviving cells form snakes, which lengthen gradually, the chromatin filling the whole interior or being more or less diffuse but localized in the middle part of the snakes. Then the chromatin separates from the cytoplasm, and takes the shape first of reticular structures, which appear as if made up of tiny mixed chromatinic threads, then of separate rectangular fragments having this reticular structure, and finally of granules, more or less closely related, arranged in squares or rectangles. At this stage new cells appear in the culture. They are large and isolated, having a sky-blue cytoplasm and two nuclear elements in the form just described, like the cells of certain young cultures. These isolated cells are found in increasing numbers in aging cultures, and there is a parallel decrease in the number of snakes.

If such ultraviolet-treated cultures are strongly illuminated for about an hour,

soon after irradiation, and then subcultured, a high percentage of the cells recovers (A. Kelner, *Proc. Nat. Acad. Sci.*, vol. 35, pp. 73-79, 1949). In strain B/r, after this light treatment followed by 2-hour culture in the dark in aerated broth, a great many cells begin to form snakes, in which the nuclear substance very often appears as contiguous granules, placed in an axial line. As the snakes lengthen, the nuclear granules are either in an axial line or scattered; exceptionally, they are in two parallel rows. Very few of the cells have the appearance of the dead cells, filled with diffuse chromatin, commonly seen in the same culture* without light treatment. Strain B on agar, given similar treatment, shows a few dead cells with diffuse chromatin, a very few small cells with central, more or less globular nuclear elements, and a large number of snakes, which lengthen progressively, and which have the same nuclear structure as the snakes that form in the absence of light treatment. Later, isolated cells are seen.

OBSERVATIONS ON BACILLI

Formation and germination of spores, and first cellular divisions in a new culture, were observed in *Bacillus mycoides*, *B. cereus*, *B. megatherium*, and *B. macerans*. In *B. cereus*, at the beginning of sporulation (16- to 24-hour culture), the nuclear substance, in almost all cells, appears in the form of several scattered granules. The number and placement of these granules are not constant; perhaps they are linked together, but if so their ties are not visible with the methods used. The one situated nearest an end of the cell becomes the nucleus of the spore. It gradually enlarges, stretching into a short rod; at the same time a homogeneous and dense cytoplasm, stained pink with Giemsa, forms around it and finally takes the ovoid shape and the

dimensions of a mature spore. The cytoplasm then changes its staining characteristics and stains sky blue; the spore is enclosed in a refractive membrane; and the nuclear element moves to the periphery, taking the shape of a short curved rod (in side view) or ovoid ring (front view), or of two or three closely related granules. During this process, the cytoplasm, nuclear substance, and lipid globules that were in the sporangium outside the spore gradually disappear—perhaps assimilated by the spore in formation. The spore is then liberated. In certain species (*B. mycoides*, for example) spores remain permanently inside a remnant of sporangium membrane, which shrinks so that only two short finger-like appendages are seen at the poles of the spore.

During germination, the spore becomes larger, and the nuclear element moves in from the periphery to the center, assuming the shape of a wavy filament or of several closely related granules, which often divide into two symmetrical parts before the

young cell emerges from the spore coat. A second, and sometimes a third, nuclear division often occurs before the formation of a visible transverse membrane inside the rod. A small granule of nuclear substance, which stains Feulgen-positive, is sometimes seen discarded on the empty spore coat; probably it is a remnant of the nuclear substance of the sporangium.

During the first divisions in a new culture of *Bacillus*, the rate of nuclear division is more rapid than the formation of transverse membranes and lengthening of the cell, so that very short cells are formed. Often their width is greater than their length, and consequently the nuclear element is in the form of transverse rods, unless deformed by near-by elements. These transverse rods divide lengthwise, often assuming V or U shapes, and the halves are pulled apart in the direction of the long axis of the cell, as happens in all bacterial divisions. The nuclear element is rarely seen in the shape of an axial thread during the early divisions.

INTRACELLULAR GROWTH AND GENETICS OF BACTERIOPHAGE

A. H. DOERMANN AND CAROLYN F.-R. DISSOSWAY

The biosynthesis of virus particles presents to the investigator a challenging, but extremely complicated, problem. Nevertheless, significant advances have been made in the past few years, especially where bacteriophage has been used as the experimental organism. The finding that genetic recombination is a predictable phenomenon, which occurs when related phage particles are growing in the same bacterial cell (Hershey and Rotman, *Genetics*, vol. 34, p. 44), is perhaps the most notable achievement so far. Not only is the result of fundamental interest in itself, but it also affords to phage-synthesis studies a specificity that is possible only by ap-

plication of the sensitivity of genetic identification.

The approach to the problem of bacteriophage growth that we are trying to develop is one which will make possible the combined application of a genetic and biochemical attack. With this plan in mind, two methods were developed whereby bacteriophage-infected cells of *Escherichia coli* can be disrupted and made to liberate their phage contents at any time during the phage life cycle (see Year Book No. 47, 1947-1948). These methods have been extended and utilized in several ways during the past year. The cyanide-lysis technique has been modified in such a way

that it can now be used to study the phage contents of single bacterial cells at any stage of the reproductive cycle. This modification and the original method have been applied to the study of genetic recombination, with the hope of finding some clue to the mechanism involved.

In addition, another problem has been encountered, which at first sight does not seem related to the plan of investigation mentioned above, but which is nevertheless expected to show a connection with it after fuller study. A genetically unstable phage type has been isolated and preliminary experiments have been made in a study of the mutability pattern of the phages derived from this stock.

TIME OF OCCURRENCE OF GENETIC RECOMBINATION

Hershey and Rotman have shown that when bacteria are mixedly infected with several T2 particles of the types rh^+ and r^+h , the progeny from single bacteria contain all four combinations of these factors, viz., rh^+ , r^+h , rh , and r^+A^+ . Their studies of about twenty independently arising r types have shown that they fall into three categories with respect to amount of recombination with the h locus. With one type there is about 2 per cent recombination. Two others yield approximately 35 per cent recombination. With a third, larger, group, about 15 per cent recombination is found. Our first question concerned the stage of phage growth at which genetic recombination takes place. It seemed likely that recombination might occur only late in the latent period, when the infected bacterium contains numerous new phage particles. Such a result would suggest that recombination takes place between the newly formed phage particles. It appeared possible, however, that the first newly formed phage particles might

already be recombinants, which would suggest that mixing of genetic material must take place even before the presence of phage particles can be demonstrated.

The experiments to test these alternatives were made with mass cultures of stocks received from Hershey. They were carried out in the manner described by Hershey and Rotman, except for incidental technical changes. The phages selected for crossing were T2Hri₃A⁺ and *TiHr⁺k*, for which Hershey and Rotman found about 2 per cent recombination. Bacteria that had been mixedly infected with these phages were subjected to the cyanide-lysis procedure at intervals after infection. Platings were made from the lysed aliquots against mixed indicator strains that permit identification of all four types of phage expected to result from this cross. The results of experiments indicate that, at a time when there are only 2.3 completed phage particles per bacterium, there is already as high a proportion of recombinants as later. It is therefore clear that recombinants occur with about the same probability among the first-formed phage particles as among those formed later in the latent period, indicating that genetic mixing takes place before the completion of any of the phage particles.

DISRUPTION OF SINGLE BACTERIA BY CYANIDE

To make a detailed genetic analysis, it is often important to observe the progeny of single crosses separately. Similarly, in studying the growth and genetics of bacteriophage it is essential in many cases to have data on the progeny from single bacteria. The next problem attacked, therefore, was that of devising a technique whereby single cells might be disrupted and thereby induced to liberate their phage contents. The method finally used is pat-

terned after the original technique of Burnet (*Brit. Jour. Exper. Pathol.*, vol. 10, p. 109, 1929), as modified by Delbrück. It is similar to the cyanide-lysis procedure except for the following details: The medium used is broth made according to Hershey and Rotman. Infected bacteria are distributed by drops to individual tubes, each tube having a probability of about 0.25 of receiving one infected cell. At a designated time the tubes are chilled and cyanide is added. After lysis has been induced, the entire individual samples are plated against mixed indicator strains.

In these experiments, T2Hris and T2HA were again used in mixed multiple infection. The results will first be analyzed on the basis of total yield of phage per cell, and then on a genetic basis. Only those bursts that contained more than one infective center were included in the analysis. Those containing a single particle were omitted for several reasons. Some of them were undoubtedly due to unadsorbed particles of the infecting phage population which escaped antiserum inactivation. Those stemming from infected bacteria fell into two groups, namely, those actually containing a mature phage particle at the time of addition of cyanide, and those not containing one. T6 was omitted from these experiments for technical reasons, and the bacteria lacking a mature phage particle would not be lysed by the cyanide alone. They would thus make a plaque on the plate, because the cell would recover from the cyanide there, resume synthesis of phage, and lyse. To omit the single plaques from the Poisson analysis seemed justified inasmuch as they would not materially affect the estimation or the distribution of the other bursts.

Five experiments were made, and 159 of 558 tubes were found to contain more than one phage particle. By totaling the Poisson analyses of the individual experi-

ments it was found that theoretically 119 of these were single bursts, 18 were double bursts, and 2 were triples. The data indicate a bimodal distribution, corresponding roughly to the bursts from single bacteria and from the accidental doubles. In a general way, it may be said that there is great variability in the amount of intracellular phage growth accomplished in the individual bacteria at a given time. Since the adsorption period was only 2 minutes, and since approximately 10 phage particles were adsorbed in that time, the difference among individual bacteria cannot be ascribed to a difference in the time of infection alone.

These results agree fairly well with those obtained by Delbrück with the virus Ti (*Jour. Bacteriol.*, vol. 50, p. 131, 1945) when he studied the phage yields from individual bacteria allowed to lyse normally. A more appropriate comparison can be made with Hershey and Rotman's results from crosses with T2H. The histogram of 100 of their single bursts lysing normally shows a distribution more concentrated around the mean. Our results show a considerably wider distribution in the relative sizes of yields from individual bacteria, with a concentration of bursts less than the mean. This difference can be explained on the hypothesis that phage growth proceeds linearly in individual bacteria, but that there is a spread in the time at which the first mature phage particles appear in the individual bacteria. If the growth of phage in our bacteria were allowed to proceed up to the time of normal lysis, both the mean and the individual yields would be moved along the abscissa, resulting in a more concentrated distribution of the bursts. This hypothesis would also predict that a curve describing intracellular phage growth in mass cultures should bend upward during the first portion of the rise, since one would add linear rates from in-

dividual bacteria starting at different times. This is precisely what was observed in the earlier experiments (see Year Book No. 47)-

The results of these single-burst experiments add detail to the picture of intracellular phage growth suggested by the mass-culture experiments. It is now clear that the phage obtained in the mass-culture experiments is not derived from a small number of easily disrupted bacteria, but from approximately 100 per cent of the infected cells.

CORRELATION IN THE OCCURRENCE OF ALTERNATE RECOMBINANT TYPES

The single-burst data already described were also studied in an attempt to determine whether the occurrence of one recombinant is correlated with the occurrence of the alternate type. Hershey and Rotman studied crosses between representatives of the three groups of *r* mutants and the *h* mutant. Only in the crosses involving *h* and *r*₇ where about 15 per cent of the progeny were recombinants, was a significant positive correlation found. It appeared surprising that no correlation could be detected between *h* and the more closely linked *ris*, where only 2 per cent recombination was found. It seemed possible that an early correlation might have been obliterated by growth of either recombinant during the latent period. Our experiment could shed some light on this explanation, and the progeny of the single bursts were therefore grouped in four genetic classes, *rh*^{*}, *r*^{*}*h*, *rh*, and *rVi*⁺. Of the 139 samples plotted, the classification of plaques was not dependable in 19 cases, and 12 other bursts contained only one of the parental types. These cases were therefore excluded from the analysis. The remainder were classified, and fell into the grouping shown in table 3. The amount of correlation may

be estimated in two ways. First, the correlated absence of recombinants may be studied. From the table it is seen that 77 of the total of 108 bursts lacked the *r*^{*}*h*^{*} recombinant, whereas 65 lacked *rh*. The number expected to lack both simultaneously, on a random basis, is the product of the above ratios, which is 0.43. This predicts that 46 bursts should show neither

TABLE 3

SINGLE BURSTS FROM CROSS T2Hr₁₃ x T2H/J,
DESIGNED TO TEST CORRELATION IN THE
OCCURRENCE OF ALTERNATE
RECOMBINANT TYPES

Type	Frequency	Type	Frequency
<i>Orh</i> : 0 -f + ...	53	3 <i>rh</i> : 1 j- + ...	1
1 <i>rh</i> : 0 -f + ...	12	2 <i>rh</i> : 2 -f + ...	1
<i>Orh</i> : 1 -f + ...	9	1 <i>rh</i> : 3 -F + ...	2
2 <i>rh</i> : 0 -f + ...	10	3 <i>rh</i> : 2 -f + ...	1
1 <i>rh</i> : 1 -f + ...	7	2 <i>rh</i> : 3 -f + ...	1
<i>Orh</i> : 2 -f + ...	2	4 <i>rh</i> : 2 -f + ...	1
3 <i>rh</i> : 0 -f + ...	2	3 <i>rh</i> : 3 -f + ...	1
2 <i>rh</i> : 1 -f + ...	1	2 <i>rh</i> : 4 -f + ...	1
1 <i>rh</i> : 2 -f + ...	1	6 <i>rh</i> : 1 -f + ...	1
<i>Orh</i> : 3 -f + ...	1		
Total recombinants are 75 <i>rh</i> : 50 ++			

recombinant. The number found, which was 53, is higher, but not significantly so. The result indicates that the presence or absence of one recombinant type is independent of the presence or absence of the alternate type-

On the other hand, the correlation coefficient, *r*_{xy}, between *rh* and *r*⁺*A*⁺ may be calculated. Hershey and Rotman have already shown that a correlation exists between the size of individuals and the number of recombinants in them. For this reason the coefficient of correlation must be calculated using the numbers of recombi-

nants as fractions of the individual burst sizes. When r_{xy} is calculated in this way, no correlation can be detected. The conclusion is that a reciprocal exchange in the later stages in the development of the virus particle is highly improbable. It is nevertheless possible that a reciprocal exchange occurs earlier, and that the correlation is effaced by subsequent reactions which complete the phage particles at random.

From Hershey and Rotman's genetic experiment taken together with the present results, we can draw one conclusion: that phage particles do not reproduce by division as unicellular organisms do. This conclusion is reached as follows. We now know that the first completed phage particles in mass cultures have about the same probability of being recombinants as the particles found later. If division is the mechanism of phage reproduction, recombinants should develop into large clones in those single bursts where they occur early, since an early recombinant should grow into more through subsequent divisions. Clones, however, were not observed by Hershey and Rotman, nor have they been seen in these experiments. The recombinants in Hershey and Rotman's experiments were distributed at random among the bursts.

In agreement with this conclusion is the fact that it is not possible to recover active phage from the infected bacteria during the early part of the latent period. Even the infecting phage particles can no longer form plaques if the cell is disrupted during the first third of the latent period. If reproduction of phage is by simple division, at least one phage particle should be available at all times during the latent period.

A GENETICALLY UNSTABLE SERIES OF PHAGE TYPES

The standard procedure for making bacteriophage stocks with a relatively low

frequency of mutant types is to infect a bacterial culture from a plaque that has arisen from a single phage particle. The lysate obtained in this manner is generally pure, except for a small number of mutant types that invariably arise in handling numbers of this order of magnitude. This procedure was applied to a peculiar scalloped plaque that appeared in an experiment where *Tqr* was used. The resulting lysate was not pure, however, but contained less than 90 per cent of the plaque type that was sought, the remainder being a mixture of other types. The procedure was repeated several times with the same result. Serial plaque isolations were also made, with no better success. It appeared that a mutable phage type was under observation, and on this hypothesis the situation was investigated further.

The first problem was one of identification and differentiation of the several plaque types arising in the plating from a scalloped plaque. The technique used was to suspend an entire plaque in broth, dilute appropriately, and then plate by the agar-layer method. Platings were made of seven apparently different plaques coming from the phage population of a single scalloped plaque. Of these, five proved to be differentiable on the medium used. The other two were probably examples of one of these types. Those differentiable were numbers 1, 2, 5, 6, and 7. They are described in table 4. Stocks were made by infecting bacterial cultures from plaques of these five types.

To verify the fact that these strains were T4 mutants, host-range experiments were made. Bacteria resistant to standard T4 were also resistant to these types, and T4-sensitive bacteria were sensitive to them. To obtain more critical evidence, stocks of types nos. 6 and 7 were compared serologically with the original T4r. They were found to be qualitatively and quantitatively identical with T4.

The composition of the phage populations in plaques of the various types was investigated to learn something about the pattern of mutability among the types of this group. This was done by assay of plaques of the various types described in table 4, followed by classification of at least 100 plaques from each population. The results are summarized in the last column of table 4. From these it can be seen that all five types are different with respect to the populations of their plaques. Type

That this is, in fact, an important consideration will be clear from subsequent data.

During one-step growth studies, differences in rate of adsorption were noted among these T4 types. To study this in a more precise way, a further experiment was made. It is known from the experiments of T. F. Anderson (*Jour. Bacteriol.*, vol. 55, pp. 637, 651, 659) and of M. Delbriick (*Jour. Bacteriol.*, vol. 56, p. 1) that the adsorption of certain strains of T4 is

TABLE 4
CHARACTERISTICS OF THE *Hqr* UNSTABLE SERIES OF PHAGES

Type no.	Approximate plaque diameter (mm.)*	Appearance of plaque	Cofactor requirement†	24-hour plaque contents‡
1	2-3	Clear halo	None; indole-sensitive	Nos. 1, 2, (5)
2	3.5-5	Turbid halo	Broth constituent; indole-sensitive	Nos. 2, (5, 7)
5	3.5-5	Turbid, speckled halo	Not tested	Nos. 1, 2, 5, 7, (6)
6	3-4	Turbid, scalloped halo	Broth or hydr. casein constituent; indole-sensitive	Nos. 1, 2, 5, 6, 7
7	1-1.5	Very narrow halo	Calcium; indole-resistant	Nos. 1, 2, 5, 7

* Absolute values depend on plating conditions and therefore vary considerably from plate to plate. The relative plaque sizes are nevertheless quite dependable.

† Based on preliminary data only.

‡ Parentheses indicate that these types occurred rarely in the plaques assayed.

no. 2 is relatively stable, in the sense that its plaques contain few phage particles of the other types. No. 1, on the other hand, is unstable, since its plaques invariably contained type no. 2 in addition to type no. 1. The other three types are even less stable than no. 1, since their plaques contain phage particles of several other types. If the comparison were based on the percentage of mutants present, the order of stability would be slightly altered. It should be noted, however, that these data may not reflect relative mutational instability, but rather may indicate differences in relative selective advantage of the various types during growth of the plaque.

markedly influenced by the presence of tryptophane, indole, and calcium. To discover whether the adsorption cofactor problem had any bearing on the present case, a preliminary test was made to see whether cofactors influenced adsorption here. A mixture of two of our T4 types was added to a large excess of bacteria in the presence or absence of the substances to be tested. Measurements were made of the percentages of each of the phage types that were adsorbed. Although the results are insufficient at this time to draw any specific conclusions about the cofactor sensitivity of the various types, it is clear that some of the substances tested do influence

the rates of adsorption of the different phages to different degrees.

All plaque analyses described so far were carried out on plaques incubated 20 to 24 hours at 37° C. In the light of information about the effect of adsorption cofactors, it seemed desirable also to study the younger plaques, where the cofactor condition of the plates had not been subjected to so much metabolic alteration. Plaques of T4 are first visible after 3% to 4 hours' incubation at 37° C. The youngest plaques that can be analyzed, therefore, are 4-hour plaques. The most significant factor in the results is that in all cases the 4-hour plaques contained only an insignificant proportion of mutants as compared with corresponding older plaques. It is furthermore significant that in the assays made on type no. 6 plaques of various ages, the percentage of mutant types is correlated with the age of the plaque. The results seem clearly to indicate that the great variety of phage types in the 20- to 24-hour plaques is in large part due to a selective mechanism operating during the growth of the plaque. Since the titer of the plaque from 8 hours to 20-24 hours increases only three- to fourfold, whereas the percentage of mutants increases more than thirty fold in comparable cases, the actual survival or the selective growth of the mutants must play an important role in their relative accumulation.

One technically useful result of these experiments is that it is now possible to prepare stocks of the various types with a

relatively high degree of purity, by infecting a bacterial culture with a 4-hour plaque rather than with an older one. In this way stocks of no. 6 with less than 0.2 per cent mutants have been prepared. It is hoped that these types will now become useful genetic markers for more advanced experiments.

Before the effect of selection among these phage types was discovered, the great variety of types arising from one phage particle in a single plaque seemed best explained by extreme mutability at certain genetic sites in the phage particle. To discover whether the observed mutability is due to instability in specific loci, or whether the hereditary material of these phages is generally unstable, tests were conducted to see whether hereditary antigenic alterations might also occur. The technique was to inactivate aliquots of a stock of type no. 6 with anti-T4 serum to a survival of 10^{-2} to 10^{-6} . Stocks were made from plaques resulting from the surviving phage particles. The rates of serological inactivation of these stocks were compared with the rate of inactivation of the original type no. 6. No significant differences were found in testing fifteen stocks derived in this way. These results suggest that the antigenic properties of these phages must be quite stable, or else that changes become manifest only after the accumulation of many changes, each of which is too small to be detected by these tests.

ORGANIZATION OF THE CHROMOSOME

B. P. KAUFMANN, M. R. MCDONALD, H. GAY, N. C. OKUDA, J. M. PENNOYER, AND S. BLOWNEY

In our approach to problems of the nature and specificity of action of genes in higher organisms, we have continued to focus attention on details of chromosome organization. These studies, originally

formulated at the level of descriptive cytology, have in recent years been supplemented by experimental procedures involving ionizing radiations and chemical mutagens—used independently and in

combination with nonmutagenic agents, such as near infrared radiation—and purified enzymes. All these approaches have been employed in our studies during the past year, primary emphasis being placed on the cytochemical methods. Progress of the work has been facilitated by a research grant from the Division of Research Grants and Fellowships of the National Institutes of Health, U. S. Public Health Service.

CYTOCHEMICAL STUDIES

The cytochemical studies initiated three years ago (Year Book No. 45, 1945-1946) were projected on the assumption that precise information concerning fundamental patterns of cellular organization could be obtained by using purified enzymes in combination with various staining procedures. Since our interest in the gene focused attention on the nucleic acids and proteins, it was necessary to accumulate a stock pile of purified nucleases and proteases; and this requirement in turn led to the development of methods for the elimination of proteolytic contaminants from crystalline ribonuclease (Year Book No. 46). About a year ago Dr. M. Kunitz, of the Rockefeller Institute for Medical Research, published a method for preparing crystalline desoxyribonuclease; and application of his method has enabled us to obtain preparations of this enzyme that are free of measurable traces of proteolytic contaminants. These two nucleases, together with the proteases, trypsin, chymotrypsin, and pepsin, constitute the group of purified enzymes used in our cytochemical studies.

In employing the enzymes cytochemically it is also essential to maintain an extensive series of controls to determine the influence of all variables capable of influencing their hydrolytic activity or interfering with the staining reactions (Year

Books Nos. 46, 47). Only by maintenance of such controls has it been possible to attribute the results obtained to the specific action of the enzyme molecules on substrate molecules. The extreme care required is illustrated by an analysis of the factors involved in the process of dissolution of cells that follows their digestion in trypsin.

The course of tryptic digestion. It has been reported by a series of observers that either crude or purified preparations of trypsin degrade proteins and lead to disintegration of the cell. This type of result is readily demonstrable, as is shown in plate IA. This section of a root tip of onion was treated with trypsin in 0.05 M phosphate buffer, pH 6, for one hour. No such dissolution occurred in the cells of an adjoining section of this root tip that served as a control and was treated only in the buffer. Because of previous experience with the complicating action of buffers in cytochemical reactions (see Year Book No. 46), it seemed essential in an analysis of the course of digestion by trypsin to determine to what extent cellular dissolution was referable to the presence of electrolytes rather than to the primary action of the enzyme in disrupting peptide linkages.

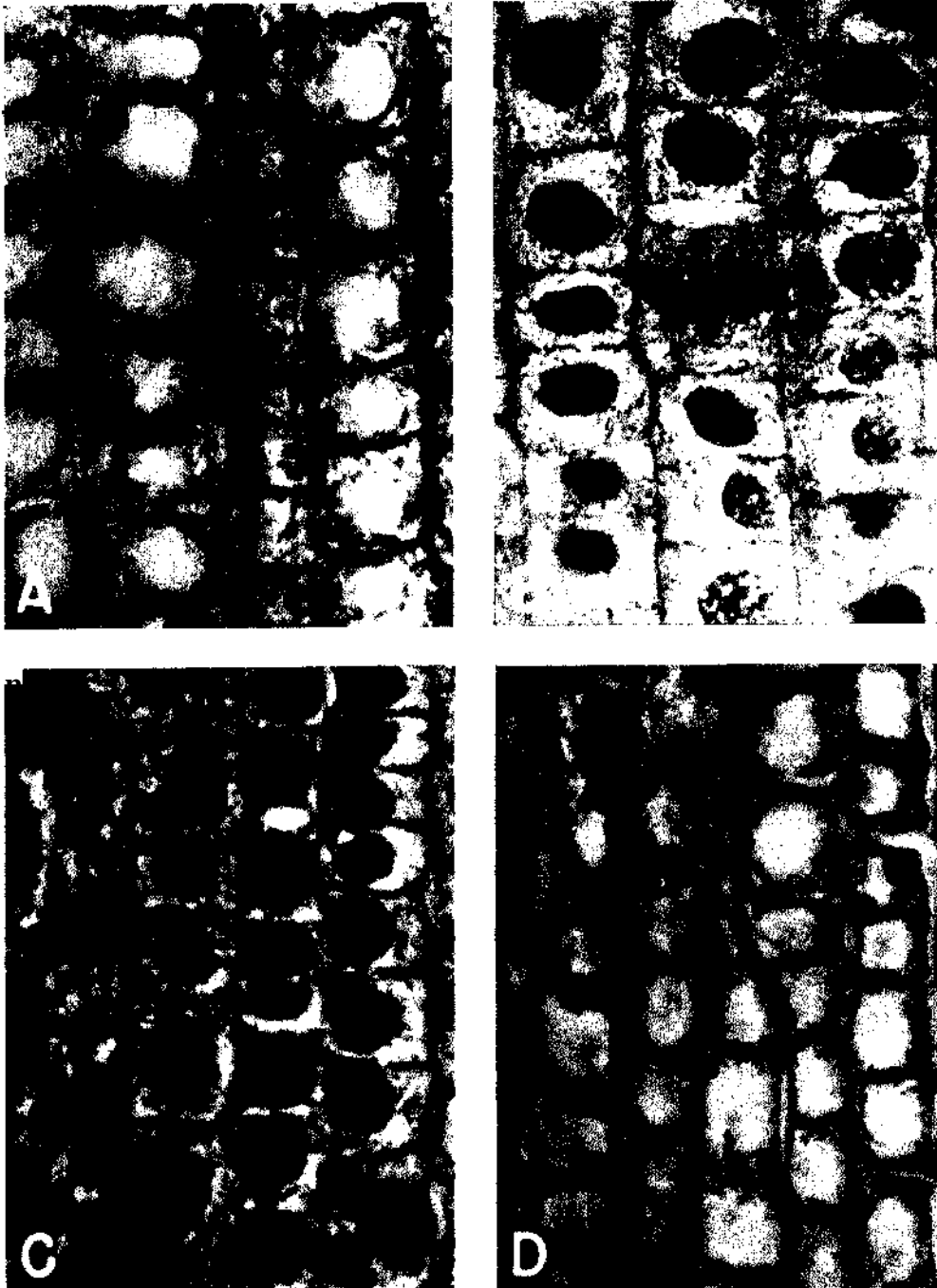
As a first step, it was necessary to determine whether trypsin causes cellular dissolution if used in the absence of electrolytes except for the traces necessary to adjust the pH. It was found that treatment of acetic-alcohol-fixed smears or sections for long periods of time with aqueous solutions of trypsin at pH 6 does not lead to cellular disintegration (pi. iB). That trypsin is active in such solutions is suggested by increase in the stainability of the treated cells with pyronin and other basic dyes. One possible explanation of this increase is that pyronin-stainable nucleic acid is released by tryptic degradation of ribonucleo-

protein (Year Book No. 47). Aqueous solutions of trypsin also reduce the stainability with acidic dyes of the protein released by the action of ribonuclease on ribonucleoprotein (cf. Year Book No. 47). Additional evidence that aqueous solutions of trypsin are not impotent was provided by experiments in which treatment with such solutions was followed, after thorough rinsing, by treatment with 0.05 M phosphate buffer or by 0.04-0.10 M sodium chloride. Dissolution of cellular contents occurred rapidly under these conditions. Obviously the electrolytes play a decisive role in the process; but since treatment with buffers or salt was followed in all cases by prolonged washing in water, further experiments were required to determine the dependence of dissolution on removal of water-soluble materials. Accordingly, sections of root tips were treated with aqueous trypsin, then with phosphate buffer. If they were then transferred to 0.1 M hydrochloric acid before rinsing in water, the contents of the cells were preserved without apparent distortion (pi. iC); but if they were rinsed in water before the treatment with hydrochloric acid, the cellular contents were not detectable (pi. iD).

This phenomenon is strikingly demonstrated by using the phase-contrast microscope, which permits direct observation of gross alterations as they are induced by the various reagents. Immersion in aqueous trypsin (0.1 mg. per milliliter at pH 6 for 1 hour) does not effect any discernible structural alteration in salivary-gland chromosomes of *Chironomus* larvae (blood-worms). Even after prolonged washing in wafer, the precise pattern of banding is clearly defined*. Upon subsequent addition of buffer or sodium chloride solution (in the concentrations indicated above), there occurs an immediate swelling of the chromosomes and separation of the bands

into their component chromomeres. If, at this stage, aceto-orcein is added, the chromosomes contract, and their bands once more become clearly defined and assume the purple color of the orcein stain. If, on the other hand, rinsing in water follows treatment with the buffer, the swelling continues until adjacent chromosomes adhere and their contents become disorganized. Continued washing leads to complete dissolution of cellular materials. It is apparent from this series of observations that the action of trypsin *per se* does not lead to the type of disintegration of substrate materials that has been attributed by other workers to specific disruption of peptide linkages. This in turn suggests a re-examination of the widely accepted concept that protein alone serves to maintain the form and structure of the chromosome. Aside from this application to problems of immediate importance in our cytochemical studies, these observations suggest a method of attack on more general problems of enzymatic digestion by resolving the process into its component phases.

Enzymatic dissection of the chromosome. Recent investigations, including those conducted in this laboratory (Year Books Nos. 46, 47), have indicated that the chromosomes of higher organisms contain histone and nonhistone proteins, and ribose and desoxyribose nucleic acids. Our cytochemical studies have indicated that ribonucleic acid is separable by enzymatic hydrolysis from a tryptophane-containing protein (Year Book No. 47); and chemical studies by various workers have shown that desoxyribonucleic acid is extractable from the chromosome in association with histone. Use of purified nucleases and proteases, independently and in combination with each other, or with various chemical procedures, should provide further information about patterns of association of nucleic acids and proteins as they exist separately and in



Tryptic digestion of onion root-tip sections. 7-micron paraffin sections of root tip of onion, fixed in acetic-alcohol, stained in methyl green-pyronin. X 860.

- A. Nuclear disintegration as produced by a solution of trypsin in buffer.
- B. Cells visibly intact after treatment with aqueous solution of trypsin.
- C. Retention of gross nuclear structure after successive treatments in aqueous solution of **trypsin**, in phosphate buffer, and in 0.1 M HCL
- D. Nuclear disintegration produced by successive treatments in aqueous solution of trypsin, phosphate **buffer**, water, and 0.1 M HCL



Pepsin digestion following removal of nucleic acids. 7-micron paraffin sections of root tip of onion, fixed in acetic-alcohol, stained in fast green. x860.

F. Extraction of all nucleic acids by treatment with 0.3 N trichloroacetic acid for 15 minutes at 100° C.

F. This section treated as F, followed by pepsin digestion.

G. Degradation of nucleic acids by successive treatments with ribonuclease and deoxyribonuclease.

H. This section treated as F, followed by pepsin digestion.

combination within the fixed cell, and should also indicate whether or not their presence is essential for maintenance of structural integrity. It must be kept in mind that cellular materials are preserved by cytological fixatives without gross distortion of the spatial relations that exist in the living cell, and thereby provide a useful basis for initial studies of the chemical organization of the living system.

Trypsin presumably attacks peptide linkages involving basic amino acids that are present in both histones and nonhistone proteins. Chymotrypsin also seems to attack linkages common to both types of proteins, since the action of this enzyme in disrupting cellular organization has been found to parallel that reported above for trypsin. Differences that we have detected in potency and rate of action of the two enzymes promise to furnish information about the specific action of each on histones and other proteins. The evidence now available indicates that disruption of linkages in either type of protein by either of these enzymes (in concentrations as high as 1 mg. per ml.) does not destroy structural continuity of the chromosome unless treatment with the enzyme is supplemented by treatment with a solution containing electrolytes. The importance of different electrolytes in this process is now under investigation.

Pepsin is another proteolytic enzyme that has been used extensively in cytochemical studies. It effects a marked shrinkage of chromosomes without effacing their pattern of structural organization. Using the phase-contrast microscope, we have observed that pepsin in hydrochloric acid (3 mg. per ml. in 0.02 M HCl at 37° C.) will reduce salivary-gland chromosomes of *Drosophila* in 2 hours to about one-tenth of their original volume. On the basis of our studies and those of Mazia and his associates, it appears that the action of pep-

sin on fixed preparations consists primarily in degradation of the nonhistone, or tryptophane-containing, protein. If so, this substance represents a much higher proportion of the chromosome than would be expected from analysis of chemically isolated materials. From the standpoint of structural continuity, the histones and the nucleic acids surviving peptic digestion constitute a clearly defined although markedly shrunken chromosome.

Nucleases—that is, enzymes that degrade nucleic acids—also serve to reveal patterns of structural organization. Ribonuclease reduces stainability of ribonucleic acids with basic dyes, and also degrades ribonucleoprotein to release a protein that stains with acidic dyes (Year Book No. 47). Such treatment does not destroy chromosome structure; nor is it destroyed if treatment with ribonuclease is followed by treatment with pepsin.

Desoxyribonuclease eliminates stainability of chromosomes by the Feulgen method, indicating that this enzyme degrades desoxyribonucleic acid. The structure of the chromosome is not impaired thereby, since it is clearly defined when stained with acidic dyes. Structural continuity is also maintained if all nucleic acid is removed by consecutive treatments with ribonuclease and desoxyribonuclease, or by extraction with trichloroacetic acid at 90° C. (pi. 2G, E). If, however, treatment with pepsin follows either trichloroacetic acid extraction (pi. 2F) or treatment with ribonuclease and desoxyribonuclease, or even treatment with desoxyribonuclease alone (pi. 2H), dissolution of nuclear contents ensues. These results might seem to suggest the existence in the chromosome of two separable complexes, one ribonucleoprotein, the other desoxynucleohistone, either of which is capable in itself of maintaining the integrity of the chromosome. As was indicated in the experi-

ments using hot trichloroacetic acid or the nucleases in succession, however, both complexes may be degraded without effacing the chromosome.

The obvious conclusion from this series of experiments is that the proteins form an interrelated system in the chromosome, and that the nucleic acids are intimately linked with the proteins and perhaps with each other. On the basis of evidence now available, no single protein or nucleic acid may be regarded as the fundamental structural component of the chromosome.

RADIATION STUDIES

Our pioneering studies with near infrared radiation led to the discovery that wave lengths centering around $i p$ can modify the frequency of X-ray-induced chromosomal rearrangements, but not of gene mutations (Year Books Nos. 44-47). With the prospect that near infrared radiation might serve, when applied to organisms in which cytological analysis is not feasible, to distinguish between induced genetic changes that are attributable to chromosomal alterations and those that are not, a series of experiments was undertaken to determine the effects of near infrared rays and X-rays, used independently and in combination, on bacterial cells. In efforts to determine the nature of the "sensitizing" action of near infrared radiation, experiments were also initiated using systems less complex chemically than those of the living cell, namely, solutions of purified crystalline enzymes.

Response of the bacterium Escherichia coli to X-rays and near infrared rays. A series of studies was carried out, with the assistance of Miss Helen Cuneo, to compare the rates of mutation of the B/r strain of *E. coli* from the normal bacteriophage-sensitive condition to a phage-resistant state after treatment with X-rays

alone and after treatment with X-rays preceded by near infrared rays. As a prerequisite to this comparison it was necessary to determine the rate of survival of bacterial cells treated either with near infrared rays or with X-rays. Exposure to near infrared radiation (for periods of either 3 hours or 24 hours at 25° C.) of a culture of *E. coli* in a medium inadequate to permit growth (the solution of salts used in the M-9 medium) did not significantly alter the number of living cells or affect the proportion of mutants as compared with those of a control culture maintained in ordinary light at the same temperature. It thus appears that near infrared radiation alone has no lethal action and no mutagenic potency.

Treatment with X-rays kills bacteria in such proportions that exponential survival curves are obtained. It was found in a series of experiments that essentially a straight-line relationship was obtained when the logarithm of the surviving fraction of bacteria was plotted against the dose of X-rays, but that the slope of these survival curves varied greatly from experiment to experiment. When inocula from the same slant were grown under identical conditions for 48 hours, centrifuged to provide cultures containing approximately 10^{10} bacteria per milliliter, and irradiated under identical conditions at the same intensity, the inactivation doses of the separate cultures (amounts of radiation required to reduce the survivors to 36.8 per cent of the initial number) ranged between 8000 and 21,000 roentgen units. The basis of this variability remains to be determined, but apparently involves biological factors as well as those attributable to dosimetry and technical procedures.

Failure to establish a uniform survival curve has complicated the process of determination of mutation rates among the bacteria surviving X-ray treatment. Only

by assay over a wide range of dosage levels in any one experiment has it been possible to secure the required information. Two methods of measuring mutation rate were employed, namely, the zero-point technique developed by Demerec and Latarjet, and the liquid-culture, end-point technique developed by Newcombe (Year Books Nos. 45, 47). The results obtained by Demerec and Latarjet suggested that mutation rate is proportional to X-ray dosage. The results obtained in our studies were too variable to provide significant statistical verification of this interpretation; in some experiments the mutation frequency was significantly higher than in untreated controls, whereas in others it was not.

The mutation rates in the bacterial populations treated with near infrared rays plus X-rays and in those treated with X-rays alone were not appreciably different in paired experiments. Again, there was considerable variability from experiment to experiment, and more data must be amassed to permit reliable statistical analysis. Inferences concerning the relation of X-ray-induced mutants to chromosomal aberrations in *E. coli* cannot be made until such information is obtained.

The effect of X-radiation on dilute solutions of crystalline trypsin. It has been known for many years that the activity of enzymes can be destroyed by X-radiation. Large doses were necessary, however, for such destruction, and it was therefore generally believed that enzymes were too insensitive to radiation to account for the radiation effects noted in the living cell. In 1940, however, W. M. Dale found that "the effect of X-rays on aqueous solutions of enzymes and of other biologically active compounds depends on the concentration and purity of such solutions, and that doses of as little as 50 roentgens can produce marked effects when concentrations of the order of those occurring in living

cells are irradiated." Work in several other laboratories has since confirmed these general conclusions. Dale postulated that enzyme molecules are affected by the ionizing radiation, not directly, but indirectly through collision with a labile product resulting from ionization of the water. Very little is known about the action of infrared radiation on enzymes. It has been reported as destroying urease, stimulating starch amylase, and having no apparent effect on pepsin. Unfortunately, the experiments on which these reports were based were done with impure enzyme preparations. In our work we have used purified enzymes prepared in our laboratory. The work with near infrared radiation is still too preliminary to warrant even tentative conclusions. We shall therefore report at this time only the results obtained with X-rays on solutions of crystalline trypsin.

Crystalline trypsin was chosen for the original studies for several reasons. In the first place, it can be accurately assayed, permitting precise reproducibility of results. It is stable at pH 2.4, thus eliminating any complicating secondary effects due to spontaneous inactivation. Finally, it appeared to be a suitable enzyme for comparison with carboxypeptidase, the only purified enzyme on which extensive quantitative data were already available, because both enzymes are primarily peptidases, their source is beef pancreas, and their molecular weights are similar (trypsin = 36,500; carboxypeptidase = 35,000).

The effect of X-radiation on solutions of crystalline trypsin in 0.005 N hydrochloric acid has been determined under a variety of conditions, such as varying the radiation dosage while the concentration of trypsin remains constant, varying the concentration of trypsin while the dosage remains constant, and varying both the concentration of trypsin and the dosage, the ratio

of the two being constant. Inactivation has been observed with doses as low as 100 roentgens. As has generally been found for proteins, however, the greater the initial concentration of trypsin, the larger is the dose of radiation necessary for comparable percentages of inactivation. For any given initial concentration of trypsin, the decrease in tryptic activity with increasing amounts of radiation is exponential. It is therefore essential, when comparing various experiments, to do so at a constant level of inactivation. For convenience both of calculation and of comparison with other published data, this level has been taken as 63.2 per cent, the amount of radiation necessary to produce this percentage of inactivation being known as the "inactivation" dose. Calculations of "inactivation" doses show that they increase with increasing initial concentrations of trypsin, but that the increase is not directly proportional in the range of trypsin concentrations so far studied (1.2-344 $\mu\text{g. per ml.}$). This means that in this range of concentrations the ionic yield—i.e., the number of molecules destroyed per ion pair—is not constant. For example, under otherwise identical conditions, the ionic yield for a 9×10^{-8} molar solution of trypsin (3.29 $\mu\text{g. per ml.}$) was found to be 0.0074, whereas for a 9.4×10^{-6} molar solution (344 $\mu\text{g. per ml.}$) it was 0.0376. Drops in ionic yields at low solute concentrations have been noted in other laboratories in studies of the effects of X-rays on oxalic acid, methyl alcohol, glutathione, tobacco mosaic virus, and rabbit papilloma virus. Such results would be expected, according to the "indirect action theory"¹ of the effects of radiation, if the solute concentration is so low that an appreciable proportion of the total number of "active radicals"¹ combine with one another rather than react with the solute molecules. Absolute ionic yields therefore

cannot be stated for radiation effects in very dilute solutions, unless one has ascertained by experiments over a wide range of solute concentrations that they are constant or can be extrapolated from the data. Unfortunately, this has not generally been done, and many of the values for ionic yields given in the literature may therefore be misleading. Barron *et al.*, for example, state that the ionic yield for the inactivation of trypsin by X-rays is 0.025. This value was determined, however, from one experiment at one concentration (50 $\mu\text{g. per ml.}$) and can apply only to that specific case.

Dale noted in his original studies (1940-1943) on the inactivation of carboxypeptidase by X-radiation that, over a wide range of dosage (50 to 400,000 r) and enzyme concentrations (calculated by us from his data to be approximately 1.6 to 540 $\mu\text{g. per ml.}$), the number of enzyme units inactivated by a given number of roentgens and for a given percentage of inactivation was constant. As noted above, in experiments using a similar range of enzyme concentrations (1.2 to 344 $\mu\text{g. per ml.}$) and of X-ray dosage (100 to 100,000 r) no constant value was found for trypsin. This indicated a marked difference between the two enzymes. Since our experiments were completed, however, Dale and his co-workers (1949) have published extended data covering an extremely wide range of carboxypeptidase concentrations, namely from 5 to 150,000 $\mu\text{g. per ml.}$ Here they report that for concentrations below approximately 200 $\mu\text{g. per ml.}$ the ionic yield decreases markedly with decreasing concentrations, although at higher concentrations it is approximately constant and equal to 0.18 enzyme molecule inactivated per ion pair. They obtained similar results with alloxazine adenine **dinucleotide**.

X-rayed 0.005 N hydrochloric acid was found to have no effect on the activity of

solutions of crystalline trypsin. A solution of trypsin, dissolved in hydrochloric acid that had received 35,000 roentgens immediately before use, when assayed after 4 hours at 25° C. had 623 X io⁶ trypsin units (denatured hemoglobin); whereas control solutions in hydrochloric acid that had not been X-rayed had 616 X io⁶ units. Such a solution of trypsin would have lost 84 per cent of its activity had it received this amount of X-radiation directly.

There appears to be no delayed effect, such as R. S. Anderson found with pepsin, nor is there any spontaneous recovery either in the light or in the dark. For example, a solution of trypsin (33 ug. per ml.) that received 18,000 r had, when assayed immediately after radiation, 285 X io⁶ activity units. After 1, 5, and 24 hours at 0° C. in light and in dark, the assays were 285, 285, and 283 X io⁶ units, respectively, for light, and 285, 285, and 286 X io⁶ units for dark.

The rate of inactivation of crystalline trypsin by X-radiation was found to be independent of the intensity at which the radiation was delivered. Inactivation doses for a solution of crystalline trypsin containing 30.4 µg. per ml. were 21,500, 21,200, 20,750, and 21,500 r with rates of delivery of 581, 264, 180, and 129 r per minute, respectively.

All the experiments reported thus far have been carried out at pH 2.4, the point of maximum stability for trypsin. Preliminary experiments indicate that the hydrogen ion concentration of the solution being irradiated affects the amount of inactivation. As yet, however, the optimum pH for X-ray inactivation of trypsin has not been established.

The nature of the change in the trypsin molecule responsible for the loss of activity brought about by irradiation is unknown. It is hoped that an extension of these data

to include trypsinogen, the precursor of trypsin, may shed some light on this problem, as well as on the even more basic problem of what group or groups in the trypsin molecule are responsible for its specific action.

Studies combining treatments with near infrared radiation and nitrogen mustard. As reported in preliminary form in Year Book No. 47, near infrared radiation, when used before nitrogen mustard in treatment of males of *Drosophila melanogaster*, effects an increase over the nitrogen-mustard-treated controls in frequency of chromosomal rearrangements, as determined by genetic tests for translocations between the second and third chromosomes. Posttreatment, on the contrary, does not produce any increase in frequency.

The data obtained in these studies have been subjected during the past year to critical statistical analysis. Since in the pretreatment series five separate groups of flies were exposed to the aerosol of nitrogen mustard, and since each was mated with three groups of females at 6-day intervals, fifteen samples were available for comparison between the infrared-treated flies and their controls. Disproportionate frequencies, and the interaction of the five separate treatments and the three transfers, posed a statistical problem that was solved, at the suggestion of Dr. A. E. Brandt of the Atomic Energy Commission, by use of the method of partitioning of chi square. The analysis indicates that the differences between the separate treatments and the different transfers are significant. The least-square lines derived from the* total data are shown in figure 3. These lines have essentially the same slope. Analysis reveals that the percentages of translocations represented in the experimental and control regression lines differ significantly from each other. The data thus indicate

that pretreatment with near infrared radiation effects an increase of about 50 per cent in the frequency of mustard-induced chromosomal translocations; and this increase corresponds closely to the value obtained in earlier experiments in which near infrared pretreatment was used with X-rays.

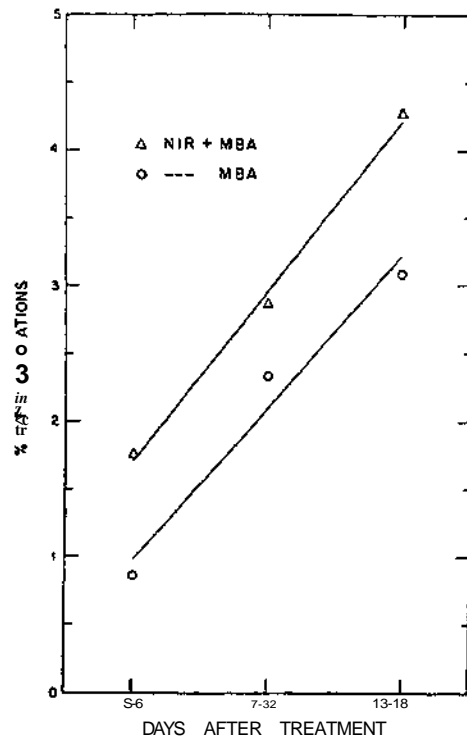


FIG. 3. Least-square lines, for pretreatment series and its controls, derived from frequencies of nitrogen mustard-induced translocations between the second and third chromosomes of *Drosophila melanogaster*. Upper line, near infrared radiation followed by nitrogen mustard; lower line, nitrogen mustard control.

Comparable statistical analysis applied to the posttreatment data indicates that the differences between successive transfers are significant, but that near infrared radiation does not significantly modify the frequency of translocations when it is used after the

nitrogen mustard. This result also agrees with that obtained in the earlier experiments in which near infrared radiation was used after X-rays.

An effort was next made to determine whether the increased frequency of translocations detected in successive transfers was due to a cumulative action of the chemical on spermatozoa that were mature at the time of treatment, or to an effect on cells that were in earlier stages of develop-

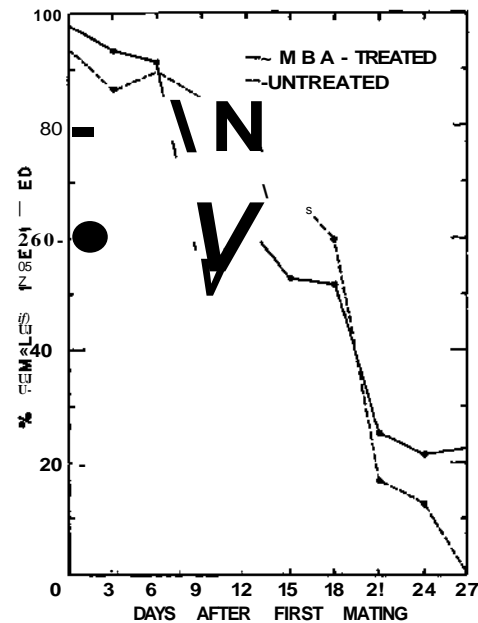


FIG. 4. Proportion of available females inseminated by nitrogen mustard-treated and untreated males at successive three-day intervals after the first mating.

ment at that time. Preliminary experiments were undertaken to determine the length of time after treatment that males remain fertile, as indicated by their capacity to inseminate females and by the progress of spermatogenesis. These studies showed that spermatogenesis is not necessarily terminated by exposure of males to mutagenically potent doses of nitrogen mustard. Cytological examination of sec-

tions of testes dissected from sexually active and inactive males throughout a 30-day period revealed dividing spermatogonia and spermatocytes and an abundance of mature spermatozoa in both. The proportion of available females inseminated by treated males is shown in figure 4. In obtaining these data, each male was mated with 3 virgin females immediately after treatment, and at the end of each 3 days thereafter. Males of the same cultures that had not been exposed to the aerosol of nitrogen mustard served as controls. A comparison of the two curves shown in figure 4 suggests that exposure to nitrogen mustard may temporarily impair sexual

activity, but that recovery occurs in a high proportion of treated males.

Additional work will be required, however, to answer the question whether the higher frequency of translocations detected in later transfers is due primarily to an effect of the chemical on mature germ cells or on immature germ cells. In the latter event, the possibility must be examined that the mustard-induced rearrangements arise in large part as chromosomal mosaics, and that the higher frequency observed in later transfers is due to the facilitation of their detection by the experimental procedure used. Experiments designed to answer this question have been initiated.

MOUSE LEUKEMIA

E. C. MACDOWELL, M. J. TAYLOR, AND L. E. LEWIS

Increasing potency of an invading pathogenic agent or transplanted tumor has frequently been observed to be closely connected with a change in the range of susceptible hosts. Thus the increase in virulence of line-I leukemia seemed to provide a valid reason for the apparent failure of the natural resistance of mice from foreign strain StoLL. During its first hundred passages, line I failed to kill any of its hosts from this strain, but by transfer 147 it was killing a large proportion of these mice.

That the line-I leukemic cells proliferated in these StoLi hosts, and were successfully carried in mice of this strain for a series of 14 successive transfers, seemed ample evidence that leukemia was the cause of death. The difference in genetic constitution between the hosts of this strain and the strain of origin provided a reasonable interpretation of the observed modifications in metabolism of the invaded lymph nodes and in cytological details of the invading cells; for upon subsequent transfer to hosts of the original strain, all the specific characteristics of line-I leu-

kemia, including metabolic rates and cytological details, reappeared. When these interpretations were proposed, the possible association of a virus with line-I leukemia was not even imagined. The two previous reports have recorded the isolation of a virus from line I and the freeing of line I from the virus, which now appears almost certainly to have been transplanted along with the leukemic cells for ten or more years.

Work of the current year with line I, freed from the virus, shows that the virus and not the increased virulence of the leukemic cells was responsible for the death of the StoLi hosts. With the virus eliminated, every StoLi (91) inoculated with line-I leukemic cells has survived, as during the earliest period of this project. But these mice are highly susceptible to the virus, which induces a more violent sickness than in C58 mice and kills every mouse. Thus, strain StoLi is killed by the virus and not by line I, and strain C58 is killed by line I but not by the virus, although an initial proliferation of the re-

spective pathogens occurs in the survivors in each case.

At the time StoLi mice were dying after inoculation with line I, the ratio of deaths in mice of the backcross generation, (C58 X StoLi) X StoLi, rose from the previous fully confirmed Mendelian 1:1 to 4:1. This year, mice (120) of this same backcross generation, when inoculated with line I free from virus, but with virulence as great as ever, once more gave the classic 1:1 ratio (50.8 per cent survived). This ratio is consistently indicated when the data are subdivided according to (1) the four types of matings (reciprocal Fi's, reciprocally backcrossed to StoLi), (2) three pairs of color genes, and (3) sex. Thus it appears that the natural resistance of StoLi to line-I leukemia, and the primary dependence of this resistance upon a recessive gene, are once again demonstrable; and it is highly probable that at no time was there a failure of this resistance.

The discovery that an unsuspected virus was responsible for changing the proportion of Hne-I survivors in a foreign strain and in a backcross does not forthwith demonstrate that all the changes in such proportions that we have observed within other lines in the course of many transfers, and all the differences between lines according to these criteria, can be explained by the intermediation of a virus. For instance, line L, which arose in strain 89 and was long carried by hosts of this strain, killed only 1 per cent of the 92 mice of strain C58 tested in the first 15 transfers, whereas the hosts of strain 89 were all susceptible throughout. With the line-L cells carried exclusively by strain-89 hosts, the proportion of susceptible C58 mice rose by degrees to 95 per cent in the course of 60 successive transfers. Considerably later, line L was found to kill 100 per cent of C58 mice, and for the last 480 transfers

this line of leukemic cells has been routinely carried by C58 mice which have maintained 100 per cent susceptibility.

We have previously reported that line L was found to be carrying a virus, and that the leukemic cells of this line had been freed of this virus. Contrary to the situation found with line I in strain StoLi, however, the elimination of the virus from line L has left the 100 per cent susceptibility of strain C58 unchanged. In this case, changes in the leukemic cells, and not the presence of a virus, were responsible for the changes in the susceptibility of strain C58.

Furthermore, lines E and H after 90-odd transfers in the hosts of the strain of origin (C58) gave susceptibility ratios, in mice of the above-mentioned backcross, that approached 1:1, thus resembling the virus-free line I. Earlier in their histories these two lines showed only 10 to 15 per cent susceptibility in the backcross.

However indicative these previous observations might be, the point seemed of sufficient importance to make a backcross test of susceptibility to leukemic cells that had passed through only a single transfer after removal from a spontaneous case of leukemia. Accordingly, a group of 102 mice of this same backcross were thus inoculated. In the case of line I, the susceptible backcross mice died at the same time and showed the same autopsy picture as the C58 controls; in this case, the intervals before death were so continuously variable and so often greatly prolonged beyond the C58 controls that no sharp line could be drawn between susceptible mice and survivors. Of the C58 controls, 14 died after 13 to 17 days and one after 45 days; of the backcross hosts, 2 died before the 20th day, and the following numbers in successive 10-day periods: 1, 3, 2, 1, 6. At 72 days, observations were discontinued and the remaining mice killed; at this

time the autopsies gave definite evidence of leukemic infiltration in 4 and probable initial stages in 2 others. Although longer observation might have added cases still more delayed, there was no approach to a 1:1 ratio of resistant to susceptible animals, and the contrast with line-I leukemia seemed sufficiently well established. Various degrees of resistance were indicated not only by the intervals before death, but also by the distribution of lesions in the backcross animals. Only 4 of these showed the general enlargements of spleen and nodes found consistently in the C58 controls. In the great majority of cases the backcross autopsies revealed a strong but highly variable concentration of leukemic infiltration in deep lymph nodes and organs (notably the ovary), whereas the superficial nodes and spleen remained relatively small. Obviously, these leukemic cells are resisted in varying degrees by most of the 50 per cent of the backcross mice that are susceptible to line-I leukemia by virtue of their genetic constitution; and it may be concluded that in regard to host susceptibility and resistance these leukemic cells, in their second passage from a spontaneous case, are intrinsically different from cells of line I.

A MILK FACTOR RESISTING SPONTANEOUS LEUKEMIA AND LENGTHENING LIFE

It has been confirmed this year that a resistance factor from mothers of the non-leukemic strain StoLi, rather than a leukemia-inducing factor from mothers of the leukemic strain C58, is responsible for the differing incidence of spontaneous leukemia among animals from reciprocal matings between these two strains. This resistance is transmitted to their hybrid young by mothers over 34 weeks old, but not by young mothers 10 to 18 weeks old. Year Book No. 46 (1946-1947) reported

preliminary results of the experiment designed to verify this mother's-age effect, which had first been recognized in an earlier experiment. The final results show that, with all fathers from the leukemic strain and each one mated to both young and old StoLi mothers, the incidence of leukemia was unquestionably lower in the hybrids from old mothers than in the hybrids from young mothers, and the lives of both leukemics and nonleukemics were lengthened. With young mothers, the final incidence of leukemia, after questionable cases were checked microscopically, was 82.6 per cent of 75 mice; with old mothers, it was 56.8 per cent of 88 mice. The young-mothers group lived from 309 to 891 days (average 595.7 days), with 32 per cent dying between 550 and 650 days; the old-mothers group lived from 546 to 1107 days (average 817.5 days), with 34 per cent dying between 850 and 950 days. The reduction in the incidence of leukemia was direct, and not secondary to the effect on longevity; for longer lives were associated with less rather than more leukemia, and the mother's-age effect on longevity was as certainly manifested by the non-leukemics as by the leukemics.

In the above experiment each mother nursed her own young, but in the next experiment, set up in 1947 (Year Book No. 46), with the same two groups of hybrids from young and old StoLi mothers, some of each group were nursed by their own mothers and some by mothers of the other age group. Although final results are not expected for another year or more, the observations up to date are highly important and significant, leaving little doubt of the validity of the general conclusion that the resistance factor from old mothers can be transmitted by nursing alone quite as effectively as before birth. The control groups from young mothers with young nurses, and old mothers with old nurses,

have closely repeated, up through the 650-day class, the curves of cumulative leukemic incidence by 50-day classes given by the earlier experiments, the gross diagnoses up to date indicating leukemia in 60.8 per cent of the entire group of 120 hybrids from young mothers and young nurses, and in 13.1 per cent of the group of 84 hybrids from old mothers and old nurses. The curve for the critical group, mice from young mothers and old nurses, almost exactly equals that for the old mothers-old nurses group, at 650 days showing leukemia in 11.0 per cent of the group of 109. In terms of those that have already died, the young-young group has yielded 81.6 per cent leukemia, and the young-old group 50 per cent leukemia; these figures are very close to the expected final inci-

dences. The difference in longevity is indicated by death, up to date, of 83.3 per cent of the young-young group against 41.3 per cent of the young-old group.

However real, the resistance to leukemia that depends on the advanced age of the StoLi mother or nurse is clearly not highly potent, in that the incidence is not reduced by even one-half. Yet the discovery of the mechanism by which this moderate reduction in incidence is brought about may well lead to further understanding, if not control, of the manifestation of an inherited tendency to leukemia. On the other hand, the discovery of the mechanism responsible for lengthening life to the extent of $\frac{1}{2}$ per cent by a factor in old nurses' milk would have considerably broader implications.

GENIC ACTION

E. CASPARI AND H. C. DALTON

Analysis of the action mechanism of individual genes has been continued. Besides the genes studied previously—that is, *a* (red eyes) and *wa* (white eyes) in the moth *Ephestia*, and *T*, *Ki*, and *Fu* (tail malformations) in the mouse—the gene $\&$ (white color) in the Mexican axolotl was included in our studies. In addition to the genetic and biochemical methods used previously, the embryological methods of explantation and transplantation were employed; and immunological methods were successfully used for the identification of genetic differences.

We wish to thank Miss Louise Pool for her valuable help in carrying out these investigations. During the summer we were assisted by Misses Louise H. Earle and Barbara C. Wolff and Mr. Noel H. Miller.

MATERNAL EFFECT OF *FU*

Fu in the mouse is a dominant gene with variable expression. As described

previously (Year Book No. 47, 1947-1948), the number of phenotypically Fused animals is lower in crosses of *Fu*/ $+$ females by $+/+$ males than in the reciprocal cross. Evidence seemed to indicate that this phenomenon might be due to the existence in different proportions of *Fu*/ $+$ animals that were phenotypically normal. This hypothesis was investigated by individual test matings of normal animals from crosses involving *Fu*.

The *Fu* strain had previously been outcrossed for 20 to 22 generations to a normal, highly inbred Bagg albino strain. Normal-tailed progeny from crosses of *Fu*/ $+$ females by Bagg albino males and Bagg albino females by $F\ll/f$ males were outcrossed to Bagg albino animals. The offspring were observed at birth and discarded, except in unusual or doubtful cases. Animals giving one or more offspring with definitely abnormal tails were counted as genetically *Fu*/ $+$, whereas animals that had at least 22 normal and no Fused off-

spring were counted as $+/+$. This limit of significance was chosen because the cross $Fu/+$ female by $-h/4-$ male gives about 30 per cent phenotypically Fused offspring. With this ratio, the probability of obtaining no $Fu/+$ offspring from a $Fu/+$ mother by chance only would be about 0.0001. Actually, in most cases more than 30 offspring were obtained.

The results of these crosses are given in table 5. The expectations given in column 4 were calculated from the number of phenotypically Fused animals obtained

studying the offspring from individual animals. All "normal overlaps" from the cross $Fu/+$ female by $+/+$ male and from the F2 gave a reasonably high number of Fused progeny, similar to that produced by phenotypically Fused heterozygotes. Six of the offspring from the cross $+•/+$ female by $Fu/+$ male, on the other hand, gave highly abnormal ratios (male no. 683: 51 normal, 1 Fused; male no. 678: 42 normal, 3 Fused; male no. 1565: 116 normal, 1 Fused; female no. 642: 43 normal, 1 Fused; female no. 685: 55

TABLE 5

PROGENY TESTS OF NORMAL ANIMALS FROM CROSSES INVOLVING *FU*

Cross	Genetically		Per cent Fused		X^2 (d.f.=1)	P
	Normal	Fused	Observed	Expected		
1. 9 $Fu/+$ X $cf +/+$	60	21	25.9	28.0	0.177	-0.7
2. 9 $+/+$ X $cf Fu/+$	45	11	19.7	10.0	7.48	<0.01
2a. 9 $+/+$ X $<? Fu/+$ (corrected)	51	5	8.9	10.0	0.175	-0.7
3. 9 $Fu/+$ X $<? F11/+$	9	6	40.0	33.8	1.54	-0.2

previously from the respective crosses, on the assumption that the actual numbers of $Fu/+$ and $+•/-$ offspring were equal, and that the deviations from a 1:1 ratio were due only to the occurrence of phenotypically normal $Fu/+$ animals. Table 5 indicates that the offspring from $Fu/+$ female by $+/+$ male crosses and from $Fu/+$ female by $Fu/+$ male crosses fitted this expectation. Progeny from the cross $+/+$ female by $Fu/+$ male included a significantly higher proportion of animals giving Fused offspring than was expected. The difference between the proportions of $Fu/+$ offspring from crosses 1 and 2 is not significant ($\chi^2=0.745$. d.f.=1, $P \wedge 04$).[^]

This discrepancy between result and expectation in progeny from the cross $-f/+$ female by $Fu/-\text{Y}$ male can be resolved by

normal, 4 Fused, all in the same litter; female no. 421: 55 normal, 2 Fused). Ratios of this type have not been obtained from other crosses involving $Fu/+$ animals, either phenotypically normal or Fused. The question arises, therefore, whether these animals actually are genetically $F\ll/+$, or whether they are $+/-f$ animals that occasionally give offspring with kinky tails.

If these animals are geotypically $Fu/+$, they may be assumed to carry modifiers that suppress the penetrance of the gene Fu . In this case, about half of their normal offspring should carry the gene Fu . Since there is no evidence for the existence of such modifiers in the Bagg albino strain, outcrosses to Bagg albinos should result in the appearance of Fused offspring in about half of the crosses. Actually, from 24

normal progeny of animals giving abnormal ratios, 450 normal and no Fused offspring were obtained. Similarly, if animals carrying modifiers leading to the suppression of the Fused character were crossed to *Fu*^{-V} animals, a reduction of the number of Fused offspring would be expected. Males 653 and 1565 were outcrossed to *Fu*⁺ females, and produced 40 normal and 16 Fused progeny. This ratio is not significantly different from that usually obtained from a cross of *Fu*⁺ female by *+/+* male.

The "Fused" offspring from animals giving abnormal ratios cannot be differentiated phenotypically from *Fu*⁺. The expression of the character is good; in one case it was even stronger than is usual in *Fu*⁺ heterozygotes. Two of these animals were reared and bred. Crossed out to normal Bagg albino females, they produced 60 normal and no Fused offspring. Thus they behaved in crosses as genetically *+/+*, and may be regarded as "Fused overlaps," that is, normal animals exhibiting the Fused phenotype.

The parents of these "Fused overlaps," which otherwise had only normal offspring, must therefore be considered as genetically *+/-K*. It may be assumed that the other 4 animals that gave abnormal ratios, whose Fused offspring were not progeny tested, were also genetically normal. If this assumption is accepted, the values in line *la* of table 5 are obtained. With this correction, the agreement between expectation and results is good. Furthermore, the difference between the percentages of genotypically *Fu*⁺ animals from crosses of *Fu*^{-h} females by *-f/+* males and from crosses of *+/+* females by *Fu*⁺ males (lines 1 and *ia*, table 5) is significant ($\chi^2=4.917$, *d.f.*=1, *P*=0.025).

Kinky-tailed animals resembling the Fused phenotype occur occasionally in

normal strains. The question arises, therefore, whether the proportion of "Fused overlaps" is greater in the progeny of normal animals from the cross *+/+* female by *Fu*⁺ male than in the normal strain. Assuming that the animals giving abnormal ratios are normal, and their phenotypically Fused offspring are "overlaps," 10 "Fused overlaps" have been obtained among 1457 offspring (0.686 per cent). The lower fiducial limit at the 1 per cent level for this ratio would be 0.331 per cent. A comparison may best be made with the offspring of normal animals from the cross *Fu*⁺ females by *-f/-b* males. Among 958 progeny of this type no "Fused overlaps" were found. It therefore appears with a high degree of significance that particular conditions exist in normal animals from the cross *-f/+* females by *Fu*⁻ males that occasionally result in the appearance of "Fused overlaps."

The results of these experiments demonstrate that the maternal effect of *Fu* is actually due to differential overlapping. *Fu*⁺ mothers influence Fused ova or embryos in such a way as to decrease the penetrance of the gene *Fu*.

TRANSPLANTATION OF EMBRYONIC TAILS IN THE MOUSE

In order to be able to analyze the action of the genes *T*, *Fu*, and *Ki*, we developed a method that allowed us to grow tails of young mouse embryos in the anterior chamber of the eye of adult mice.

The mouse embryos were timed by the vaginal-plug method. Nine to 12 days after the vaginal plug had been observed, the mothers were killed with chloroform and the uteri dissected out and kept in mammalian Ringer's solution at 37° C. The embryos were taken out of the uterus and pieces of the tail or tail bud were cut

off with glass needles. Usually two pieces were prepared from each embryo, one containing the extreme tip of the tail and the other a more proximal section. In some cases pieces were further dissected by a cut dividing the dorsal from the ventral part of the section, so as to obtain pieces that presumably lacked the neural tube and pieces that carried large amounts of neural tube.

The mice used as hosts were mostly Bagg albinos. In some cases strain-C58 and strain-89 mice were used. The hosts were anesthetized by subcutaneous injections with nembutal. Definite strain differences in sensitivity to nembutal were found; C58 mice frequently died of doses that produced anesthesia but not death in Bagg albinos. An incision was made, with an iridectomy scalpel, through the cornea near the dorsal border of the sclera. The transplant was introduced into the anterior chamber in a drop of Ringer's solution by means of a Spemann micropipette. Preliminary attempts to use a metal trocar for transferring the piece of tissue were unsuccessful, because the tissue tended to cling to the metal surface. Usually the transplant was moved, by gentle pressure on the cornea, to the ventral border between iris and cornea.

All operations were carried out under sterile conditions. The Petri dishes used for dissection of the embryos were sterilized by dry heat, the Ringer's solution in the autoclave. The metal instruments for the dissection of embryos were soaked for 14 hour in Zephiran chloride and washed in several changes of sterile Ringer's solution. The glass instruments were dipped in alcohol and washed in sterile Ringer's solution between operations. In many cases the cornea of the host was washed with dilute Zephiran chloride (1:10,000).

The explants were kept in the host for 4 to 5 days; then the host was sacrificed

and the eyes removed. The transplant was dissected out and fixed in Carnoy, or else the eye was fixed in toto. They were then sectioned and stained with hematoxylin-eosin or with azan.

In spite of the sterile precautions, the eye was frequently infected, as judged by the opacity of the cornea or lens. On the whole, 41 out of no eyes appeared to be infected. The infection did not interfere significantly with the growth of the transplant ($\chi^2=0.275$, $P<0.50$). There is no evidence that proximal pieces of the tail grew better than the extreme tail tips ($X^2=0.259$, $P<0.50$). There was no evidence of differences in survival according to the genotype of the graft (heterogeneity $X^2=2.957$, $d.f.=4$, $P<0.50$).

After 4 to 5 days in the host eye, at least some of the grafts showed definite increase in size. All of them showed a certain amount of organization, although the degree was different in different cases. In the best-developed pieces, as shown in plate 3, the neural tube, notochord, and tail gut, in the correct arrangements, could be distinguished. The mesenchyme was sometimes arranged in a structure similar to somites, lateral to the neural tube (pi. 3). The mesenchyme surrounding the notochord frequently assumed a histological structure suggestive of precartilagage (pi. 4B).

Grafts from embryos with Bagg albino normal, *T*, and *Ki* parents showed no particular characteristics of differentiation that could be associated with these genes. In grafts from embryos with *Fu* parents, however, although differentiating somite material looked healthy, the notochord and neural tube were either missing altogether or small and poorly differentiated.

Plate 4A illustrates the amount of growth attained by some transplanted tissues. The donor, from a cross of normal

by Kinky, was 9 days old at the time of operation. A small dorsal piece of the tail bud, containing practically nothing but neural plate, was allowed to remain 4 days in the host. Comparison of this neural tube with that of plate 3, which is represented at the same magnification, will give an indication of the growth in this transplant.

An apparent case of induction is illustrated in plate 4B. The graft was a proximal ventral piece of tail from a normal embryo from a mating of *Ki* by *Ki*, 11 days old. It contained somite material from the right side, probably including pieces of notochord but certainly no neural tube. After 5 days in the host the transplant, *on* sectioning, presented the appearance shown in the plate. There is visible a centrally placed notochord surrounded by concentrically arranged mesenchyme cells, which look like precartilage. Adjacent to this, at the edge of the transplant, is a tuberous structure resembling a neural tube. Although it cannot be stated with certainty that no trace of neural-tube tissue remained in the explanted piece, it appears very likely that this structure was induced by the transplanted tissue.

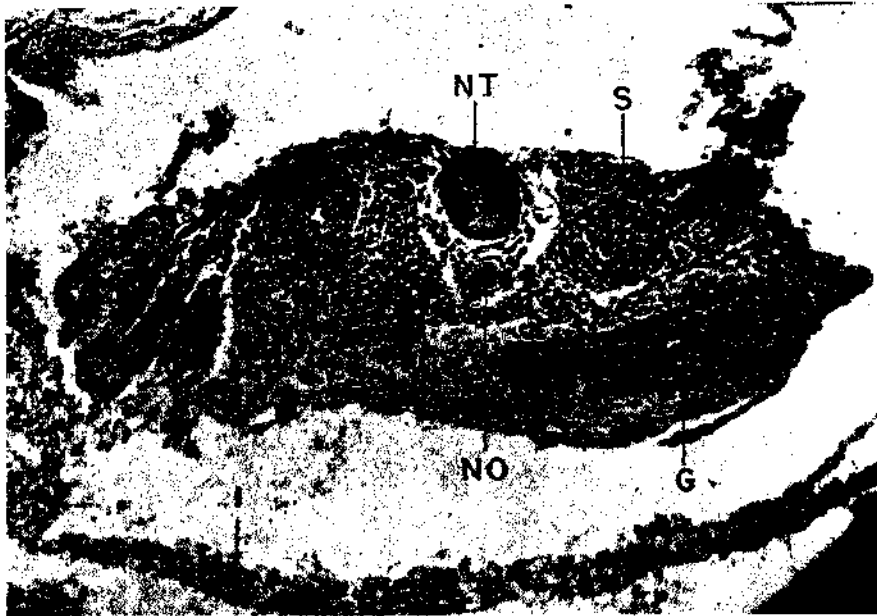
GENE ACTION IN THE AXOLOTL

Amphibian pigment cells offer advantages for the analysis of gene action, because their developmental origin in the neural crest and the subsequent establishment of the larval pigment pattern by migration through the embryonic tissues make possible two types of experimental attack on the problem. First, by explaining in hanging-drop tissue cultures dorsal pieces of neural fold from early neurulae one can obtain isolates of neural-crest material, which in the posterior trunk region consist largely of potential chromatophores. These pro-pigment cells move out from the explants onto the cover glasses, permit-

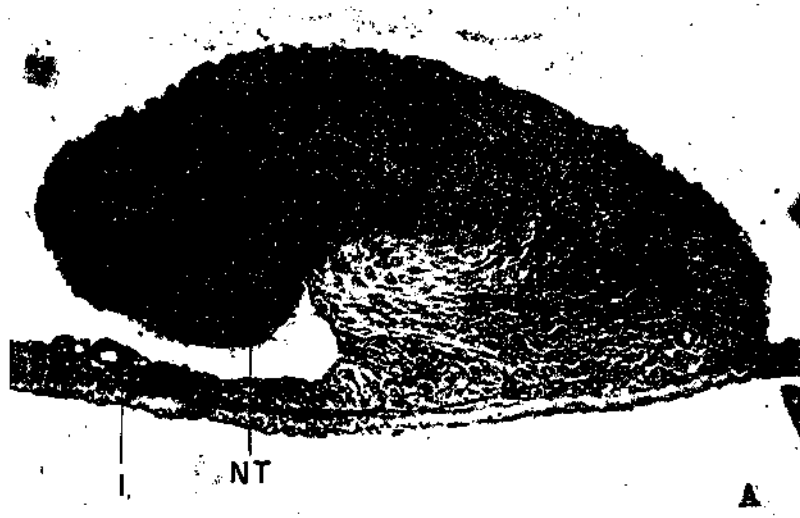
ting direct observation of their intrinsic capacities for migration, proliferation, and pigment synthesis under controlled conditions. Second, by methods of embryonic transplantation one can test reactions between potential pigment cells and their embryonic environment, observing factors extrinsic to the chromatophores themselves but important for pigment synthesis and cell migration. Combining these methods, it is possible first to localize the seat of gene action in the embryo and then to attack the problem of analyzing the nature of this action.

The genes selected for this investigation are those responsible for the pigment patterns characteristic of the white (*dd*) and black (*DD*) strains of the Mexican axolotl *Siredon mexicanum*. It has long been known that these patterns of pigmentation are associated with a single pair of alleles showing Mendelian segregation, the white condition being recessive. Embryonic transplantation experiments described in the literature have suggested that gene action in this case is mediated through the epidermis, which in the white genotype fails to provide some diffusible substance necessary for melanin synthesis. The present investigation was undertaken to determine more precisely the nature of this epidermis effect on pigment development.

Tissue-culture experiments. Before concluding that the effect of gene *d* is limited to the epidermis, it is necessary to exclude the possibility that the gene has some direct action intrinsic to the chromatophores. Several series of tissue cultures were prepared from embryos of both genotypes, involving in all over 300 explantations from the posterior trunk region of early neurulae to hanging drops in Holtfrcter's solution. All tissue cultures were maintained in a constant-temperature room



Proximal piece of tail from n-day *Ki* embryo, from mating *KixKi*; 4 days in host. *NT*, neural tube; *No*, notochord; *S*, somite; *G*, tailgut; *I*, iris. X 200.



A. Tail bud of 9-day embryo from mating normal X *Ki*. Dorsal piece of tail bud only, mostly neural plate. 4 days in host. X200.



B. Proximal piece of tail from normal 11-day embryo from mating *KixKi*. Ventral piece of tail, containing right somite and piece of notochord. 5 days in host. X 200.

Abbreviations as in plate 3

at 20° C. Melanophores of both genotypes are capable of pigment production in vitro, with *no* discernible differences between the two groups in rate or degree of pigmentation of the cells. This result was unexpected, since some diffusible substance from the epidermis was thought to be essential to melanin synthesis in the axolotl, and the melanophores on the cover glasses were not in association with epidermis. The same result was obtained in explants

to distinguish between mesenchyme cells and pro-pigment cells, some of the counts may have represented the former cell type, although it could be seen in the older cultures that the population of migrating cells on the glass was composed almost exclusively of chromatophores. The mitotic rates of the cells observed exhibited a decline from about 6 per cent at 3 days to almost zero at 8 days, mitoses being practically negligible after that time (table

TABLE 6
MITOSES IN TISSUE CULTURES FROM AXOLOTL NEURAL CREST

AGE (days)	WHITE AXOLOTL			BLACK AXOLOTL		
	Total cells	Mitotic cells	Per cent mitosis	Total cells	Mitotic cells	Per cent mitosis
3.....	1263	84	6.6	626	25	4.0
4.....	991	58	5.9	494	13	2.6
5.....	912	39	4.3	580	20	3.5
6.....	1951	63	3.2	980	17	1.7
7.....	1134	28	2.5	763	5	0.7
8.....	1208	6	0.5	203	0	0.0
10.....	732	4	0.5	550	4	0.7
14.....	1674	4	0.2	706	2	0.3
18.....	1347	8	0.6	84	0	0.0
21.....	645	0	0.0	51	0	0.0

of later series from different batches of eggs.

The studies were extended to include observations on mitotic rates and on cell migration. Samples from a series of 63 tissue cultures were fixed and stained in aceto-orcein on successive days, beginning with the third day after explanation, in order to determine whether there is any difference in inherent capacity for proliferation of cells from the two strains. The counts included only such cells of the explants as migrated out onto the cover glasses, exclusive of sheets of epithelial cells that occurred occasionally among the cultures. Since it is impossible

6). The figures for cultures of the black strain are slightly lower than those for the white strain, but not significantly so ($P < 0.20, > 0.10$). In relating these findings to conditions in the embryo, it must be remembered that the tissue-culture medium is composed only of inorganic salts and provides no nutrients to the cells, which consequently are limited to the food reserves present within them at the time of explanation. When the yolk platelets are exhausted, growth and proliferation must necessarily cease. Since, however, the larval pigment pattern is established in control embryos during the same time in which cells of the tissue cultures migrate

and produce pigment in apparently healthy condition, it seems reasonable to expect that if intrinsic differences in capacity for mitosis existed between chromatophores of the two genotypes, and were important in the development of the genetic patterns, they would show up in the mitotic rates observed. The conclusion is that potential and differentiating chromatophores of the two genotypes do not differ in mitotic capacity when isolated from the embryo.

To obtain a measure of migratory activity, the following procedure was applied to the same series of tissue cultures stained

emerge as salient features: Considerable variance was exhibited by all groups into which the data were broken down. As a summary of the genotypic group characteristics, the mean values of tissue-culture measurements of area per culture, number of cells per culture, and cell density are presented in table 7. The average values for the white group are higher than the corresponding figures for the black group. The group mean difference between area measurements is not significant, but there appears to be little doubt that there is a real difference in number of cells per cul-

TABLE 7

MEAN VALUES OF TISSUE-CULTURE MEASUREMENTS

	White (35 cultures)	Black (23 cultures)	<i>t</i>	P*
mm. ² /culture.	2.211± 0.131	1.824± 0.121	2.18	>0.05
Cells/culture.	333±21.7	215±25.3	3.58	<0.01
Cells/mm.*.157± 9.13	.126± 11.5	2.13	<0.05

* Approximation to 5 per cent and 1 per cent level computed as mean of *t* values for d.f. 35 and 23 weighted by the two variances.

with aceto-orcein. Camera lucida drawings were made of the areas occupied by migrating cells on the cover glass. These were measured with a planimeter, and the figures converted to square millimeters. The cell density of each culture was computed by dividing the total cell counts made in the mitosis survey by the area measurements. Interpretation of results must take into account the fact that chromatophores in the cultures frequently migrated on both the glass surface and the drop surface, but that all measurements of areas occupied include only the former because cells on the drop surface floated away during fixation. All figures for the black and white groups of tissue cultures were subjected to extensive statistical analysis, from which the following points

emerge as salient features: Considerable variance was exhibited by all groups into which the data were broken down. As a summary of the genotypic group characteristics, the mean values of tissue-culture measurements of area per culture, number of cells per culture, and cell density are presented in table 7. The average values for the white group are higher than the corresponding figures for the black group. The group mean difference between area measurements is not significant, but there appears to be little doubt that there is a real difference in number of cells per cul-

ture, since $P < 0.01$. The difference in density measurements is probably also significant ($P < 0.05$), but this might be expected, since these figures are derived from the cell counts, and the number of cells per culture is correlated with the cell density ($r = 0.736$ and 0.555 , for the black and white series respectively). It was possible to check on the significance of two operative variables, size of explant and age of donor, which might conceivably affect the group comparisons, because the black series contained some explants of small size (one-half and one-third of posterior trunk neural fold) as well as pieces of entire posterior trunk fold such as were used exclusively in the white series, whereas the white series contained some explants from donors with open neural folds (stage

16) as well as some from donors with closed neural folds (stage 20-21), which were used exclusively in the black series. When the data were broken down within a single genotype into two groups, on the basis of either size of explant or age of donor, there was no significant difference between the groups, a fact which indicates that the black and white genotype group differences were not due to these two experimental variables.

Further information on the history of migrating cells in the tissue cultures was obtained by averaging the figures for each day of age and plotting the values against time. The number of cells per culture and the cell density, in both black and white groups, became less with time. The area per culture, however, diminished in the black but increased in the white group. This difference may indicate a greater capacity for migration in cells from white explants as compared with black, but it may also reflect the significantly larger number of cells in the white cultures, since number of cells is correlated with area occupied ($r=0.554$ for black and white respectively). Since the group mean differences between black and white with respect to area occupied are not significant, the evidence at present does not justify the conclusion that the two genotypes exhibit any real difference in migratory capacity of the chromatophores in vitro.

Samples from another series of 63 tissue cultures were fixed in Carnoy's fluid on successive days after the third day following explantation, for detailed study of the morphology of chromatophores and of the structure and history of pigment granules. This study is still in progress, but observations so far completed fail to show any differences that might be correlated with the two genes under investigation.

In summarizing the tissue-culture ex-

periments, it may be said that the study of white and black axolotl chromatophores in vitro has shown that the genes *D* and *d* do not affect the chromatophores themselves in such a way that intrinsic differences in capacity for proliferation, migration, or pigment synthesis are demonstrable in tissue cultures of the two strains. The effects of the genes in question, therefore, appear to be mediated through factors extrinsic to the chromatophores. Evidence about the nature of this extrinsic effect on pigment development has been obtained by embryonic transplantation experiments.

Transplantation experiments. Relations between developing chromatophores and their surrounding tissues were tested by embryonic transplantations, which arranged these components of the two genotypes in different combinations. Early experiments were aimed at demonstrating differences in development of chromatophores of the same origin when in association with tissues of the black strain and of the white strain. Neural folds from the posterior trunk region of early neurulae were transplanted to the mid-ventral belly region of late neurula or early tail-bud hosts. This transplantation site was chosen to eliminate any confusion of graft chromatophores with those of the host, since the ventral belly region does not become pigmented in either strain during the period of observation of this experiment. Each donor provided one transplant in a black host and one in a white host, allowing subsequent comparison of graft chromatophores of identical genotype and age developing in the two environments. Melanophores from these grafts of both genotypes exhibited no disadvantages for melanin synthesis in association with tissues of white embryos. Both types of host permitted the differentiation of completely black melanophores. The same result was obtained when neural crest from tail-bud

embryos of both genotypes was transplanted reciprocally to the normal topographic position for this tissue. On the basis of the mechanism of gene action suggested in the literature, one would expect melanin synthesis to be inhibited in the white hosts. The evidence of these experiments, therefore, does not support this view, since there appeared to be no difference in rate or extent of pigment formation by grafted cells in the two strains.

Comparison of the extent of migration of melanophores in the two types of host, on the other hand, revealed a striking difference. Melanophores of both genotypes appeared to migrate freely beneath epidermis of the black strain but not beneath epidermis of the white strain. Evidence of this inhibitory effect on migration by white tissues appeared in both heterotopic and homotopic grafts. The neural-fold pieces grafted heterotopically into the belly region adhered both to the skin and to the gut surface, and in all cases melanophores migrated into both these regions. The areas occupied by graft chromatophores in both skin and gut were measured by means of camera lucida drawings and a planimeter. When total counts of melanophores from each graft were made and divided by the area measurements, it was apparent that the pigment cells of both genotypes developing in white hosts not only occupied much less area than cells from the same donors developing in black hosts, but also were much more closely crowded together, as if they had failed to spread to a comparable degree.

When neural crest is grafted homotopically from black to white tail-bud embryos, the epidermis lying dorsal to the neural crest is necessarily included in the graft. On the white hosts this bit of dorsal epidermis formed later a section of the dorsal fin above the grafted neural crest.

In all cases melanophores migrated extensively into this section of the fin but not into adjacent areas of host fin. Another set of operations was made in two steps, in order to obtain graft melanophores of black origin growing in the complete absence of any donor epidermis. First, pieces of dorsal epidermis overlying the neural crest of black embryos were replaced by pieces of flank epidermis from white embryos. Flank, rather than dorsal, epidermis was chosen to prevent the possibility of transplanting any adhering neural-crest cells. On the following day, the black neural crests, covered now by white epidermis, were transplanted to white embryos. In these cases, melanophores did not migrate into the skin dorsal to the grafts, but dense black lines of melanophores appeared beneath the epidermis at the edge of the graft regions. Corresponding results of grafting neural crest of white embryos to black hosts under similar conditions also suggest that the mobility of pro-pigment cells is greater in an environment of *DD* (black) tissue than in one of *dd* (white) tissue.

The view that a diffusible substance necessary for melanin synthesis is provided by black epidermis but lacking in white epidermis was based on the assumption that pro-pigment cells in white embryos migrate extensively onto the flank but do not form pigment. The following experiment was designed to investigate the validity of this assumption. Triangular pieces of epidermis were transplanted from black to white embryos, some transplants being oriented with the base dorsal and some with the apex dorsal. If epidermis from a pigmented strain permits pigment formation on a white axolotl by furnishing favorable conditions for melanization to cells normally occurring on the host flank but not normally pigmented under white epidermis, then the orientation of the

grafted triangles, provided they covered the same level of flank, would make no difference in the number of melanophores appearing beneath them. If, on the other hand, the pigment cells observed under grafted black epidermis owe their position on the flank to the removal of migratory inhibition normally encountered with the host epidermis, then triangles with the base up would be available for migration to pro-pigment cells from the length of neural crest covered by the base of the triangle, whereas triangles with the apex up would be available for migration to cells from a much more restricted length of neural crest. These conditions would be reflected in different densities of melanophores beneath grafts of the two types. The process of graft healing and subsequent growth of the embryos rotated some of the triangles, so that some were later not precisely oriented with base or apex up. As a measure of the avenue open to migrating cells moving into the graft area, the ratio of the top length of the graft to the area of the graft was chosen. Graft areas, as indicated later by the distribution of melanophores, were measured by means of camera lucida drawings and planimeter, and melanophores appearing beneath the entire epidermis of the graft were counted. When the density of melanophores was plotted against the ratio of top length to area of the epidermis grafts, there was indicated an obvious correlation, as shown in figure 5.

The results of all experiments support the idea that in the white and black strains of the axolotl, the genetic differences in pigmentation are mediated through differences in the tissue environment and not in the chromatophores themselves, the white pattern resulting from an inhibitory effect concerned, not with conditions for melanin synthesis, but with the migration of pro-pigment cells.

INVESTIGATIONS ON AN ENZYME SYSTEM OXIDIZING TRYPTOPHANE TO KYNURENIN

In *Ephestia*, the gene *a* inhibits the oxidation of tryptophane to kynurenin. Attempts to demonstrate an enzyme catalyzing this activity in *Ephestia* homogenates have been essentially negative. It was therefore decided to study this enzyme system in mouse liver.

Homogenates of mouse liver were prepared by grinding in 0.1 molar phosphate

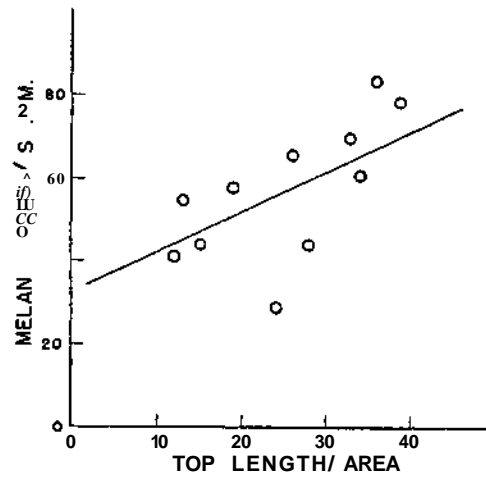


FIG. 5. Regression of melanophore density on ratio of top length to area in epidermis grafts.

buffer, pH 6.8, at 0° C. in Potter Elvehjem homogenizes. The oxygen uptake of homogenates in the presence and absence of tryptophane was determined in a Warburg apparatus at 37° C. At the end of each experiment, tryptophane was determined by the method of Bates, and kynurenin by the method of Otani and Nishino. For each experiment, therefore, there are three sets of data to establish the activity of the enzyme.

With this method it has been shown that oxidation of tryptophane to kynurenin does occur in mouse-liver homogenates. The enzyme concerned seems to be present la

low concentration, since 50 per cent homogenates are necessary to obtain strong activity. Twenty per cent homogenates gave only 3 per cent of the activity of 50 per cent homogenates, and no activity could be detected in 10 per cent homogenates.

Some properties of the enzyme system were studied in 50 per cent homogenates. Its optimum pH is between 6.7 and 7.1. It loses activity on standing, so that after two days at 0° C. only 65 to 70 per cent activity is left. Dialysis against water at 0° C. for 16 hours does not affect the activity. The enzyme system was found in liver only; 50 per cent homogenates of mouse lung, kidney, spleen, and testicles were inactive.

When liver homogenate is centrifuged, activity is found both in the sediment and in the supernatant. After centrifuging for one hour at 18,000 r.p.m., 55 per cent of the original activity is found in the supernatant. After fractionated precipitation with (NHU⁺SCX and subsequent dialysis against water for 16 hours at 0° C, all activity is found in the fraction precipitated at one-third saturation with (NEUJaSO*. Globulins prepared in this way oxidize tryptophane to kynurenin at an optimum pH of 6.8 to 7.0. The oxygen-consumption curve is characterized by a lag period after addition of tryptophane, during which no increased oxygen uptake occurs. This lag period lasts from 20 to 40 minutes. The need for cofactors is demonstrated by the fact that the activity of these preparations is considerably increased by addition of Mg⁺⁺ ions and of a Kochsaft prepared from mouse liver. During the reaction, CO₂ is released.

These **experiments** have suggested two possible reasons for the failure of *Ephestia* homogenates to show oxidation of tryptophane to **kynurenin**. An inhibitory effect of *Ephestia* homogenates on mouse-liver

homogenates was described in Year Book No. 47. Evidence obtained recently indicates that slight activity may be present in the globulin fraction obtained from *Ephestia* pupae, but not in that obtained from larvae. This would indicate that the enzyme system may be more concentrated in pupae than in larvae, in agreement with the fact that most of the pigment production takes place in the pupal stage.

Serological differences between a and a Ephestia.* Evidence was reported last year that the gene *a*, besides affecting pigment metabolism, also causes the formation of qualitatively different proteins. This assumption has been further investigated by serological methods. Rabbits were immunized by five subcutaneous injections at 6-day intervals with 0.9 per cent NaCl extracts from homogenized *Ephestia* larvae. Three rabbits were immunized against *a⁺a⁺* and two received extracts from larvae of an *aa* strain that had been made isogenic with the *etc?* strain by outcrossing for 8 to 9 generations. Seven days after the last injection, the rabbits were bled and the serum obtained.

The sera were tested against antigens from both strains by means of the precipitin reaction. The antigens used in this case were cleared by centrifugation at 12,000 r.p.m. for 1 hour. The supernatant was of a clear greenish color, but had a tendency to form a black precipitate on standing. The antigens were therefore used immediately after being prepared, and compared on the basis of their nitrogen content, which was determined by a micro-Kjeldahl procedure.

No differences in the reaction of the sera against homologous and against heterologous antigens were found by simple dilution. Three of the sera that had relatively high titers (between 1/10,000 and 1/100,000) were more thoroughly tested by the optimum **flocculation** method. **Dif-**

ferent dilutions of antigen were mixed with a constant dilution of serum, and the time of the first appearance of visible flocculation was determined for each tube. The ratio of serum to antigen in the tube showing fastest flocculation was designated as optimal proportion. In table 8 the optimum proportion for the sera tested is expressed as milligrams of antigen nitrogen per cubic centimeter of antiserum. The numbers in parentheses indicate the number of determinations performed.

TABLE 8

OPTIMUM PROPORTIONS FOR THREE ANTI-EPHESTIA RABBIT SERA WITH THE HOMOLOGOUS AND HETEROLOGOUS ANTIGENS
(Optimum proportion expressed as milligrams antigen N reacting optimally with 1 cc. of serum)

Serum	Antigen	
	a^+a^+	aa
1 (anti- a^+a^+)	0.282 (3)	0.379 (4)
3 (anti- $\&^+\&^+$)	0.280 (4)	0.379 (3)
6 (anti-flo)	0.206 (3)	0.138 (2)

The table indicates that in every case the sera showed a higher titer when tested with the homologous than when tested with the heterologous antigen. In repeated determinations, some variability of the optimum proportion values, expressed in milligrams N, was found; but in not a single case did the ranges for the heterologous and the homologous antigens overlap.

The sera were absorbed with the heterologous antigen by incubating antigen and serum at optimum proportions for 1 hour at 37° C. and leaving in the cold for 24 hours. After centrifugation, the supernatant sera were tested against the homologous and heterologous antigens. Sera 1 and 6 still gave reactions with both anti-

gens after absorption, but higher titers with the homologous than with the heterologous antigen. After a second absorption, these two sera did not react with either antigen.

Serum 3, on the other hand, lost its ability to form a precipitate with the heterologous aa antigen after one absorption at optimum proportions. It still reacted with the homologous a^+a^+ antigen in a 1:25 dilution (0.0055 mg-N). This absorbed serum, which formed a specific precipitate with $etc?$ antigen, was used to determine the chemical nature of the active antigen. Antigen fractions were obtained from both $etc?$ and aa saline extracts, by half and full saturation with (NH^4SCX) and subsequent dialysis against H_2O . The serum gave a precipitate only with the fraction obtained by half saturation of a^+a^* extract with (NHU^4SO^*) . All other preparations failed to react.

These experiments demonstrate that antigenic differences exist between a^+a^* and aa *Ephestia*. They furthermore indicate that at least one of the substances responsible for these differences is a protein belonging to the globulin fraction.

SEROLOGICAL STUDIES WITH THE BRACHYURY MOUSE

The positive finding of serological differences between tf^+ and a *Ephestia* suggested the possibility that similar serological differences might be found for mouse genes. The gene T (Brachyury) was used. Testicles and spleens from freshly killed T/H- mice were ground up in physiological saline and extracted three times with saline in the cold. The pooled extracts were injected into rabbits. Five subcutaneous injections were made at 6-day intervals. Sera were obtained on the tenth day after the last injection. The sera were then tested against antigens obtained from

males of the genetic constitutions $T/+$ and $+/+$, which had been made isogenic by outcrossing for 18 generations. Two different antigens were used: the first saline extract, and a second saline extract obtained by treatment with saline of the sediment from the first extraction. It is assumed that the first extract contained easily soluble proteins, including the serum proteins from the blood left in the organs, whereas the second extract contained substances that dissolved with more difficulty, probably including small particles. These two types of antigen were prepared from both testes and spleens of both strains, and kept at 0° C. after preservation with merthiolate 1/10,000. Their nitrogen content was determined, and they were adjusted to contain equal amounts of nitrogen.

In precipitation tests the sera did not show any differences in titer when tested with the homologous and heterologous antigens. Absorption experiments with the first antigen of the $+/+$ strain were unsuccessful, except in the case of one anti-spleen serum, which reacted with the homologous antigen. After absorption with the second antigen of the heterologous strain, three sera (two against testicles, one against spleen) turned out to give precipitates with the homologous but not with the heterologous antigen. Serological differences between $T/+$ and $+/-f$ animals are therefore clearly indicated.

Serological differences between $T/+$ and $+/\sim f$ testes were also demonstrated by complement fixation. The same sera were used as for the precipitation tests, after inactivation for 30 minutes at 56° C. The antigens were adjusted to contain 0.05 mg. N. Different dilutions of sera were used, and incubated in the presence of complement (2 units) for 30 hours at 37° C, and for 30 minutes at room temperature. At the end of this period, the

indicator system, consisting of 2.5 per cent sheep erythrocytes and 2 units of hemolysin, was added, the tubes were incubated for 30 minutes at 37° C, and the degree of hemolysis was recorded. The tubes were then kept for 24 hours in the refrigerator. At the end of this period, the tubes were centrifuged, the supernatant discarded, and the sedimented blood corpuscles suspended in 5 cc. of 0.9 per cent saline. The saline suspension was then read in the Klett-Summerson colorimeter with a blue filter. The degree of lysis was recorded in terms of the percentage of red blood corpuscles found as compared with the saline control.

The results of one of these experiments are recorded in figure 6. The curves obtained in this way for the second antigen from $+/+$ and from $T/+$ are obviously different, the serum reacting more strongly with the homologous than with the heterologous antigen. This is apparent not only from the titer for complete lysis, but particularly from the general shape of the curves. More complement appears consistently to be fixed by $T/+$ antigen than by $+/+$ antigen at the same serum dilutions. The same result has been obtained with two other anti-T testicle sera, tested with the second antigen. On the other hand, no differences were found in the same sera when they were treated with the first saline extracts of $T/+$ and $+/+$ testicles.

The anti-T testicle sera were also tested against antigens obtained from $Ki/+$ animals. Ki is a gene situated close to T on the same chromosome, having similar though not identical phenotypic effects. It appears from the curve of figure 6 that $Ki/+$ is intermediate between $T/+$ and $\bullet f/+$. This result is also evident in the other two anti- $T/+$ testicle sera tested. It therefore appears that connected with the gene Ki there is an antigenic structure similar to but not identical with X. The

results of the experiments with *Ki* antigens seem to indicate a physiological relation between the closely linked genes *T* and *Ki*.

Absorbed sera proved to be highly anti-complementary when tested by means of the complement-fixation method.

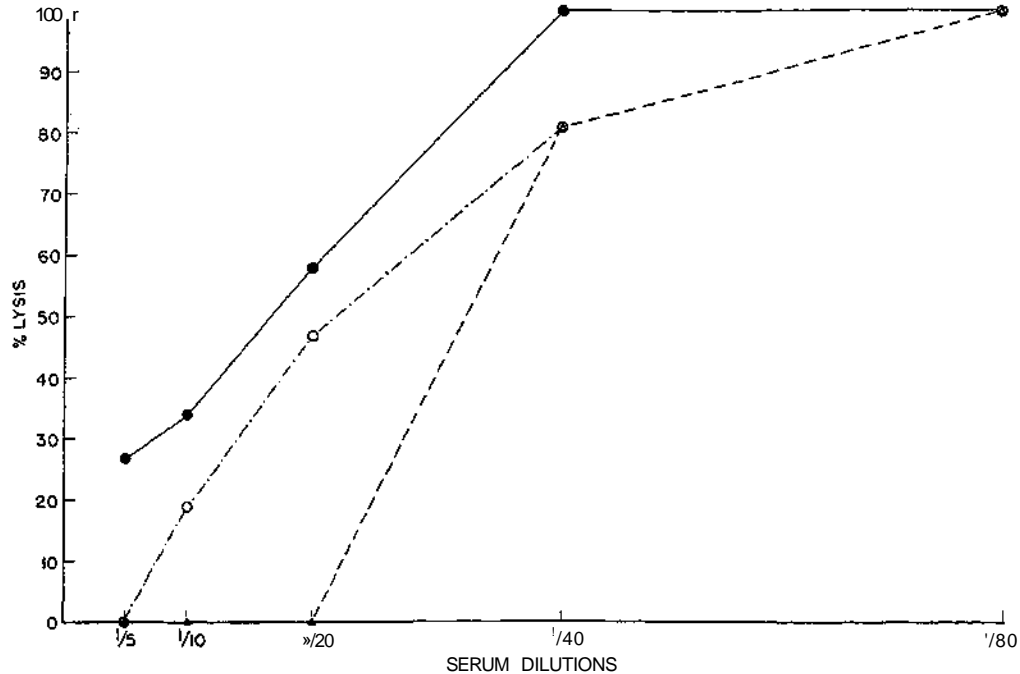


FIG. 6. Complement fixation of an anti-T testicle serum with "second" antigens from *T*, -f, and *Ki* testicle antigens, adjusted to contain 0.05 mg. N each. Abscissa: serum dilutions. Ordinate: per cent hemolysis, determined by nephelometric measurement of nonhemolyzed corpuscles. \bullet — \bullet = *T*/+; \circ — \circ = *Ki*/+.

GENETIC STRUCTURE OF NATURAL POPULATIONS

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Most biologists agree that adaptation to the environment is the principal driving force of organic evolution. But modern genetic thought visualizes the relations between organism and environment in a way quite different from environmentalistic theories of the nineteenth century, which have recently become a topic of renewed discussion because of the polemics aroused by Lysenko and his partisans. Living beings are not passively molded by physical agencies, as mechano-Lamarckists believed. Nor can a species change by exertion of its

will, as supposed by psycho-Lamarckists and finalists. Moreover, organisms are not altered by a kind of sympathetic magic, which makes them able to "select" useful and to reject useless materials from changed environments, as imagined by Lysenko. It is the view of a majority of evolutionists that mutation and Mendelian recombination continually produce innumerable genetic materials, some of which are more and others less suitable for perpetuation in various environments. The available genotypes are then adjusted by

natural selection to the opportunities available in the world.

To say that evolution is brought about because organisms are changed by environment is inexact. Organisms change in the process of becoming better able to survive and reproduce in the environments in which they live. Evolution is a response of the organism to the challenge of the environment. And this challenge does not arise from physical conditions alone, but also from interactions with other organisms that share the same physical environment. It is probable, at least in higher organisms, that the biotic environment is more important in evolution than are physical conditions in the narrow sense.

CO-OPERATIVE STUDIES ON TROPICAL DROSOPHILAS

The above conception of the relations between environment and evolution suggests new types of study. Genetic experiments are done mostly with domestic animals, cultivated plants, or species otherwise associated with man, such as commensals, weeds, or pests. Many organisms domesticated by or living with man have important technical advantages for use as experimental materials. But to understand the evolutionary process as a whole, work on such species is not sufficient. We must examine the causal links connecting the evolutionary patterns of different organisms with the biota of which these organisms are constituent parts. Interrelationships between living beings and the main types of environment that exist on our planet are to be studied.

Comparison of genetic population structure in related species living in temperate and in tropical climates seems especially promising. In any temperate or cold climate, sharp seasonal changes in the environment occur every year. Any popula-

tion of organisms living under such conditions must evidently be adapted to cope with a succession of sharply different environments. Tropical climates in general permit the environment to remain relatively more uniform throughout the year, and may seem to demand less adaptive versatility from the inhabitants. Tropical biota, however, include greater numbers of animal and plant species than is the case in temperate or cold lands. Thus members of tropical biotic communities meet a great variety of challenges, to which they may respond by adaptive evolutionary changes. The evolutionary process in the tropics, taken as a whole, may have a faster tempo and a greater creativeness than in temperate and cold climates.

A program of comparative studies on population genetics of species of *Drosophila* in temperate and in tropical climates was initiated some seven years ago (see Year Books Nos. 43, 1943-1944, and 47, 1947-1948). In 1943, the writer, in cooperation with Dr. C. Pavan, of the University of São Paulo, made an exploratory survey of species of *Drosophila* that occur in the state of São Paulo and near Belem in the state of Pará in Brazil. Two species, *Drosophila willistoni* and *Drosophila pro saltans*, were chosen as materials for further and more intensive study. The former is the commonest species in most of Brazil; it is also ecologically most versatile, since it occurs in a great variety of environments and feeds on many species of fruits. In contrast, *D. pro saltans* is a rare form, ecologically specialized; thus far it has been found in large numbers only in a few localities, in the state of Maranhão and on the island of Marajó. Preliminary genetical work on these species, necessary to make them available for experimental studies of the genetic population structure, has been carried out at Columbia University by Mr. B. Spassky, Mr. S. Zimmering, Pro-

fessor A. G. L. Cavalcanti, and the writer.

From August 1948 to June 1949, inclusive, a group of investigators, assembled at the laboratory of Professor André Dreyfus, at the University of São Paulo, carried out a program of orientation studies on population genetics and ecology of tropical species of *Drosophila*, especially *D. willis-toni* and *D. prosaltans*. The group included, besides Professor Dreyfus, Drs. C. Pavan, A. Brito da Cunha, and E. Nascimento Pereira, of the University of São Paulo; Professor A. G. L. Cavalcanti and Miss Ch. Malogolowkin, of the University of Brazil, Rio de Janeiro; Dr. A. R» Cordeiro, of the University of Porto Alegre, Rio Grande do Sul; Ing. Agr. M. Wedel, of the University of Buenos Aires, Argentina; Dr. Hans Burla, of the University of Zürich, Switzerland; Mrs. N. Dobzhansky, and the writer. Mr. B. Spassky remained at Columbia University in New York, but participated in the cooperative program by furnishing certain mutant strains and carrying out many experiments. The work of the above group was made possible by financial assistance extended by the University of São Paulo, the Carnegie Institution of Washington, and the Rockefeller Foundation. Brazilian military and civil authorities, in particular General Newton Cavalcanti, Brigadier General Eduardo Gomes, the governors and their aides in the states of Goyaz, Amazonas, Paraná, Rio Grande do Sul and the territories of Rio Branco, Acre, and Guaporé, Dr. Felisberto de Camargo, director of the Instituto Agronomico do Norte at Belem do Pará, and other officials and private persons too numerous to be named, greatly facilitated the field work by providing airplane and other transportation and by extending many valuable courtesies, which made the travel and collection in remote parts of Brazil a success as well as a pleasure.

COLLECTING JOURNEYS

The first task was to collect material for cytological studies and for genetic experimentation and to make field observations on ecology of *Drosophilas* that inhabit the principal climatic and vegetational zones of Brazil. The regions in which collections were made are shown by black circles on the accompanying map (fig-7)-

Three stations were established in the state of São Paulo, at which collections were made at approximately bimonthly intervals. One of them, in coastal rain forest near Vila Atlantica, is sufficiently warm and humid throughout the year to permit the maintenance of flourishing *Drosophila* populations. The second, near Mogi das Cruzes on the plateau, has a cool and fairly dry winter season; and the third, near Pirassununga in the interior of the state, is hot and humid in summer but dry in winter. These three stations formed a climatic gradient, whose influence on *Drosophila* populations was studied.

The collecting expeditions to localities more remote from the laboratory in São Paulo were as follows. From October 29 to November 10, 1948, Dr. Pavan and the writer visited the central part of the state of Goyaz, and made collections at Monjolinho (near Anapolis) and at Palma (Paraná, see map). This is a zone of *crrado* savanna and of gallery forest vegetation, warm throughout the year but with pronounced wet and dry seasons, the latter causing reduction of the abundance of most species of *Drosophila*. From January 5 to 26, Professor Cavalcanti and the writer collected in the vicinities of Cruzeiro do Sul and Palraares, territory of Acre, and at Porto Velho, territory of Guaporé. This is a zone of exuberant forest vegetation of Amazonian type (*hylaesa amazonku*); despite variations in the amount of precipi-

tation at different seasons, temperature and relative humidity remain continually favorable for *Drosophila* breeding. Between February 22 and March 5, Dr. Pavan and

most of the year there is almost no precipitation and the vegetation dries out and loses its foliage, as it does in winter in temperate climates. These conditions are

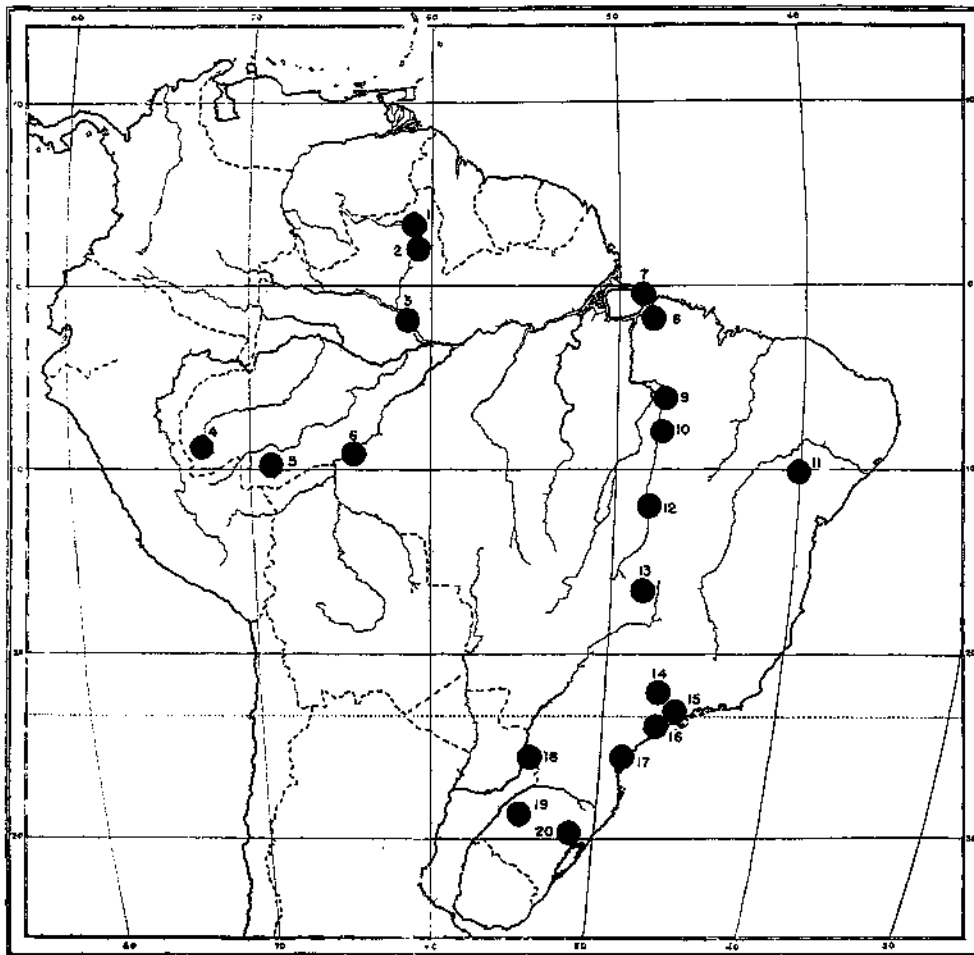


FIG. 7. Map of South America, showing the location of the places where samples of *Drosophila* populations were taken. 1, Savanna of Rio Branco; 2, Rio Mucajaf, territory of Rio Branco; 3, Rio Negro, Aniazonas; 4, Cruzeiro do Sul, Acre; 5, Palmares, Acre; 6, Porto Velho, territory of Guaporé; 7, Marajó Island; 8, Belem, Pará; 9, Imperatriz, Maranhão; 10, Carolina, Maranhão; 11, Catunf, Bahia; 12, Paranã (Palma), Goyaz; 13, Monjolinho, Goyaz; 14, Pirassununga, São Paulo; 15, Mogi das Crazes, São Paulo; 16, Vila Atlantica, São Paulo; 17, Paranaguá, Paraná; 18, Iguassu National Park* Paraná; 19, Santo Angelo, Rio Grande do Sul; 20, Reuter, Rio Grande do Sul.

the writer made an excursion to Rio Salitre and to Catuni, near Bomfim in the *caatingas* in the state of Bahia. The *caatingas* are characterized by the sharpest seasonal climatic chintzes possible in the tropics;

close to the limit of endurance for *Drosophila*, and only a few species are able to withstand the rigors of the *caatingas*.

Dr. A. da Cunha, Mrs. Dobzhansky, and the writer visited the state of Paraná, and

made collections at Paranaguá on the Atlantic coast, and at the Iguassu National Park at the boundary with Argentina and Paraguay, between March 22 and April 2. The conditions at Paranaguá are, on the whole, similar to those at Vila Atlantica (see above). The Iguassu region has a forest of tropical aspect, but the temperatures are low in winter and frosts occur. A relatively small variety of species of *Drosophila* was encountered in the region. From May 18 to 23, Dr. Cordeiro and the writer visited the state of Rio Grande do Sul and collected at Santo Angelo in the western part of the state. Dr. Cordeiro made a collection at Reuter in the south central part at an earlier date, in January. The state of Rio Grande do Sul is outside the tropical zone and has cool winters. A rather large number of species of *Drosophila* was nevertheless encountered in the collections made in that state.

Between April 12 and May 10, Dr. Pavan and the writer collected in the savannas of the territory of Rio Branco and at the margin of the forest zone at Rio Mucajai in the same territory, on the lower Rio Negro in the state of Amazonas, and near Belem in the state of Pará. The savanna of Rio Branco has a long dry season, and tree vegetation occurs there chiefly in the form of gallery forests along streams and near marshes. Rio Negro has exuberant rain forests and an equable hot and humid climate. Mucajai is probably intermediate climatically. The Belem region, despite its having a drier and a wetter season, is probably as favorable for the maintenance of large *Drosophila* populations as is the Rio Negro region; and a large variety of species, many of them new to science, was encountered there. Collections were made near Belem also in September and October 1948 by Dr. Pavan and the writer, in June 1948 by Dr. Pavan, and in July-September 1949 by Dr. Pavan and the writer, so that

information was obtained about the status of *Drosophila* populations at different seasons.

From July 29 to August 13, Dr. Pavan and the writer traveled in the state of Maranhão, and collected in the vicinities of Carolina and Imperatriz. This region is transitional between the savannas (*campos cerrados*) of central Brazil and the Amazonian rain forests of Pará. Pronounced wet and dry seasons occur yearly. A gradual change in the composition of the *Drosophila* fauna was observed, from that previously found in the state of Goyaz to that living near Belem. Between August 27 and September 5, Dr. Pavan and the writer visited the estates belonging to the family Tocantins Penna in the northeastern part of Marajó Island, state of Pará. Despite its relative proximity to Belem, this part of Marajó has extensive grass-covered swamps, alternating with rather open forests growing on higher ground; wet and dry seasons are clearly differentiated; and the *Drosophila* fauna proved to be unique because of a high frequency of *D. prosaltans*, which is rare in most other parts of Brazil. Finally, Dr. Warwick Kerr very kindly collected and sent to us a sample of the *Drosophila* population from the vicinity of Santa Cruz de la Sierra, in Bolivia, in July 1949.

The collecting journeys made from August 1948 to September 1949 in the Brazilian territory entailed between 27,000 and 28,000 kilometers of airplane travel alone. The number of *Drosophilas* of various species collected and classified during this time was close to 110,000 individuals.

DIVERSITY OF SPECIES IN TROPICAL ENVIRONMENTS

Perhaps the most striking and significant difference between *Drosophila* communities in tropical and in temperate countries

is that the former, as a rule, contain more species than the latter. For example, at Chitina, Alaska, the writer collected at least a thousand *Drosophila*s, all of which belonged to a single species, *D. athabasca*. Collection with the aid of fermenting banana bait in a favorable locality in California mountains is likely to yield, among some hundreds of individuals, only 5 to 10 species. Localities are common in which only 2 or 3 species, or even a single species, are found. Furthermore, regardless of the number of species caught, one of them, *D. pseudoobscura*, is in many localities more common than all others combined. By way of contrast, collection on banana bait in Brazilian forests usually yields more than 10 species, and may reveal more than 30 in a single neighborhood less than one square kilometer in area. Moreover, several species may be common in such collections, none of which is dominant. Only in those tropical environments that are intrinsically unfavorable for *Drosophila* does the number of species become reduced and one or two species become dominant. This occurs, for example, in the peculiar desert regions of northeastern Brazil called *caatingas*, where the heat and aridity of a prolonged dry season appear to be beyond the toleration limit of all except a few species. *Drosophila nebulosa* is the unchallenged dominant in the *caatingas*, as it is also during the dry season of the year in the savanna (*campo cerrado*) of central Goyaz.

Drosophila species that are attracted to banana bait subsist in nature mainly on fermenting fruits, tree sap, and fallen flowers, which in tropical forests often ferment on the humid and well shaded ground. For insects that feed on substances of this kind, tropical forests evidently offer more diversified and favorable conditions than do tropical savannas, or temperate—particularly cold—environments. It may

be noted, however, that many other groups of organisms likewise show greatest proliferation of species in tropical lands. Thus the astounding diversity of species of trees found growing in small areas in Brazilian forests was noticed and commented upon by the early explorers. In co-operation with Dr. George Black, of the Instituto Agronomico do Norte, and Dr. Pavan, counts were made of trees with diameters of more than 10 cm. at chest height on two one-hectare (100 X100 m.) plots near Belem do Pará. One of these plots in a periodically inundated forest (*igapó*) had 60 species among 564 individual trees. The second, on higher land (*terra firme*), had 87 species among 422 trees.

Tropical environments thus contain a greater variety of ecological niches (biological opportunities) for living beings than do temperate-zone environments. To occupy an ecological niche, however, an organism must be adapted for it, that is, must possess a combination of physiological and morphological traits that make maintenance and perpetuation of life possible. Different combinations of traits are needed for efficient exploitation of different ecological niches. To conquer the ecological niches available in a given region, life must accordingly evolve a variety of genetic constitutions proportionate to the variety of biological opportunities. This can occur in two ways. First, numerous species may be formed, each adjusted to a single ecological niche or to a few similar ones. Second, a relatively small number of species may become polymorphic, that is, each composed of a variety of adaptively different genetic types. It appears that both ways have been followed in the evolution of *Drosophila*, and that a greater variety of species, and a greater polymorphism within some of these species, have evolved in the tropics than in temperate countries.

LOCAL AND SEASONAL VARIATIONS IN
RELATIVE FREQUENCIES OF
DROSOPHILA SPECIES

A community of many related species, each adapted to a slightly different set of environmental conditions, represents a sensitive system, which can be expected to react to even slight changes in the environment. *Drosophila* populations of tropical forests show this lability in several different ways. In experiments made by Burla, Brito da Cunha, Cavalcanti, Pavan, and the writer, similar bait was placed at 10-m. intervals along 200-m. transects in what appeared to be a reasonably uniform forest at Horto Florestal near São Paulo. *Drosophila* flies that came to each bait were collected separately and classified as to species. The results showed that the different species are far from being uniformly distributed over the territory sampled. Instead, nuclei of high population density are scattered through the forest, separated by areas where flies are rare or even absent. Furthermore, the localization of these nuclei is by no means the same for different species. A neighborhood that is evidently attractive for *D. willistoni* may contain few individuals of *D. griseolinata*, and vice versa. This microterritorial differentiation breaks up a community consisting of many competing species into tiny colonies with a much smaller number of competitors.

The formation of nuclei of population density for each species is caused by the fact that different species are attracted preferentially to different food substances. This can be shown if, instead of collecting *Drosophila*s on uniform bait placed at different points, one finds them on different natural baits located as close together as possible. On the ground in tropical forests one often finds fallen and fermenting fruits or flowers of one species of tree, and a short distance away fruits or flowers of

another species. Collections made on such natural baits by Pavan, da Cunha, and the writer disclosed two interesting facts. First, different species of *Drosophila* show preferences for different foods. Within a distance of some ten meters, hundreds of individuals of a *Drosophila* species may congregate on one and largely ignore another natural bait. Second, such preferences do not reach the stage of rigid dietary specialization, and a kind of fruit avoided by a species of *Drosophila* in one place may be relatively well attended in another place. Just what causes these variations in attractiveness of the same kind of fruit is not clear. Possibly some microclimatic differences between places in which the fruits happen to be located are effective, and the yeasts and other microorganisms that make the fruits ferment may well be different and may attract or repel different *Drosophilas*. However that may be, most or all species of *Drosophila* (except the fungus feeders, which we did not attempt to include in our studies) can utilize a great variety of foods, and hence are competitors in nature. The competition is alleviated, however, by different food preferences, which may permit some species to be temporary monopolists in some parts of the environment.

Seasonal changes in the environment occur in tropical forests, even in regions such as the coast of São Paulo or the equatorial rain forests near Belem do Pari, where temperature and humidity are favorable at all times for the development of plants as well as of *Drosophila*. For example, different species of trees come into flowering and fruiting at different seasons. Although such seasonal changes may seem small compared with those in temperate climates, they profoundly affect the *Drosophila* populations.

As indicated above, *Drosophilas* were

collected at approximately bimonthly intervals, chiefly by Pavan, da Cunha, Burla, and the writer, at three stations in the state of São Paulo. At each of these stations, about a dozen places were marked, so that banana bait was always placed in the same positions. The flies collected were classified as to species. The relative frequencies of various species at each collecting station changed greatly from season to season. Thus, *D. willistoni* and its sibling species *D. paulistorum* were common at all stations during the summer, at times even reaching the status of dominants; but during the cooler part of the year they became relatively less common than certain other species (*D. simulans* at Pirassununga, *D. mediotriata* at Mogi das Cruzes, *D. capricorni* at Vila Atlantica). Distinct seasonal changes in relative frequencies of *Drosophila* species were observed also at Belem do Pará in the equatorial zone.

A problem of much interest is whether these seasonal changes are cyclic; that is, whether the same relative frequencies of the different species will recur in each locality year after year. One can well imagine that a system composed of some twenty species, ecologically rather similar and hence competing, may be so sensitive to environmental variation that climatic differences between years will make repetition of the same set of relative frequencies improbable. This problem must be settled by future observations. Thus far we possess only some data collected by Dr. Pavan in 1946 and 1947 at Mogi das Cruzes. Comparison of these earlier collections with those made in 1948-1949 at similar seasons indicates quite considerable differences in the composition of the populations. Unfortunately, the earlier collections were not made at precisely the same neighborhoods as the later ones, and hence the disturbing element of territorial variation is not excluded*

DIFFUSION RATE AND POPULATION DENSITY

The fact that uniform bait may attract quite different collections of species of *Drosophila* when placed at points only some 10 m. apart suggests that the flies do not travel over long distances, but come to bait chiefly in the immediate vicinity. This inference was checked by experiments on *D. willistoni* carried out by Burla, da Cunha, Cavalcanti, Pavan, and the writer in the vicinity of São Paulo.

The technique of such experiments, worked out previously for *D. pseudoobscura* (see Year Books Nos. 39-47), consists in releasing at some point in a natural environment a known number of flies marked by an easily recognizable but innocuous mutant trait, and then recording the numbers of marked and wild flies captured on bait placed at regular intervals at different distances from the point of release. Mathematical methods applicable to the resulting data were developed by Professor Sewall Wright, of the University of Chicago, for the experiments on *D. pseudoobscura*. Professor Wright has very kindly checked also the calculations made for *D. willistoni*.

The diffusion rates of flies in a two-dimensional environment are measured by the variance of the distribution of the marked flies in the territory around the point of their release at different times after their liberation. In *D. willistoni*, as well as in *D. pseudoobscura*, this variance increases with time much faster at high than at low temperatures. Temperatures of about 15° C. are limiting, since at lower temperatures the flies remain about stationary. But above this limit, *D. willistoni* shows migration rates of a lower order of magnitude than does *D. pseudoobscura*. In other words, *D. willistoni* is even more inclined to form semi-isolated local populations or colonies than is *D. pseudoobscura*.

The same experiments used to study the migration rates of the flies yield data from which the population densities of the wild flies in the experimental field can, granting certain assumptions, be deduced. Such deductions indicate population densities of wild *D. willistoni* between 10 and 28 flies per 100 sq. km. in the experimental fields near São Paulo. Population densities of all species of *Drosophila* in the same fields can be estimated, with less assurance than for *D. willistoni* alone, at between 60 and 139 flies per 100 sq. m. Population densities of *D. pseudoobscura* and related sibling species in California mountain forests have been estimated, during the most favorable season of the year, at from 0.4 to 10.0 flies per 100 sq. m.; and this species and its relatives are usually more abundant than all other species in the same localities. The conclusion is justified that the aggregate population densities, of *Drosophila* are appreciably greater in tropical forests than in the forests of California.

CONCEALED GENETIC VARIABILITY IN THE
CHROMOSOMES OF *DROSOPHILA WILLISTONI*
AND *DROSOPHILA PROSALTANS*

Representatives of a species of *Drosophila* collected in nature are usually rather uniform in their externally visible traits. The morphology of each species appears to be fairly well stabilized, and deviations from the norm are adaptively undesirable. An exception to this rule is the fairly common and widespread Brazilian species *D. polymorpha*, which shows a very considerable variation in the color pattern of the abdomen. This case, studied in detail by da Cunha, proved to be a clear instance of balanced adaptive polymorphism. The forms having very light and very dark abdomens are homozygotes, whereas the intermediates are heterozygous for a pair of alleles of a single

gene. The heterozygotes are adaptively superior to both homozygotes, (cf. discussion of chromosomal variability in the next section).

Studies made by several investigators, chiefly on European and American *D. melanogaster* and *D. pseudoobscura*, have disclosed that flies of these species found in nature are often heterozygous for various recessive mutant genes. These genes, when homozygous, cause the death of all or a part of their carriers (lethals, semi-lethals, subvitals), as well as sterility, modifications of the development rate, and various morphological abnormalities. The heterozygotes for these recessive genetic variants, however, are "normal" flies in every respect. These variants are thus concealed in heterozygous condition, and form a store of potential, rather than actually expressed, variability.

We have extended studies of the above type to the Brazilian *D. willistoni* and *D. prosaltans*. Strains have been synthesized in the laboratory, chiefly by Mr. Spassky, which have the second or third chromosomes (autosomes) "marked*" by various mutant genes and inverted sections. By making appropriate crosses of flies collected in nature to these "marked" strains, it is possible to obtain, in the third filial generation, flies that carry the same wild chromosome twice (are homozygous for it). If the chromosome in question causes, when homozygous, a reduction of the viability of its carriers, certain deviations from normal Mendelian segregation ratios result in the cultures. Inspection of the homozygotes makes possible the detection of morphological abnormalities; and breeding tests show whether these homozygotes are fertile or sterile.

Drs. Pavan, Malogolowkin, Cordeiro, and Wedel, Mrs. Dobzhansky, Mr. Spassky assisted by Mrs. Spassky, and the writer carried out analyses of the second and

third chromosomes in population samples of *D. willistoni* collected in various parts of Brazil. Professor Cavalcanti took charge of experiments of a similar nature with *D. prosaltans*, which, being a rare species, was found mostly as isolated individuals. The results of these rather large-scale and very laborious experiments cannot be reported yet. One can state, however, that the store of concealed genetic variability found in the natural populations of the two above-named species in Brazil is at least as great as it is in the temperate-zone species studied previously. Genetic variants of diverse kinds have been encountered.

CHROMOSOMAL VARIABILITY IN *DROSOPHILA* *WILLISTONI*

Contrasted with the constancy of external traits in natural populations of most species of *Drosophila* is the great variability of structure of their chromosomes. This variability consists chiefly in inversions of chromosome segments. Individuals having two chromosomes of a pair with like gene arrangements (inversion homozygotes) and with unlike arrangements (inversion heterozygotes) are encountered in nature. Flies of the same species carrying chromosomes of different types interbreed at random. Experiments and observations made on *D. pseudoobscura* have shown that the chromosomal polymorphism is adaptive and that it is balanced (see Year Books Nos. 40-47). Inversion heterozygotes, with a single known exception, have adaptive values higher than homozygotes. This being the case, natural selection maintains the polymorphism in natural populations, and yet permits these populations to react by rapid alteration of their genetic composition to even small and temporary changes in the environment. Chromosomal polymor-

phism is, consequently, a part of the actual rather than of potential variability, even though this variability happens to find its expression, in *Drosophila*, in physiological rather than in visible morphological traits.

Samples of natural populations of *D. willistoni* from different parts of Brazil were brought to the laboratory at São Paulo, and the larval salivary-gland chromosomes were studied in their offspring by da Cunha, Burla, and the writer. A chromosomal polymorphism more extensive than that known in any other species of *Drosophila* was disclosed. Inversions were found in all the chromosomes—the autosomes as well as the sex chromosomes. At least 34 different inversions were encountered (as compared with 20 in *D. pseudoobscura*, chromosomally the most variable species hitherto known, in which 15 of the inversions are concentrated in a single chromosome, the third). Taking the Brazilian populations as a whole, individuals that carry no inversions (inversion homozygotes) are definitely less common in nature than are inversion heterozygotes. One of the larvae examined was heterozygous for 16 inversions, which seems to be the highest degree of inversion heterozygosity found thus far in any organism.

Although there is no direct proof that the chromosome polymorphism in *D. willistoni* is balanced and adaptive, this may be accepted as the most likely working hypothesis, by analogy with other species studied in this respect. Chromosomal polymorphism may be regarded, then, as a means whereby the species becomes capable of occupying and exploiting efficiently a variety of ecological niches in the environment in which it lives. The amount of this polymorphism in populations that inhabit different climatic regions of Brazil becomes an interesting problem for study, and efforts have been made to approach the problem from several angles. The re-

suits are not ready to be reported in detail. One fact is clear enough, however; namely, that inversion heterozygosis is more frequent in some parts of Brazil than in others. Thus far, the minimal value was observed in a sample from the *caatingas* in the state of Bahia, where the average number of inversions carried by an individual in the heterozygous state is about 0.8. The maximal value, about 9 heterozygous inversions per individual, occurred in samples from the savanna-gallery forest region of central Goyaz. A high degree of inversion heterozygosis was found also in samples from the tropical rain forests of Acre, Guaporé, and Rio Negro, whereas the rain forests near Belem do Pará have relatively few inversion heterozygotes.

COMPARISON OF CHROMOSOMAL VARIABILITY IN DIFFERENT SPECIES

Drosophila willistoni is the commonest species of the genus *Drosophila* in many parts of Brazil. Collection on different natural baits (see above) showed that this species is also very versatile ecologically, in the sense that it is found on a great variety of foods. It is tempting to correlate these facts with the great amount of chromosomal polymorphism present in the species; and in this connection it is logical to inquire whether, in other species of *Drosophila* that are ecologically less versatile and less common than *D. willistoni*, chromosomal polymorphism is proportionately less. A study of chromosomes was accordingly undertaken, by Professor Dreyfus on *D. nebtidosa*, by Professor Cavalcanti on *D. prosaltans*, by Miss Pereira on *D. sturtevantii* by Dr. Burla on *D. annulimana* and related forms, and by da Cunha, Burla, Pavan, and the writer on *D. paitlistorum*, *D. equinoxialis*, *D. tropicalis*, and other species. The work has not been completed yet, but it is safe to say that no other species so far studied approaches *D. willistoni* in frequency of inversion heterozygosis.

Comparison of *D. willistoni*, *D. pauUstorum*, *D. equinoxialis*, and *D. tropicalis* is particularly instructive. These species are very closely related and are morphologically almost indistinguishable (see below). *D. willistoni* has the widest geographical distribution: it extends from central Mexico and the southern tip of Florida, southward to at least the Rio Grande do Sul in Brazil and the territory of Misiones in Argentina. As stated above, it is in many places the commonest species of *Drosophila*, and it shows the greatest chromosomal polymorphism. *D. pauUstorum* has a somewhat narrower distribution: it is known from equatorial Brazil (territory of Rio Branco, state of Para) down to the coast of Sao Paulo and Parana. Within this distribution region it competes in commonness with *D. willistoni* and is, in fact, more numerous than the latter in some places (Belem do Para) or in some seasons (in summer on the coast of Sao Paulo). Its chromosomes show a variety of inversions second only to that in *D. willistoni*; the average number of heterozygous inversions per individual, although varying from region to region, is fairly high. *Drosophila equinoxialis* and *D. tropicalis* are thus far known to occur only in the basins of the Amazon and the Tocantins—from Belern do Pará to Rio Branco and Rio Negro, and from central Goyaz to Guaporé and Acre. Not only are the distribution regions of these species included among those of *D. willistoni* and *D. pauUstorum*, but even where *D. equinoxialis* and *D. tropicalis* do occur they have rarely been found to be as abundant in their rivals. Only a few Inversions have been observed in *D. eqtti-noxialis* and *D. tropicalis*, and the fre-

quency of heterozygous inversions per individual is distinctly low.

SIBLING SPECIES

Mass collecting of *Drosophila*s in different parts of Brazil led, as was to be expected in a tropical land whose *Drosophila* fauna had been only superficially studied, to the finding of many new species. Unfortunately, our program of research left little time for strictly taxonomic work. Some taxonomic situations had to be straightened out, however, before other types of work could proceed. One such situation arose with the discovery that the flies originally classified as *D. willistoni* actually belong to four distinct species, which are very similar in morphological traits. Following Mayr, such morphologically similar species are called sibling species. Burla, da Cunha, Cordeiro, Malogolowkin, Pavan, and the writer submitted the four sibling species of the *willistoni* group to a comparative morphological, genetic, and cytological analysis.

The four species, *D. willistoni*, *D. paulistorum*, *D. equinoxialis*, and *D. tropicalis*, are reproductively isolated populations, which cannot exchange *genes* with one another. If females of any one of these species are kept with males of any of the other three, few or none of the females are inseminated. This sexual isolation is relatively weaker when *D. paulistorum* males are used; that is, *D. paulistorum* males are accepted more readily by females of the other species than are other heterospecific males. Regardless of whether or not some interspecific matings occur, however, no hybrid offspring are produced.

Whether this is due to early death of the hybrid progeny or to failure of the heterospecific sperm to consummate fertilization is not known. In any case, the reproductive isolation is complete.

Apart from crossing experiments, a safe method of identification of the four species is examination of their salivary-gland chromosomes. Five chromosome strands are present in the salivary-gland nuclei of each of the four species. Four of these strands have disk patterns sufficiently similar in all species so that they can easily be recognized and homologized. The fifth strand, which corresponds to the third genetic linkage group (the third chromosome), is rather similar in *D. paulistorum* and *D. equinoxialis*, but quite differently built in *D. willistoni* and *D. tropicalis*. Examination of the third chromosome is therefore sufficient for identification of the species, except that *D. paulistorum* and *D. equinoxialis* have to be distinguished by less striking differences in other chromosomes.

A detailed comparison by Burla of the external morphological traits of the four species, and a comparison of the genitalia made by Malogolowkin, disclosed several small differences which, after some practice, suffice to distinguish the species. These distinguishing traits are subject to some geographic variation within species, however, which somewhat complicates the situation. Thus, whereas *D. willistoni* from southern Brazil always differs from *D. tropicalis* in the position of the anterior scutellar bristles, *D. willistoni* from equatorial Brazil shows a variation that makes this trait no longer reliable.

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DIVISION OF HISTORICAL RESEARCH

Cambridge, Massachusetts

A. V. KIDDER, *Chairman*

In archaeology it is always difficult, often impossible, to adhere to a prearranged schedule of field work. The archaeologist never knows what lies underground: its quantity, the state of its preservation, or the often entirely unexpected leads and problems it may open up. Also, he must be prepared temporarily to abandon any given undertaking because of some reported discovery which, for one reason or another, must at once be exploited. This is particularly true of work in the Maya area, in which so little actual excavation has been done that one can seldom predict what or how much or how little one is going to find; there is so great an amount of unexplored country that at any time may come word of a ruin of such obvious importance that it must immediately be given at least preliminary investigation.

The finding of the unique wall paintings at Bonampak, described in the last two Year Book reports, was a case in point. Another was the discovery, in 1948, of the extraordinarily rich tomb of the Miraflores phase at Kaminaljuyu, in the outskirts of Guatemala City. The clearing of this tomb not only took the time of two staff members for many weeks and further required many months for the study of the specimens recovered, but also proved that native Middle American culture had reached so high a state of both technological and social advancement at what had formerly been believed to be a still formative period that future investigation of the origin of Middle American civilization will have to be rather radically reoriented.

The Division's troubles at Kaminaljuyu did not end with 1948. The upper benches of the tomb, which lay several meters down

from the top of a 20-m. mound, could not be entirely excavated during that year without burrowing too deeply for safety into a tremendous overburden of hard-packed earth. So, with labor kindly supplied by the Government of Guatemala, the off season was employed in cutting it away, with the result that a second tomb, set somewhat higher, came to light. The extremely difficult task of its excavation and the recovery of its many mortuary offerings was carried out by Mr. Shook.

To Mr. Shook also belongs the credit for identification of a hitherto unrecognized stage of culture older than Miraflores but younger than one we have called Las Charcas. The original find, a burial accompanied by much pottery, was made in the side of a road-cut west of Guatemala City in the Department of Sacatepequez; this name has been assigned to the phase. Subsequently, excavation for the storage tank of a gasoline station near Chimaltenango yielded a further large Sacatepequez collection.

Study of the enormous amount of Miraflores material from Kaminaljuyu, of the Sacatepequez specimens, and of a rich new Las Charcas find has shown that these three phases were sequent stages in the (we believe very long) development of an important pre-Classic highland culture that culminated in Miraflores. The forerunners of that culture, even in Las Charcas times far from primitive, are still unknown, and we cannot as yet explain the apparently abrupt and certainly very great change marked by the opening of the Classic Esperanza phase at some time during the early centuries of the Christian Era.

Establishment of the Las Charcas-

Sacatepequez-Miraflores sequence contributes significantly to knowledge of Guatemala highland prehistory. From the broader view of the history of art and technology, its potential value is great, for it provides a large and fully documented body of data to supplement our scanty knowledge concerning such aspects of cultural growth as developments in the techniques of pottery making and the life histories of decorative styles. Much of our theorizing on these matters has been highly speculative, because based on series of specimens whose relative ages are uncertain. The only weakness of the present sequence—and it is of course a very serious one—is due to our ignorance of the amount of time that elapsed between earliest Las Charcas and latest Miraflores. For all we now know, it may have been two hundred or a thousand years. But there is hope that further perfection of methods for employment of radioactive elements for determination of the age of archaeological specimens may eventually permit reasonably accurate dating.

A second unplanned investigation was made necessary by road work at Asuncion Mita in eastern Guatemala, where in the elimination of a curve some large mounds were being cut down. In one of these, previous digging by pot hunters had exposed an earlier structure containing vaulted chambers. Mr. Strömsvik accordingly went to Asuncion Mita in April to make record of such parts of the building as had been exposed. Both architecture and pottery seem to indicate connections with Copan during the so-called Acropolis Period. During his visit, Mr. Strömsvik took opportunity to reconnoiter the region, mapping and collecting potsherds from a number of other sites.

The final season of Mr. A. L. Smith's survey of the Guatemala highlands was devoted to the Departments of Quiche,

Alta Verapaz, and Chimaltenango. Mr. Smith revisited Nebaj, Quiche, where he made remarkable finds in 1946 and 1947. This year a tomb, located in 1947, was excavated. Among the mortuary offerings of pottery and jade was a vessel, apparently a trade piece from Alta Verapaz, which is believed to date from the early years of the Late Classic Period. If this is confirmed, it will show that part at least of what we have considered the Early Classic Period of Nebaj was contemporaneous with the beginning of the Late Classic of Peten; in other words, that we must recognize a certain amount of cultural lag in the highlands.

After a survey of the San Andres Sajcabaja region of Quiche, where a number of large sites were mapped, Mr. A. L. Smith, accompanied by Mr. R. E. Smith and Dr. Stephen Borhegyi, proceeded to Alta Verapaz. Sites in the neighborhood of Coban and in the upper and middle drainage of the Rio Polochic were examined. Some of these contain remarkably fine construction in which very large and accurately cut stone slabs were used. Potsherds were scarce, but those that were recovered and certain vessels from a tomb make it evident that the ruins in question date from the Late Classic Period.

Finally, A. L. Smith visited the ruins of Mixco Viejo in the drainage of the Rio Grande. From this extensive site, known to have been occupied at the time of the Spanish conquest, he recovered a large collection of potsherds illustrating the hitherto little-known wares of the sixteenth century.

Mr. E. M. Shook, as already stated, excavated a second rich tomb in the great Miraflores phase mound at Kaminaljuyu. The specimens from the tomb, hundreds of thousands of potsherds from the fill of the mound, more thousands from various other mounds at Kaminaljuyu that are

being cut down for brickmaking, and still others from road operations to the west, are being classified and studied by Mr. Shook and the Chairman.

The most important field operation of the period under review was the Campeche expedition of Dr. George W. Brainerd and Mr. Karl Ruppert. Past work of the Division had developed a sound framework of knowledge relative to much of the southern area of the Maya and to northern Yucatan. There existed, however, an intervening area that was but sketchily known and little understood, our lack of knowledge being particularly acute in respect to ceramics. Although this little-known area stretches across the peninsula from coast to coast, it seemed of particular importance to gain knowledge of the so-called Rio Bee and Chenes areas of eastern Campeche. By making a survey of the pottery of these areas it was hoped that relationships might be established with the Peten region to the south and Yucatan to the north, and that the cultural development of Yucatan might thus be anchored more firmly to the relatively well dated cultures of the south. Such linking up of southern and northern areas seemed of particular importance before embarking upon any new operations in Yucatan.

By arrangement with the University of California at Los Angeles and the Southwest Museum, Dr. Brainerd obtained leave from those institutions for a period of six months to carry on this work for the Division. He was assisted by Mr. Ruppert. During the winter and spring they conducted excavations at the Chenes sites of Santa Rosa Xtampak and Dzibilnocac, and at a number of Rio Bee locations centering about the site of Xpuhil. At the time of writing this report we record that the materials collected are being analyzed; the study has progressed far enough, however, to indicate that the Chenes-Rio Bee archi-

tectural styles and the associated pottery are in part contemporaneous with the Puuc remains in Yucatan and with the Late Classic (Tepeu) in the Peten. The Peten association should provide approximate dating, in terms of the Maya calendar, of Chenes-Rio Bee and Puuc remains, a dating that has at times been hotly debated. There is every reason to hope that the work of Brainerd and Ruppert has made an important advance toward solving the relative chronology of the northern, intermediate, and southern areas, and that the work also will throw light on cultural influences between areas.

Laboratory and desk work again occupied a large proportion of the efforts of the staff. Miss Shepard has continued her work in ceramic technology. A special project dealing with pottery of the southwestern United States and designed to acquaint archaeologists more generally with the applications and significance of ceramic technological data is nearing completion. Miss Shepard has also given time to the preparation of a ceramic handbook for the use of archaeologists. Miss Proskouriakoff has brought to completion her initial studies of Maya sculpture. It is anticipated that this work will go to press by the end of this year. During the period under review Mr. Thompson completed and submitted the manuscript of the introductory volume of his studies of Maya hieroglyphic writing. This volume is now in press. Similarly completed and in press is Mr. A. L. Smith's work on the excavations at Uaxactun. Prior to entering the field with Dr. Brainerd, Mr. Ruppert continued his study, preparation, and arrangement of unpublished materials dealing with earlier activities of the Institution at Chichén Itza. Dr. Morris made further progress toward the publication of his researches in Southwestern archaeology. Although by

far the greater portion of his efforts was devoted to administrative duties, Dr. Pollock gave some time to his study of Yucatan architecture.

Dr. Norman A. McQuown, by arrangement with the Department of Anthropology of the University of Chicago, again gave part of his time to the Institution for researches in Maya linguistics. Dr. McQuown spent five months in Guatemala, working mainly on the Mam language, but giving some attention to other highland Maya languages, to Xinca, and to arranging for collaboration with a number of individuals in the field. Dr. McQuown's field trip marks the end of active participation by the Institution in the field of Maya linguistics. It is hoped, however, that the work will continue under the auspices of the Department of Anthropology of the University of Chicago.

Mr. Roys spent two months in Yucatan in continuance of his studies of native Maya literature and the history of the Maya area. As in the past, he devoted much of his time to field research bearing upon the political geography of the region at the time of the Spanish conquest. He also gave special attention to the search for conquest-period sites that might merit particular attention under the Division's proposed program of operations in Yucatan.

Most of the above-noted activities have been devoted to winding up the over-all study of the Maya and to clearing the deck for intensive research on the archaeology and history of northern Yucatan. In focusing its attention on this field, the Division is returning to the area in which, over forty years ago* opened the long and fruitful career of Sylvanus Griswold Morley as a Mayanist.

With Morley's death on September 2, 1948, the Maya, ancient and modern, lost

their most tireless and effective advocate. His whole adult life was dedicated to the furtherance of Maya research. That he was able to accomplish so much was due to a unique combination of scholarly ability, skill as a promoter, unbounded energy, and limitless persistence. His firsthand contributions as explorer, recorder of texts, and student of the hieroglyphs were outstanding. His driving enthusiasm resulted in the entrance of Carnegie Institution into the Maya field. He lost no opportunity to induce agencies to co-operate or to undertake independent investigations. He was thus largely responsible for the fact that so many persons have been able to devote themselves to Maya studies.

Morley early realized that in the last analysis any branch of research is made possible only by popular understanding of its aims and appreciation of its value. He was therefore tireless in publicizing the Maya by lectures and writings. With the same end in view, and also to preserve for the future the finest examples of Maya architecture and sculpture, he inaugurated at Chichen Itza and continued at Quirigua and Copan the custom of stabilizing and repairing excavated buildings and re-erecting fallen monuments. This has greatly helped to stimulate interest in their antiquities on the part of the governments of the countries in which these ruins are situated, and has led to the preservation of much that might otherwise have been lost.

Morley's scientific, practical, and promotional accomplishments were many. But, in the long run, undoubtedly the greatest was his success in inculcating confidence in the good faith of American scientific agencies and in bringing about the close and cordial relations, both personal and intellectual, that now exist between those scholars of the United States

and of Latin America whose common interest is in the prehistoric past of the New World.

Shortly after the close of the period under review, Dr. George Sarton will retire from the staff of the Division. His retirement will terminate an association of thirty-one years. Dr. Sarton's unceasing labors in the history of science have resulted in transforming a previously unorganized and largely unrecognized field of study into a recognized discipline. Though his retirement will mark the end of the Institution's activities in the history of science, his efforts have assured a continuing interest, both in this country and abroad, in this field of learning.

On June 11, 1949, the Chairman received the honorary degree of Doctor of Science from the University of Michigan.

GUATEMALA HIGHLANDS

EDWIN M. SHOOK

The cutting away of Structure E-III-3, Kaminaljuyu, to obtain material for bricks continued throughout the past year. The great mound, as previously reported (Year Book No. 47, pp. 215-217), is the largest individual structure of some two hundred which make up the ruins on the southwest edge of Guatemala City. The salvaging of the invaluable archaeological material and data from the excavations was possible through the co-operation of Sr. Moises de Leon, superintendent of the government brick factory. He not only placed several laborers under our supervision, but permitted his workmen to be shifted here and there on the mound to facilitate the recording of the exposed remains.

Information was gathered on the architectural development of Structure E-III-3 from a small semicircular adobe unit about 2 m. high (no. 1) through six successive major additions. The final stage (no. 7)

was a massive, flat-topped, rectangular pyramid over 20 m. in height and measuring 70 by 90 m. at the base. Ceramic material obtained from the fill of each unit indicated that the long sequence of architectural activities took place during the Early and Middle Miraflores phases of the Pre-Classic Period. The lavishly stocked Tomb 1, reported in Year Book No. 47, had been cut through the top of Structure 5. A similarly constructed and equipped tomb, no. 2, was found during the past field season. It had been dug through the top of Structure 6, the next pyramid, which completely encased Structure 5 and Tomb 1.

Tomb 2 occupied a position just west of and higher than Tomb 1, its east wall actually penetrating the upper west bench of the older tomb. Although less carefully constructed and less richly furnished, Tomb 2 provided more information because it had not been disturbed by the brick-factory workmen as had Tomb 1. Prior to the roofing of the tomb with timbers, the principal individual, a mature adult, had been laid horizontally, head to the south, on a low wooden table or platform resting on the tomb floor. The body was completely painted red and probably dressed or wrapped. Two children, about eight years of age, had been sacrificed and placed in an extended position on the tomb floor just west of the principal body. Another skeleton, that of a young adult, lay extended, face downward, on one of the east benches above the roof of the tomb. Over one hundred pottery vessels, some containing ash and charcoal, had been placed on the surrounding benches after the timber roof was laid.

In contrast with Tomb 1, only a few pottery vessels were found on the tomb floor. Here, however, were most of the nonpottery objects: mica sheets, sting-ray

tails, fossil fish teeth, stuccoed containers (possibly gourds), quartz crystals, water-worn stone pebbles, basalt implements, bone spatulas and ornaments, mosaic mirrors of pyrite and of crystalline hematite, obsidian pellets and flake-blades, tubular jade beads, mosaic sets, and a mask or headdress heavily incrustated with jade elements. The last was found face down off the southeast corner of the wood platform that had supported the principal body. The decay of the platform, or a fragment of falling roof timber, may have rolled the object to the tomb floor. The backing of wood and leather, we believe, was still firm when this occurred, and the heavy jade elements remained in position until falling material settled around and covered the whole. This earth fortunately maintained the original form of the object fairly well after the backing rotted, and the recording and removal of the jade elements more or less in their original positions were possible. The headdress or mask has been reconstructed by the artists of the Archaeological Museum of Guatemala.

After Tomb 2 was roofed, additional offerings were placed on the benches and the large space was filled with earth from the roof level to the floor of Structure 6. An adobe floor then covered the entire tomb area, permitting the use of the pyramid top again. At the same time, we think, the last great increment to **E-III-3** was started. Prior to the completion of Structure 7, which added some 4 m. to the *total* height of the mound, Tomb 2 was re-entered *unit* partially rifled. The looters disturbed only the principal skeleton, from the pelvic region to the head; we found none of those bones in place. We did find scattered human bones and teeth in earth mixed with red paint, well above the tomb floor, apparently from the main skeleton *set aside* by the looters. The jade ornaments necklace, *and* pendants

which normally would adorn a person of such obviously high rank were entirely missing. The jade-incrusted headdress and beads lay oil to the sides of the wood platform and were overlooked by the looters, perhaps because they were covered by debris.

The evidence strongly suggests that only one generation (twenty-five years or less) elapsed between tombs. The occupant of Tomb 2 may have been the immediate successor of the individual buried in Tomb 1. Some striking changes had taken place in Kaminaljuyu in those few years. A higher percentage of Usulután ware vessels was found in Tomb 2, many of them similar in shape to fine, incised red ware bowls, with everted rims and three sharply pointed nubbins. The latter vessels, although among the most common in Tomb 2, did not occur in Tomb 1. They apparently developed during the interim for specialized ceremonial uses, and often are found containing ashes and charcoal. As a result of the burning, the bowls' interiors frequently are blackened. Tomb 2 contained no "mud" pots or frog effigy vessels of fine red ware, as did Tomb 1, no stone vessels, mortars, or "mushroom" stones. The less care shown in the construction of Tomb 2, the fewer offerings, and the evidence of looting may indicate that the principal individual buried there had not been so successful a ruler or priest as his predecessor, or that economic conditions at the time of his death were more severe than at the beginning of his rule.

Structure E-III-3 is only one of a number of ancient structures being dismantled in Kaminaljuyu. Periodic visits to the various excavations helped to gather much information **that** otherwise would have been lost. Fortunately, one of the principal mound groups has been set aside through the civic interest of the owner,

Sr. Arturo Samayoa, to be preserved as a national monument. The National Institute of Anthropology and History has appointed a caretaker, whom we have trained, to maintain the monument and to collect archaeological material as it is excavated elsewhere in Kaminaljuyu.

Modern road construction and trenching for pipe lines led to more discoveries in the past year that amplified knowledge of the oldest known ceramic phase in the Guatemala highlands, the Las Charcas, and of the subsequent Sacatepequez phase. Both of these predate the long Miraflores phase. An ancient pit, containing a rich deposit of apparently pure Las Charcas material, was found in Colonia Progreso, southeast of Mound B, Kaminaljuyu. The material, sealed by a heavy layer of obsidian flakes, included a wide range of ceramic types illustrating many specialized features. Among the latter were spouted and shoe-shaped vessels, effigy monkey heads broken from vessel walls, small solid crudely hand-modeled monkey figurines, two cylindrical and two stemmed flat seals or stamps. Also found were many burned adobe fragments bearing impressions of leaves, corncobs, and other vegetable objects, carbonized seeds of several fruits, and ashy remains of woven fibrous material. The Colonia Progreso find represents the first large lot of Las Charcas material recovered by a controlled excavation.

Further search along the newly constructed Roosevelt Highway west of Guatemala (see Year Book No. 47) disclosed more deposits of the Sacatepequez phase, usually in deep rectangular or circular bottle-shaped pits. The pits occur sporadically along the road from San Bartolome Milpas Altas to Chimaltenango. Archaeological sites with mounds were located in the vicinity of the pits, but direct associations were difficult to ascertain, because the surface material collected from

these sites shows that occupancy continued into later periods. The ruins, in order along the highway, are Xaraxong, Chacaya, Santa Maria Cauque, Manzanales, Los Pinos, San Roberto, Rio Sumpango, Tejar, Tejarcito, Santa Fe, El Rancho, and Santa Sofia. The last six sites are in the Chimaltenango Valley; the others, in the Rio Sacatepequez Valley.

Advantage was taken of an opportunity offered by Mr. Hugh Craggs, of Guatemala City, to accompany him on an automobile trip through southeast Guatemala, Salvador, and Honduras. A brief reconnaissance was made of eastern Salvador, the coastal plain of Honduras north of the Gulf of Fonseca, and the highland valleys of Zamorano, Tegucigalpa, and Comayagua. The archaeological museums of Comayagua and Tegucigalpa and the ruins of Tenampua and other sites were visited.

Firsthand observation of the topography and climatic conditions of eastern Salvador and of southern and central Honduras directs attention to the importance of the natural intercoastal corridor running from the Pacific coast via the Rio Goascoran Valley to the upper Comayagua, thence to the Ulua Valley and north coast of Honduras. Through this natural funnel must have flowed not only aboriginal commerce, but forces affecting the cultural development of much of southern Mesoamerica. Eastern Salvador and the upper Comayagua Valley have a concentration of ancient remains showing occupancy over the known range of pre-Columbian history. It is most desirable that a study be made of the Rio Goascoran drainage from the Pacific lowlands to the upper Comayagua Valley.

Shook also visited the archaeological zone of Chalchuapa, where the Salvador Government for several years has carried on intensive excavation and repair of the

principal structures, under the able direction of Mr. Stanley H. Boggs.

In southeast Guatemala a record was made of the small, hitherto unreported site of San Juan Las Minas, 1 km. west of Asuncion Mita. The extensive, well known ruins of Asuncion Mita, south of the modern town, have been noted for their excellent state of preservation, the quality of the thin-slab masonry, and the corbeled arch used by the builders to roof their structures. This is the most southern site on record where the Maya-type vault was utilized. Despite the importance of these ancient buildings, they were being destroyed to obtain stone for road construction. Shook submitted a report to the Institute of Anthropology and History which curtailed the quarrying of the site except for the main structure, already beyond saving. Strömsvik later visited Asuncion Mita and obtained as full a record as possible of what remained (see p. 231).

In 1945 the Guatemala Government began an agricultural colonization project in the southeastern corner of the Department of Peten. Poptun, a small village near the headwaters of the Rio Machaquila, was selected for the permanent site of the colony and renamed Poptun. The operation entailed intensive land clearing for agricultural experimentation, surveys, building of the physical plant, and opening of a highway to Cardenas on the Rio Sarstoon. The road, to be completed in 1949, is being constructed from Poptun south through San Luis and north from Cardenas. Since 1945, reports have been received repeatedly from individuals and through the local press of archaeological discoveries made in Poptun, Cardenas, and along the highway. This past year the governor of the colony, Col Ernesto Alvarez, invited the Institute of Anthropology and History and Carnegie Institution

to visit Poptun and report on the finds. Lie. Adolfo Molina, then Director of the Institute, R. E. Smith, and E. M. Shook went to Poptun in November and spent four days recording the cultural material, and mapping and photographing the archaeological sites.

Five ancient sites—Los Cimientos, Hortaliza, Sabana, Poptun, and Petensuc—were studied briefly during the visit. They lie roughly in an east-west line, north of the modern settlement, between the drainage of the San Pedro and Machaquila Rivers. The remains of Poptun are the best preserved architecturally. They undoubtedly represent what was the region's civic and religious center. There are three known groups, South, Central, and North, connected by a broad artificial causeway with a low masonry parapet on each side.

The North Group consists of five rectangular platforms of fairly well cut stone masonry on top of a leveled and terraced limestone hill. The southern base of the hill also was terraced, but no masonry stairway connected the lower with the upper terraces. Apparently a pathway zigzagged up the steep hillside to the structures on top. The causeway from the South and Central Groups joined the great basal terrace of the North Group. Here a single plain stela of limestone was erected.

No evidence was found in Poptun or in the small outlying sites that the Maya here used the typical vault or even masonry buildings. All the masonry noted in the area was for substructures, terraces, and low walls bordering courts, terraces, and causeways. The substructures were low rectangular platforms to support houses and temples constructed of perishable materials.

Poptun evidently was never a major center. It may have served the civic and religious needs of a comparatively small

population scattered widely throughout the area. Although there is evidence from the cultural material of occupation from Pre-Classic (Chicanel) to Late Post-Classic time, the period of greatest activity was during the Late Classic (Tepeu).

Engineers of several American oil companies have for the past two years been making an intensive topographical survey of the Department of Peten. They have discovered many hitherto unreported archaeological sites and caves containing ancient cultural material, and deposited their data, maps, and specimens with the Division. Dr. Barnum Brown, of the Sohio Oil Company, made important discoveries along the Rio de la Pasion from the junction of the Santa Amelia to the confluence of the Rio Salinas or Chixoy. He recovered quantities of petrified bones of Upper Pleistocene fauna on bars and barrier reefs along the Pasion. They had been washed out from deposits somewhere on an eastern tributary of the stream, possibly the Rio Santa Amelia. One bone fragment, as yet unidentified but thought to be that of a sloth, has three sharp V-shaped cuts on the unbroken external surface. These cuts, according to Dr. Brown, were made in living tissue. If the cuts were made by man, as Dr. Brown thinks, this is the first evidence found in Guatemala of man associated with now extinct fauna.

Dr. Brown also discovered an artificially built island in the Rio de la Pasion, above the ruins of Seibal, and a previously unreported sculptured stele at the latter site. He collected large samples of pottery from various points along the river, including Seibal and Altar de Sacrificios. The ceramic material shows a range of occupancy in southern Peten from the Chicanel phase of the Pre-Classic through the Early and Late Classic periods.

Two efforts were made during the spring

of 1949 to locate the conquest town of Nito, an important commercial center of the Indians. The town was conquered by Gil Gonzalez in 1524 and renamed San Gil de Buena Vista. Apparently the site proved unsuitable to the Spaniards and they soon abandoned it for a new location on the Rio Duke near the sea. The Spanish name, often reduced to San Gil, as well as the Indian name Nito, continued to be applied synonymously to the new town site where, in April or May 1525, Cortes arrived after his extraordinary march overland from Mexico to Honduras. Cortes found his countrymen starving at San Gil and shortly after his arrival he moved them to Honduras. Since the abandonment in 1525, both sites, San Gil near the sea and Nito the original Indian trading town, have been unoccupied and their exact locations lost. It is of historical significance to place these sites and especially to obtain from Nito data on cultural material anchored firmly in Christian chronology.

A launch and other facilities were generously provided by the United Fruit Company through the courtesy of Mr. William Taillon and Mr. Edward Farnsworth. The latter accompanied Shook on the second expedition. Two areas were intensively investigated. The first was the south side of the Golfete, the body of water connecting Lake Izabal with the Rio Dulce, which existing information suggested as the most likely area in which to find Nito. Several days were spent along this shore, penetrating the dense tropical jungle wherever possible and pushing a dugout canoe up the tributary streams. Finally an elderly native of unusual intelligence was encountered. He knew the area well, including a navigable stream, an abandoned property, and a site with mounds, all still known as San Gil. He had discovered and removed a frag-

mentary bronze bell from the site about eighteen years ago. The bell was later seen and recorded by Shook, and efforts were made to have it sent to the Guatemala Museum.

We followed the Rio San Gil upstream for approximately 3 km. from the Golfete. Only the first kilometer was navigable in a dugout canoe, the next 2 km. having been blocked by fallen trees and by sand bars, but the entire distance would be navigable by canoe if the stream channel were cleared. At the 3-km. point two shallow, swift-running branches join to form the Rio San Gil. Here the land is level, fertile, and at present heavily overgrown with dense tropical rain forest. This latter condition so obscured the area that, though we searched over a wide zone, we were unable to locate the mounds where the bell had been found. The general locality, Shook nevertheless believes, must have been the site of Nito. Strong evidence for it is the survival of the name San Gil for the place, the river, and a high mountain rising immediately behind, as well as availability of a fine water supply and *access* by canoe to the Golfete.

The second area investigated extends about 4 km. along the south bank of the Rio Duke, from its mouth opposite Livingstone to the foot of the mountain range paralleling the coast. Here undoubtedly was the later settlement of San Gil de Rueea Vista, where Cortes found the Spaniards. Most of the 4 km. may be eliminated as unfit for settlement because of swamps and lack of fresh water. Two places were found to offer adequate facilities to sustain a small colony. One, on elevated and well drained ground 2 km. southwest and upstream from Livingstone, still is known as Buena Vista. An excellent spring of fresh water issues from the base of the hills only about 50 m. back from the Rio Duke. No ruins were visible,

but reports from the local people tell of ancient artifacts being found when the land is cleared for corn planting. The other possible location is on a high bluff at the outlet of the Rio Duke, known today as Herreria Point. A fresh water supply from two small streams is available and there is a certain amount of cultivable land. A small preconquest Indian site (Herreria) was found overlooking the Caribbean Sea 500 m. south of the Rio Duke. Broken china, crockery, and glass bottles gave evidence of occupation during Spanish colonial and modern times, but nothing definitely assignable to the early colonial period was found.

While awaiting transportation in Puerto Barrios, Shook mapped and recorded a previously unreported site called San Manuel. The ruins are situated on the south bank of Rio Cacao and are cut by the auto road to Santo Tomas. The principal structures are *so* grouped around a plaza as to form an oval, in contrast with the normal rectangular arrangement. Some forty or more mounds, the majority low house platforms, compose the site. All are substructures built of earth. The largest structure, about 5 m. in height, has its surface partially faced with water-rounded boulders. No ball court, stone sculpture, or surface pottery was found to suggest the occupation period of the site.

GUATEMALA HIGHLANDS

A. L. SMITH

The 1948-1949 field season was the final of four seasons devoted to archaeological reconnaissance in the Guatemala highlands. As in previous years, the main purpose of the expeditions was to obtain as much information as possible in a minimum period of time and with little excavation. In most cases sites were roughly mapped, detailed measurements were taken

of individual buildings, a photographic record was made, and pottery was collected. In some instances perspective sketches were made of whole sites or of individual groups. The season included expeditions to sites in five areas: Sacapulas, Nebaj, San Andres Sajcabaja, all in the Department of Quiche; various sites in the Department of Alta Verapaz; Mixco Viejo in the Department of Chimaltenango.

Mr. A. L. Smith, assisted by Sr. Gustavo Espinoza, began the season at Sacapulas and visited three sites: Chutixtiox, Xolpacol, and Chuchun. At Chutixtiox, a late hilltop site, a tomb was found associated with an early structure. Unfortunately it had been robbed in ancient times, but the excavation yielded a few objects from the tomb and from the entrance.

Xolpacol, also a late hilltop site, proved, although small, to be extremely interesting. Among its ten structures were a well preserved late-type ball court, a small round platform supporting a round superstructure with four doorways, and a low platform upon which rested twin temple mounds. The main temple mound stood in the center of a small plaza. It had been dug into and a tomb exposed (see Year Book No. 46, 1946-1947, p. 185). A very good sample of pottery was recovered from the surrounding fields and down the steep slopes which had been used as refuse dumps.

Chuchun is a small valley site. Of the eight structures noted, the most important were an open-end ball court and two fairly large temple mounds. A small surface collection of pottery was recovered*

Leaving the Sacapulas area, Smith, accompanied by Sr. Cesar Tcjeda, assistant archaeologist, and by Mrs. Smith, moved to Nebaj. For the remainder of the field season Mrs. Smith took charge of the food and the gathering of surface pottery collec-

tions. While in the Nebaj area time was found to visit Finca San Francisco, belonging to the Brol family; from here the ruins of Chikal were visited.

The major effort at Nebaj was the further investigation of the large mound excavated during an earlier field season (Year Book No. 46, pp. 185, 186). A tomb was found that proved to be of the same ceramic period as the earliest tomb previously discovered, late Early Classic or early Late Classic. The grave was relatively rich in furniture, and additional material was found in the dirt that had caved in from the sides of our original excavation. Before leaving the site, we completely filled the hole.

Two small groups of mounds just north of the main ruins of Nebaj were mapped. Both groups have open-end ball courts leading into small plazas with an altar mound in the center, the ball court being the principal structure in each group.

The site of Chikal, which is of the Huil type, namely, open-end ball court and adjoining plaza with an altar in the center of the latter, is in a very poor state of preservation. The ruins are used as a corral, and stones from the structures have been removed to build walls. No map of the ruins was made, as Burkitt had published one. No pottery was found at the site.

From the Nebaj area the expedition moved to San Andres Sajcabaja. This area had previously been reconnoitered from the air (Year Book No. 46, p. 187). Eleven sites were visited: La Lagunita, Xcpom, Xolja Alto, Xolja Bajo, Pantzac, Patzac, Llano Grande, Los Cimientos, La Iglcsia, Chuscap, and Xabaj. These were located on a tracing of a map of the municipality.

La Lagunita, a large site, consists of two groups separated by a deep ravine. Group A has fourteen structures, an enclosed court, and several plazas. In the

main plaza is a plain stela, beneath which someone had dug. Six of the mounds are large, but no traces of superstructures were found. Group B, with thirteen structures, has two possible ball courts, one with open ends, the other closed but with no apparent end zones. The rest of the structures are platforms surrounding courts or small plazas. Both groups are surrounded by deep ravines and lend themselves to defense. Pottery from the site seems to place it in the Late Classic Period.

Xepom, Xolja Alto, and Xolja Bajo are small sites. Xepom and Xolja Alto are on the tops of hills overlooking the Rio Agua Caliente; Xolja Bajo is in the river valley. Very few architectural details of interest were found and only a small amount of pottery was collected. From the sherds recovered, Xolja Alto and Xolja Bajo appear to have been occupied in Late Classic times.

The largest site visited in this area was Pantzac. The ruins are not unknown, having been mapped by Sapper and briefly described by Maudslay. Sapper's map is extremely accurate and needs few changes. There are thirty-eight mounds still in existence, but Sapper shows others which have been razed; many of the architectural details that he indicates have long since disappeared. The masonry is of well cut and faced blocks which the inhabitants now living in the ruins use to build their houses and fences. The site divides itself into about five groups containing temple mounds and long structures surrounding plazas and courts, altar mounds, platforms, and a sunken court. There are two ball courts, one with open end zones and an adjoining plaza not unlike the Huil type, another with end zones probably closed with low walls. Pottery, found in quantities in the surrounding fields, places the occupation of the site in plumbate times. Although probably not built as a defense

site, Pantzac lies *on* a plain surrounded by deep ravines which would afford easy defense.

Patzac, like Pantzac, is surrounded by deep ravines. It is a large site having thirty-four structures. These are mostly in the two main groups, the rest being in scattered groups of two and three mounds. Each of the main groups has a ball court with closed end zones. Other structures take the form of temple pyramids, platforms, and altars. Several of the structures support low walls of superstructures. The pottery collected shows that this site was occupied at the time of the conquest.

Llano Grande lies in a plain, and the site definitely was not built with any idea of defense. The ruins consist of a large rectangular platform, reached by a stairway on its south side, and supporting four mounds and a sunken court. The last, which is at the west end of the platform, is probably a ball court. To judge by the pottery recovered, the site was probably occupied during the Late Classic Period.

The best-fortified site visited in the area was Los Cimientos, completely surrounded by steep ravines, the only entrance being over a causeway. Nine structures at the highest point comprise the ceremonial center. Eight of these follow the edges of terraces and form the sides of a small plaza; the ninth structure, a temple mound, is in the plaza. Two of the nine structures are temple mounds, seven are low platforms, most showing signs that they once supported superstructures. The main feature at the site is the terracing on its east, south, and west sides. The terrace walls are built against natural levels running in all directions and varying in height. Reaching to the side of the ravine, the terraces are quite extensive and may once have been used as sites for houses of perishable material. Surface pottery is

scarce, but a small collection places Los Cimientos in the conquest period.

La Iglesia, the main structure of which has been turned into a crude modern church, has two groups. Group A lies on a large oval hill which rises about 50 m. above the surrounding plain. It consists of a rectangular court surrounded by a low wall with an entrance in the south side. All but one of four platforms in the court carried stone superstructures. The superstructure of the largest was of great interest, as the walls stood to considerable height in some places, and the method of construction could be examined in detail. A good sample of pottery of the plumbate period was obtained from this group. Group B is on the level of the plain. It is made up of a plaza with a large rectangular platform on each of three sides. No masonry was showing, and there was no evidence of superstructures.

Chuscap was visited because of a reported subterranean passage that had never been explored. It turned out to be a bottle-shaped hole in the center of a small field on a hill near the Rio Agua Caliente. In shape it resembles a *chultun*, having a narrow neck for an entrance, with stones around the top, which was covered with a stone lid. Nothing was found in it and its purpose could not be determined. No structures of any kind were found in the vicinity.

The last site visited in the San Andres Sajcabaja area was Xabaj. It is a small group of three platforms strung out along the top of a hill high above the plain. The platforms, which are almost square, still support the remains of superstructures. Two of the structures had each a small modern shrine on top, and the third had six. Several of the shrines contained small carved monuments. No pottery was recovered. According to the local inhabi-

tants, Xabaj is an important place of worship today.

The fourth expedition was to Alta Verapaz, the personnel being Mr. and Mrs. Smith, Mr. R. E. Smith, and Dr. Stephen Borhegyi. Ceramic studies were in the charge of R. E. Smith and are reported by him below. Perspective sketches of ruins were made by Borhegyi. The following sites were visited: Esperancita, Tampoma, Omaxa, Canchunac, Las Tinajas, Pueblo Viejo, Seacal, Chijolom, Chichen, Santa Elena, Chicuxab, Valparaiso, and Chin-chilla.

The expedition's first base was Finca Mocca, belonging to Mrs. Robert Hempstead. The finca lies northwest of La Tinta, a village on the Rio Polochic. The first ruin visited was Esperancita, a small site consisting of eight mounds and several terraces on top of a sloping ridge. The ridge rises in natural terraces, the upper three having been artificially leveled and faced with rough stone slabs. Large stairways lead from one terrace to another. The lower terrace supports the main group. Here six long low platforms surround a small plaza with a rectangular platform in the center. The second terrace supports a long low platform. There were no structures in the third. The platforms are not oriented to one another, as they follow the contour of the ridge. A sketch was made of this site.

Tampoma has five small groups spread out on the flat valley floor. A small river, the Tampoma, flows through the ruins to join the Polochic. The largest group has what appears to be a ball court with an adjoining plaza with long low platforms on three sides. Off to one side is a low mound with a plain stela and altar in front of it. The other groups had three or four mounds each, surrounding small plazas.

At Omaxa, close to Tampoma, are several small mounds and one large one. Un-

fortunately the site was so overgrown that no work could be done. Canchunac, west of Finca Mocca, consists of a series of terraces and one mound.

After leaving Finca Mocca the expedition moved to Finca Las Tinajas on the south side of the Rio Polochic, where two ruins, Las Tinajas and Pueblo Viejo, were visited.

The ruins of Las Tinajas, also called Tzesac and located on Sapper's map as Chacujal, are close to the Rio Tinaja, which flows into the Polochic. It is possible that this may be the Chacujal mentioned by Cortes in his fifth letter to the Emperor Charles V. It is a large site, at present badly overgrown. Twenty-six structures were found, a number of which supported the remains of stone superstructures. Two temple platforms stood in the center of a large plaza. The buildings were constructed of a hearting of clay and water-rolled stones faced with thin stone slabs laid in clay. It is worthy of note that no clay or thin stone slabs seem to occur in the vicinity of the ruins. A sketch was made of the site.

Pueblo Viejo is an extensive ruin not more than a kilometer west of the ruins of Las Tinajas. The mounds are piles of water-rolled stones with only a few instances of thin slabs around the base. It is very possible that these mounds formed a part of Las Tinajas that was never completed. No map was made of Pueblo Viejo because of its overgrown condition.

The expedition next proceeded to Finca Scamay and thence to Finca Arena!, both of which belong to Mr. George Kocster, and which lie north of the Rio Polochic not far from the village of Senahu. At Arenal two sites, Seacal and Chijolom, were visited.

Seacal lies on top of a steep hill covered with outcroppings of rock of the type used in the masonry at the site. The main

feature of this small ceremonial group is a rectangular sunken court with sides of large, beautifully cut and faced stone blocks. The court is surrounded by rectangular platforms on the ground level above it. A sketch was made of the site.

Chijolom, a small group in which the ball court is the main feature, lies in the saddle between two hills. The ball court is the open-end type and has its sides built against the two hills. On one side terraces and stairways rise farther up the hill. Beyond a rectangular platform at either end of the ball court, terraces extend down into ravines. The masonry, the most beautifully cut and fitted seen in any part of Guatemala, was laid without the use of mortar. A sketch was made of the group, showing details of stonework.

After leaving Arenal the expedition moved to Coban, once more as guests of Mrs. Hempstead. Three sites were visited: Chichen, Santa Elena, and Chicuxab.

The ruins of Chichen are about 30 m. above the north side of the Rio Chichen. Almost all the seventeen structures are oriented to one another and are grouped around three plazas. There are rectangular platforms, altar platforms, a temple mound, and two open-end ball courts. The last are very similar to the ball court at Chijolom. No evidence of superstructures was found. A sketch was made showing the entire site.

Santa Elena, south of Coban, is a small valley site of four platform mounds, three of which are oriented around a small plaza. The fourth mound lies to the north of the plaza group. Chicuxab, a three-mound site, lies in a hollow in the hills. The three platforms are almost shapeless from having been plowed over.

After leaving Coban the expedition returned to Guatemala City via Salama. Between Coban and Salama two sites, Valparaiso and Chinchilla, were examined.

Valparaiso, a small site lying north of the Rio Coban, had been visited by Shook and Smith some years ago, when a collection of surface pottery was made and the site was roughly mapped. We now attempted to obtain more pottery, but the owner was away and the resident Indian family would not permit any work.

The two groups which make up the ruins of Chinchilla are on the slopes of two adjacent hills on the north side of the Rio Frio. The site was previously seen by Dr. Kidder and at that time was in a much better state of preservation. The main group consists of five structures: four platform mounds are oriented around the four sides of a small plaza, in the center of which is a large temple platform. The other group consists of three small mounds.

The final trip of the season was by Mr. and Mrs. Smith and Borhegyi to Finca Las Pilas, belonging to Sr. Francisco Martinez del Rosal, in order to examine the ruins of Mixco Viejo, a conquest site mentioned in early accounts. The ruins have been mapped by Sapper. Mixco Viejo consists of twelve groups spread along the flat top of a ridge completely surrounded by steep ravines. It is a well fortified place which can be entered only by a steep narrow trail, easily defended. The larger* groups consist of structures surrounding, in each case, a plaza, often with a temple mound or platform in the center. The structures are not oriented to one another because they follow the edges of the ravines. In many cases the upper edges of the ravines are faced with stone. On the south side there are natural terraces where the abundance of refuse indicates that this may have been an area where houses of perishable material once stood.

The sixty-eight or more structures at the site consisted of temple platforms, long platforms with three or more stairways on

one side, altar mounds, long low rectangular platforms, twin temple platforms on a single platform, and two ball courts. The last were of the conquest type with closed end zones and a stairway at the center of each end. Although many of the structures must have had superstructures, no evidence of them was found. A sketch was made of Group B, one of the largest groups, that contained the greatest variety of structures. A large surface collection of pottery was recovered, but nothing was found in the several pits that were dug in platforms. The surface pottery contains some Early Classic, a good deal of Late Classic, and an overwhelming amount of conquest period wares. No plumbate period pottery was found.

GUATEMALA HIGHLANDS

ROBERT E. SMITH

The pottery findings of the Alta Verapaz archaeological survey were quantitatively small but typologically significant. The hilltop sites, Seacal, Esperancita, Chijolom, Chinchilla, and Samac, were especially lacking in potsherds with the exception of Seacal, which contributed about a hundred. These sites, including the ball-court site of Chijolom, probably served as shrines, and therefore ceremonial pottery normally associated with tombs and caches was mostly used. In their function the Alta Verapaz hilltop sites differ markedly from those of other sections of the country, which appear to have been fortified retreats and where quantities of utilitarian as well as more elaborate pottery are found.

A tomb with skeleton, six pottery vessels, and bone ornaments was uncovered at Seacal. Two of the vessels equate with Tcpeu 3 types. A cache in Mound 1 at Esperancita contained two pottery vessels suggesting a Tcpeu 1, 2-Chipoc-Chama 3,

4-*Alta Verapaz II* horizon. In addition, some sixty sherds were recovered from three sizable trenches. None were found on the surface. At Chijolom a trench was dug across the center of the ball court and a pit was sunk into the center of the northeast platform mound, but the only place from which potsherds were recovered was a low terrace off the southwest end of the ball court, where there was a vegetable garden. Here some thirty sherds were gathered from the surface. These indicate a *Tepeu 3* to *Tohil*, or even later, horizon. The pottery picked up from the surface of the larger group at Chinchilla included two faceted, red ware, Z-angled sherds of *Miraflores-Chicanel* type. The other eight fragments recovered were too small and weathered for identification. A tomb, excavated on *Finca Samac* many years ago, included a skeleton and five pottery vessels. One of the latter is a *Tohil* plumbate specimen which dates this grave as *Post-Classic-Tohil* phase according to Dr. Wauchope, or as *Alta Verapaz III* according to Dr. Butler. On top of the hill upon which the tomb was discovered we were fortunate in finding a ready-made cut, exposing a section filled with potsherds ranging from *Chipoc* (*Tepeu 1, 2-Chama 3, 4-Alta Verapaz II*) to *Yaqui*, the latest pre-conquest ceramic phase.

The valley sites, *Las Tinajas* and *Tampoma* in the *Polochic* valley, *Chichen*, *Chicuxab*, *Santa Elena*, and *Valparaiso* in the *Coban* area, as a rule were well supplied with potsherds. The exception was *Las Tinajas*, a large site where we dug a deep pit into the center of a long low platform mound and found a handful of unidentifiable sherds. Pits were dug throughout the main plaza, from which a total of one potsherd and an effigy-head foot resulted. At *Tampoma* two pits were dug, one into a rock-pile mound, the other under an unmarked stela. Most of the

identifiable sherds from the former belong to the *Chipoc* phase, as do those from the latter pit, which also contained jade (two tubular beads and three very thin flat perforated ornaments) and a pyrite mosaic mirror fragment. Most of the sherds from this site were picked up from the surface, an ancient river bed. Because of the water-worn or generally weathered condition of these fragments, much of the material is unidentifiable, but a quantity of *Chipoc* types were recognized. It is likely that further study will bring to light later horizons.

In the *Coban* area the largest site observed was *Chichen*. Here we dug pits into three mounds, trenched across the center of the main ball court, and collected surface pottery. Mound III contained the earliest (*Chkanel*) as well as the latest recognized (*Tohil*) pottery. Undoubtedly the site was occupied up to the conquest, and further study presumably will prove this. *Chichen* is a present-day pottery-manufacturing center. *Chicuxab* is a small site, but the surface is literally bristling with potsherds. We did no digging here, but picked up a carefully selected collection, containing largely *Chipoc* types besides a few *Tzakol*-type and *Tohil* pieces. Another small site, *Santa Elena*, close to *Chicuxab*, had an abundance of pottery on the surface and inside small platform mounds, two of which we trenched. The only *Chicanel*-type sherds were found on the surface. The mounds and surface yielded *Tzakol* and *Chipoc* types in considerable quantity and probably later types. At *Valparaiso* a surface gathering contained a few *Tzakol* and *Chipoc* types and an abundance of unidentified fragments which probably form part of later cultures.

From this survey we find that in the *Alta Verapaz* we can recognize various phases which closely parallel those suggested by Dr. Butler (*The Maya and their*

neighbors, pp. 250-267, New York, 1940). Dr. Butler's work, however, deals for the most part with the Chixoy drainage area and includes sites in the western part of the Alta Verapaz or farther west in the Department of Quiche, an area not touched in this survey. In order to keep the ceramics of the two areas separate, we are proposing new ceramic phase names for central and eastern Alta Verapaz: Chipoc (Tepeu 1, 2; Pokom; Chama 3, 4; Alta Verapaz II; Late Classic), Seacal (Tepeu 3), Samac (Tohil; Chipal 2; Alta Verapaz III; Post-Classic). The Pre-Classic and Early Classic, as well as the later phase corresponding to Yaqui, will be named when further knowledge as to the distribution of the wares of these periods in the Alta Verapaz is available.

HONDURAS AND GUATEMALA

GUSTAV STRÖMSVIK

During the past season no archaeological excavation took place at Copan, but an experiment in preserving archaeological sculpture should be recorded. Sculpture that has lain in the ground for some time is subject to minute plant growth, such as lichen and fungus, whose roots loosen the tiny particles of the surface of the sculpture; rain and wind continue the erosion. The sandstone at Quirigua and the tuff at Copan are particularly liable to this action. Some years ago S. G. Morley sprayed Stela K at Quirigua with a hardening agent, but the monument is again covered with lichen and fungus. Early this season Mr. R. E. Smith sent a new hardening agent to Copan for experimentation. The six lowest steps of the Hieroglyphic Stairway were carefully cleaned and dried; the viscous fluid was then applied with a brush. It will be some years before results can be seen.

Progress was made toward completing

a report on the three superimposed ball courts at Copan (see Year Book No. 36, 1936-1937, p. 138). Considerable work also was done in amplifying, checking, and correcting the specimen catalogue at the Copan Museum as a preliminary step in the preparation of a report on the cultural material in the Copan Valley.

In March, Strömsvik went to Guatemala for consultation with the Chairman of the Division. About this time, near the town of Asuncion Mita, Department of Jutiapa, several mounds of ruins were in danger of being razed by the construction of the Roosevelt Highway, whose route lay directly across the biggest and most important mound, locally called Cerro de Laja. Strömsvik consequently went there at the beginning of April to obtain all possible information before the mounds were destroyed and to co-operate with the Institute of Anthropology and History of Guatemala, which defrayed half the costs and supplied the services of Sr. Gustavo Espinoza, who assisted most ably in the excavations and explorations.

At the Cerro de Laja, which was our chief concern, a room filled with debris was completely excavated in order to examine the structural and architectural details, particularly the vaulting. This is the southernmost occurrence of the vault so far found in the Maya area. There can be no doubt, moreover, that the structure is of Mayaoid origin, for numerous fragments of Maya Polychrome and Copador pottery were found in the debris, thereby placing the remains in the Classic Period.

There undoubtedly are other rooms in this large mound, which will soon be removed completely by the highway work. At that time the details of construction should be recorded as they become apparent.

The countryside surrounding Asuncion Mita was given a hasty reconnaissance;

Brunton compass survey maps were made of four main ruins and some minor ones. The main groups were designated A, B, C, and D (Cerro de Laja is Mound i in Group A). In Group A lies the best-preserved ball court, from which were removed two parrot-head sculptures now to be seen in the entranceway of a house in town. These heads, like those at Copan, were cut to stand vertically in the benches. Another less well preserved ball court is located in Group C, west of the road and uphill from Group A. Groups A, B, and C are located south of town, between it and the bridge of Tamazulapa. All appear to be more or less of the Late Classic Period, in part contemporaneous with the Acropolis at Copan. These groups consist mainly of small courts each dominated by a larger structure. All constructions, of mud and slate on lava-block foundations, are oriented roughly to the cardinal points. Another type of construction seen in these groups is the tombs, of which there seem to be a great many, both sacked and untouched. All are vaulted.

Group D is located about 1 km. due north of town. It consists of a number of round, much eroded mounds, irregularly placed, built of mud with very little admixture of river boulders and no slate. Wherever a road cut through a mound, potsherds were gathered. These proved to be of Usulután ware and decoration, very similar to ceramics of the Miraflores phase in the Guatemala highlands. No polychrome pottery was recovered at Group D.

There apparently was a pre-Columbian settlement just where the town of Asunción Mita is now located. We were told by inhabitants that wherever one digs, artifacts turn up. A typical stone "yoke" and some pottery showing accentuated phallic symbols have been found here. Mexican affinity is suggested, and one wonders if the remains may be Pipil.

In reviewing the findings at Asunción Mita and vicinity, we seem to see three phases of occupation: (1) A population of Mexican stock apparently resided here at the time of the Spanish conquest, how long is unknown. (2) There was a Classic Maya phase of unknown duration, contemporaneous with one phase at Copan, when Copador pottery spread over parts of Honduras and El Salvador. That was the high point in the Maya culture. (3) There apparently also had existed an earlier settlement, characterized by earth mounds and Usulután pottery, and probably contemporaneous with the Miraflores phase in the Guatemala highlands.

CAMPECHE

GEORGE W. BRAINERD

The purpose of the field work undertaken in Campeche this year was the linking of the ceramic sequences of Yucatan and the Peten region of Guatemala by excavation at a series of intermediate points in the area lying between them. The work was in charge of Dr. George W. Brainerd, who took six months' leave of absence from the University of California at Los Angeles and from the Southwest Museum for this purpose. Mr. Karl Ruppert, who was thoroughly familiar with the southern part of the area to be worked, was the other member of the expedition.

Previous architectural exploration of this intermediate territory between the northern and central Maya ruin areas had been done by Ruppert (*Archaeological reconnaissance in Campeche, Quintana Roo, and Peten*, Carnegie Inst. Wash. Pub. 543), and by Dr. H. E. D. Pollock (Year Book No. 35, 1935-1936, pp. 122-125). The standing ruins in the Chenes, which is the northern part of this area, are distinctive and are similar in many particulars of style to those of the southern part of the area,

which has been called Rio Bee after the site of that name. These two architectural styles (or, perhaps better, this joint style) are in turn easily distinguishable from that to the north in the adjoining Puuc area of Yucatan, and from that of the central or Peten Maya area which adjoins the Rio Bee area to the south. The explored Chenes and Rio Bee sites are separated by about 100 km. of unexplored area which presumably also contains ruins of Chenes-Rio Bee architectural style.

Three sites were tested ceramically this season: Santa Rosa Xtampak and Dzibilnocac in the Chenes region, Xpuhil in the Rio Bee area. Collections were also made at other sites within 30 km. of Xpuhil. Approximately three months were spent in excavation. The next three months were spent in Merida in recording the collections, to be followed by a month at Los Angeles in analysis and preparation of a report. During the time in Merida Mr. R. E. Smith gave two weeks to the identification of Peten influence and trade in the collections. This procedure was of great benefit in establishing approximate chronological equivalence between the Campeche ceramics and the sequence at Uaxactun in the Peten, where stratigraphic phases have been fixed in time by Maya dates on accompanying monuments.

The determination of the correct time relations between the ruins of the Peten and Yucatan areas is of importance both in the telling of Maya history and in the dating of the Maya culture in terms of the Christian calendar. The Chenes-Rio Bee area, intermediate between the two, is the likeliest place to look for datable connections between them. Its study is also important in the determination of the nature and direction of cultural influence at all periods between Yucatan and Peten.

All three sites dug showed evidence of occupation ranging from prc-stela (Forma-

tive) times until somewhat before the period of Toltec influence at Chichen Itza. In each site, however, one period predominated in the pottery, presumably that of the standing architecture of the site. This period in all cases was characterized by a predominance of the Medium Paste Slateware which also predominates in the Puuc ruins and others of the Classic Period in Yucatan. The occurrence of this pottery at Xtampak in quantity in the terrace underlying stelae, two of which bear Maya dates of approximately 9.16.0.0.0, gives a contemporaneous date for this ceramic horizon in the Maya calendar. This dating by sherd material was reinforced by the finding of cache pottery of Classic ware in the stela platform. The terminal dates of the period are harder to work out, but some information should appear from the detailed analysis not yet begun. At present there is a suggestion that the occupation of the Puuc sites was somewhat later than, though overlapping in time with, that of the Chenes sites. The beginning date of this pottery period is also not closely fixed as yet. The absence in all three sites of clearly defined deposits between the Classic and the underlying Formative suggests either a period of abandonment or a longer use of the pottery we call Classic. At various sites in Yucatan a series of ceramic phases between Formative and Classic have been found and designated by the general term Developmental. Yucatan Developmental pottery occurs very sparingly in the Chenes sites tested.

Dating In the Maya calendar of Chenes architecture at Xtampak is given confirmatory evidence by Smith's classification of the Peten tradewares. The majority of polychrome tradeware sherds from each site tested falls into the Uaxactun subphase Tpcu 2, which Smith dates 9.13.0.0.0-9.19.0.0.0. Thus the dating of the Chenes and Rio Bee architectural styles falls well

within the Maya Initial Series Period. The coming detailed analysis of these collections should limit more closely the dating of the Yucatan periods in reference to the period of these sites.

The degree of cultural similarity among the material of the Puuc, Chenes, Rio Bee, and Peten areas is useful in evaluating Maya cultural development. Both Chenes and Rio Bee pottery of the Classic Period show preponderant percentages of Medium Paste Slateware, the chief Yucatan ware of this period. This ware is distinctive from that of the Peten. The pottery of each of these three Slateware areas, however, is easily distinguishable from that of the others by differences in detail of vessel forms. The finer monochromes of the Rio Bee area are very similar to certain wares of their corresponding horizons in the Peten, whereas the Chenes fine monochromes resemble those of the Puuc area. These degrees of relationship seem at least roughly to parallel the architectural relationships which have been observed.

An additional finding of interest is that of a new Formative phase which seems to precede the Late Formative apparent in nearly all Yucatan sites thus far sampled. The Yucatan Late Formative, as well as that found in the Campeche sites this year, bears certain marked similarities to types of pottery found in the Chicanel phase at Uaxactun, according to the findings of R. E. Smith. There are also sporadic resemblances to the earlier Mamom Uaxactun phase. At Dzibilnocac and Xtampak were found deposits of a phase seemingly earlier than Late Formative and showing ware and form resemblances to the early deposit found at Maul under a late Formative deposit (see Year Book No. 41, 1941-1942, p. 255). At Dzibilnocac the deposit of this new phase includes some Late Formative pottery; at Xtampak the diagnostic Late Formative wares are absent.

This period may be provisionally called Middle Formative; the Mani deposits, Early Formative. The Middle Formative phase shows less similarity to Chicanel than does the Late, but no similarity to Mamom. The Early Formative seems to show no resemblances to any pottery of other areas thus far known.

The dating of the Early and Middle Formative periods of the northern Maya area is not at present a profitable subject even for conjecture. The Middle Formative collections are small; the Early Formative, although larger, are limited almost exclusively to water jars. The distinctive character of these periods is striking, but further excavation is imperative before they can be properly placed. The study of the cultures sampled this season has scarcely been initiated.

CERAMIC TECHNOLOGY

ANNA O. SHEPARD

Work in ceramic technology has been concentrated on two principal projects during the current year: the preparation of a general book on ceramics for archaeologists, and a special study in the Southwestern field. The purpose and plan of the ceramics book has been stated in an earlier report (Year Book No. 46, 1946-1947, p. 190). The Southwestern study, also mentioned previously (Year Book No. 47, pp. 219-220), deals with the brown wares of the southern part of the area, the so-called Mogollon pottery.

The age and the place in Southwestern culture history of the Mogollon people has been a central theme of controversy among archaeologists for a number of years. One school has maintained that the Mogollon constitutes a distinct basic culture which influenced the better-known Pueblo development to the north; the other school regards the Mogollon as merely a variant

Pueblo culture. This question of basic cultures is one of definition and does not directly concern the technologist, whereas the record of contacts between peoples and their interactions is one of primary interest to him because it is preserved in no small measure by pottery. Furthermore, the interpretation of ceramic characteristics is one of his direct concerns and responsibilities. Brown pottery is an important diagnostic of the southern culture, and the contrast of its color with that of Pueblo pottery in the north, which is whitish or gray, is generally considered indicative of a fundamental difference in preferences and practices of the Mogollon potters. Quite aside from the question of how much weight should be given pottery color in evaluating a culture, there is need of reviewing the factors which affect the color of fired clay. Composition of clay and method of firing, or, stated in other terms, natural resources and a trait of material culture, are both primary factors. Stressing the latter to the neglect of the former leads to erroneous conclusions. If the same kinds of clay had been available throughout the Southwest, the color difference in the pottery of the two regions would reflect the potters' choice of clay and method of firing and could be considered an independent culture trait. But this is not the case. In much of the northern part of the territory there are extensive formations of Cretaceous age which abound in good-quality, buff-firing clay. These formations are absent throughout most of the southern part of the area, where the common clays are red-firing. To overlook this fact magnifies cultural differences. Likewise the idea that brown wares are earlier and more primitive than grays and that similarity of color indicates relationship arises from failure to recognize that the most widely distributed and abundant clays are red-firing. The Pueblos

of the north were favored in their ceramic resources and in consequence developed a nearly unique class of primitive pottery.

Evidences of contact between Mogollon and Pueblo peoples consist largely in trade pottery: black-on-white in southern sites, and red, brown, and smudged in northern. The latter have been identified as Mogollon only by surface appearance. One of the purposes of the Southwestern technological project has been to determine as far as possible the source of all supposed Mogollon intrusives and to consider the bearing of technological features on the question of the influences of the two peoples on each other. It has been necessary first to define the range of paste variations in the Mogollon area as far as collection and survey permit. This work has in itself direct archaeological applications. Since the two principal types of Mogollon pottery, red and brown, are undecorated and lack distinct, variable features of workmanship, varieties peculiar to certain localities or periods cannot be recognized by simple inspection, and consequently pottery does not serve as fully as in some areas to correlate occupations and to establish time sequences. This is a serious disadvantage, because the pottery was made over a large area and during a considerable time span. If it is to be broken down into spatial and temporal varieties, this must be done primarily on the basis of body composition. Pottery from the Petrified Forest region illustrates how paste analysis may add detail to the picture and alter interpretations. Sherds from twenty-one sites surveyed by the Laboratory of Anthropology included a brown ware defined as a homogeneous type which upon petrographic analysis was found to include four different pastes identified by temper. Of the four tempers—sand, sherd, altered feldspar, and volcanic rock—the last named occurred in only one specimen, which was

doubtless an intrusive from the south. The principal pastes, sand- and sherd-tempered, show sufficient difference in distribution to suggest that they were made by different peoples or at slightly different times, a possibility considered by the Museum of Northern Arizona in selecting sites for excavation in the region this summer. A clear-cut and readily recognized temporal difference in paste occurs in the Forestdale region, where most of the pottery is sand-tempered, but an early variety from Bluff Ruin is tempered with crushed diabase.

Collections studied to date, in addition to those mentioned, are from Dr. Emil Haury's excavation at Point of Pines, Dr. Paul Martin's from the SU site, the major excavated sites of the Mimbres region, and San Simon to the southwest. Collections from some sixty sites covered by Peabody Museum's survey of the north central and northeastern part of the area are now in process of analysis.

The project has been a venture *in* cooperation, since we have depended for pottery on a half-dozen institutions, and many of the archaeologists representing them hold sharply opposed views. Both samples and data on provenience and frequency of occurrence have in every case been supplied freely and generously, and there has been no reluctance to loan even rare sherds. The study has also been greatly facilitated by a gift of 333 analyzed petrographic sections from Mr. Harold Gladwin, of Gila Pueblo, and Mrs. Nora Gladwin Fairbanks. The collection was prepared and studied by Mrs. Fairbanks for Gila Pueblo and includes a large series from Magellan sites.

A short study of Mesa Verde pottery from the excavations of Dr. Deric O'Bryan was undertaken because of its close similarities to La Plata pottery analyzed for Dr. Morris some years ago. The study clarified the relationship of pottery types

and showed distinct fluctuations in classes of paste which, when correlated with other archaeological data, may have a bearing on the shifts of peoples within the region. One interesting result was the discovery that a considerable proportion of the pottery, including nearly all the earliest, was tempered with crushed andesite, a source of which was found in an early gravel sheet on the Mesa. That Basket Maker III potters should search out this deposit and select from it the igneous rock would indicate that they came to the Mesa from a region, such as the La Plata Valley, where it was the custom to temper with crushed rock instead of sand, the common temper of this period.

LINGUISTIC RESEARCH

NORMAN A. MCQUOWN

Dr. McQuown's primary purpose in his trip to Guatemala in the summer and autumn of 1948 was to educate himself with respect to the phonemics and a good part of the morphology and syntax of the Mam language. Among his secondary purposes were: to familiarize himself with the phonemics of the other Guatemalan languages in which Andrade made phonograph recordings (Quiche, Kanjobal, Quekchi); to do the phonemics of Cakchiquel, for which Andrade gathered extensive written texts (transcribed by Juan Resales); to take a look at Xinca, in order to get an outside check on Maya-non-Maya cultural contacts; and to make arrangements (both in Guatemala and in Mexico) for utilizing, so far as possible, the Maya materials already collected, or in process of being collected, by missionaries working with these languages in the field, and to ensure their collaboration and aid in further work on the languages of the family. A certain measure of success was attained in all these undertakings.

Five months were spent in Guatemala and Mexico, and the items listed were accomplished:

(1) Three and a half months were spent in Guatemala City, working on Mam, with an informant generously furnished by the Instituto Indigenista; and one week was spent with Mr. and Mrs. H. D. Peck, at Txol Be, San Juan Ostuncalco. (a) A Mam grammar and dictionary file of some 7500 entries was accumulated, (b) The phonemics and a considerable portion of the grammar of Mam was worked out. (c) Some ten Mam texts were collected incidentally, in the process of teaching the informant to write with a phonemic alphabet, (d) The Instituto Indigenista was provided with a Mam alphabet, (e) Mutual orientation sessions were held with the Pecks, in which the problems of Mam phonemics and grammar were discussed. (f) Arrangements were made with the Pecks, with the informant, and indirectly with Mr. and Mrs. Edward Sywulka, to collaborate in the preparation of a Mam dictionary, (g) Arrangements were made with the Pecks to complete the transcription and translation of Andrade's 300 Mam recordings (all material to be phonemically transcribed according to a system worked out on this trip); they have already made great progress, and should be able to complete the work in another year, (h) Arrangements have been made with Mr. Edward Sywulka to have access to his master's dissertation, ^UA morphology of the Mam language,¹¹ recently completed at the University of Oklahoma. The continued collaboration of Mr. Sywulka and of the Pecks in working out unsolved problems of Mam grammar will probably make further actual field work on Dr. McQuown's part unnecessary for completing the descriptive phase of the work on Mam.

(2) Two weeks were spent in becoming familiar with the phonemics of the Quiche,

Kanjobal (Jacalteco), and Quekchi languages, in each of which Andrade made a fair number of recordings (55, 37, and 23, respectively), (a) Two days were spent in Chichicastenango, working on Quiche, with three informants from Chichicastenango and one from Totonicapan. A vocabulary of 1000 items was taken and a tentative sketch of the phonemics was worked out (subsequent work in the Instituto Indigenista with another informant from Totonicapan verified the results of the first sketch and, in addition, established beyond question the phonemic status of the short and long vowels). Brief checking was done on a preliminary transcription of the Quiche recorded materials prepared by Andrade (the newly discovered feature of vowel length will make retranscription of all materials necessary). The Instituto Indigenista was provided with a Quiche alphabet; on the previous trip to visit the Pecks, arrangements were also made with Mr. and Mrs. Stanley Wick, working with the Quiche of San Cristobal, for future collaboration in all aspects of the work on Quiche, (b) Two days were spent in Huehuetenango, working on Kanjobal (Andrade's Jacalteco) of Santa Eulalia, with the informant of Mr. and Mrs. Newberry Cox, at present in San Miguel Acatan. A vocabulary of some 1500 items, a text, and some paradigmatic materials were recorded; a tentative sketch of the phonemics was worked out; vowel length was not found, but subsequent work with other Guatemalan Maya languages (Quiche, Cakchiquel, and Quekchi) which have it makes a recheck desirable (although there is evidence that some of the Chiapas languages do not have phonemic vowel length and that Kanjobal may belong with them rather than with the other languages in Guatemala). The collaboration of the Coxes will make this recheck possible without further field

work; they can also be counted on for help in the transcription and translation of that part of Andrade's recorded material which is as yet unworked. The Instituto Indigenista was provided with a Kanjobal alphabet, (c) Two days were spent in San Juan Chamelco, working on Quekchi, with the informant of Mr. and Mrs. William Sedat. A vocabulary of some 1000 items was recorded; a tentative sketch of the phonemics was worked out (phonemic vowel length was definitely established). The collaboration of the Sedats can be counted on in the future, for the working out of the grammar, and the transcription and translation of that part of Andrade's materials which is as yet unworked. The rechecking of the already transcribed materials will in the case of the Quekchi involve a minimum of effort, since Andrade indicated in these materials a fair portion of the long vowels. The Instituto Indigenista was provided with a Quekchi alphabet.

(3) One week was spent on the phonemics of Cakchiquel; one day was spent in San Pedro Yepocapa, working on the local dialect. A vocabulary of some 750 items was recorded, and a beginning was made toward teaching the informant to write; a tentative phonemic sketch was worked out. Subsequently, three days were spent in Panajachel, working on a slightly divergent dialect, with five informants. A vocabulary of some 1300 items was recorded; a tentative phonemic sketch was worked out (subsequent work in the Instituto Indigenista with an informant from Comalapa, and in Mexico with Juan Rosalcs of Panajachel, confirmed the sketch, and established both short and long vowels as phonemic). The Instituto Indigenista was provided with a Cakchiquel alphabet. Juan Rosales, in view of the establishment of vowel length as phonemic, will have to recheck the texts

written by him, indicating long vowels where they occur.

(4) The extra-familial check was accomplished in two days, which were spent in Chiquimulilla, working on Xinca, with an informant from Santiago and one from San Sebastian. A vocabulary of some 750 items was recorded and a tentative sketch of the phonemics was worked out. The Instituto Indigenista was provided with a Xinca alphabet. Subsequent inspection of the Xinca vocabulary showed some dozen loan words, some from Nahuatlan languages, some from Maya; consideration of the Maya loans ("bean," "mat," "custard apple," "witch doctor," "ladino") shows that they were borrowed from Mam or from Kanjobal (or from one of the Chiapas languages), indicating a previous more northerly location for the Xinca; comparison of "bean" (from Maya) with "corn" (either a loan from Lenca or a loan into both Xinca and Lenca from a third language) would indicate that "bean" was acquired considerably later than "corn," and was introduced to the Xinca (and apparently to the Lenca and other Middle American groups) by the Maya. These "explanations" are only conjectures, of course, but they indicate how extremely fruitful further work might be. Xinca is rapidly going out; someone should do a thorough study before it becomes extinct.

(5) The fifth purpose was accomplished, by and large, in the personal meetings with individual missionaries. Collaboration has been secured with the Pecks and the Sywulkas for Mam; with the Coxes for Kanjobal; with the Wicks for Quiche; with the Oughtens for Ixil; with the Sedats for Quekchi; with Antonio Goubaud and the workers of the Instituto Indigenista for Cakchiquel, Quiche, and Mam; with the workers of the Summer Institute of Linguistics in Mexico for all the Maya languages there. In Mexico, ma-

materials for volume 26 of the University of Chicago's Microfilm Series on Middle American Cultural Anthropology (texts and dictionaries in Choi, Tojolabal, and Tzotzil) were obtained from the Summer Institute and were filmed; other Maya language materials of Summer Institute workers will be added to this series from time to time.

HISTORY OF THE MAYA AREA

RALPH L. ROYS

During the past year *The prophecies of the Maya tuns or years in the Books of Chilam Balam of Tizimin and Mani* has been completed for publication, and some time has been spent in reading the proofs of this Contribution and the *Guide to the Codex Perez*.

The source material for the history of Mayapan has been assembled and translated into English from the Spanish and Maya, where such translations were not already available. The sources quoted are accompanied by commentaries, in which they are compared and discussed and some of the more important implications are noted.

There is fairly good evidence that the area ruled by Mayapan included most of the Yucatan Peninsula north of Campeche, although the evidence for the northeast and east coasts is conflicting. Nothing has been found about Campeche in this connection.

Taken as a whole, the references to Mayapan indicate the need of a new interpretation of the source material. In the past, historical reconstructions have been based principally on the chronology of the Maya Chronicles in the Books of Chilam Balam, but with unsatisfactory results. Uxmal, Chichén Itza, and Mayapan were long considered to have been contemporary capitals in Yucatan, but archaeol-

ogists have found that their more important building activities occurred at three different periods in the order named. J. E. S. Thompson has noted the weakness of this chronology, and a comparison of all the historical sources suggests that the accounts by Gaspar Antonio Chi offer a framework in which Landa's narrative and the episodes related in the Chronicles could more easily be reconciled with the archaeological findings. This compilation also presents evidence that the important Xiu family became a political factor in northern Yucatan only in the late fourteenth or early fifteenth century, and not in the eleventh century as previously believed.

The late winter and spring were spent in Yucatan continuing previous studies of the political geography at the time of the Spanish conquest (Year Book No. 36, *Wlfj-Wdfj** pp. 23²4; No. 38, 1938-1939, pp. 252-253). One purpose of the present survey was to see what indications exist that the modern towns are situated at conquest sites of the same names; another was to locate historic sites that are no longer shown on the maps.

A search was also made for conquest sites which would be likely sources for the recovery of late preconquest and early colonial pottery. Although ceramic sequences have been established from very early times down to the fall of Mayapan, about the middle of the fifteenth century, it has been difficult to find sherds which could be identified as belonging to the period following that time. It seems possible that this is partly due to a Spanish policy of replanning the Indian towns, by which it is known that the houses were concentrated nearer the church and central plaza and for the first time arranged along regular streets with smaller yards and gardens than previously. Several centuries of occupation in colonial and modern times

have further aggravated this disturbance of the topsoil containing the sherds which had accumulated during the last pre-Spanish period and that of the conquest. For this reason particular attention was paid to historic sites from which the people had been removed at a fairly early period. A few of these—Hunacti, Calotmul, and Cauich—had been located during previous visits to Yucatan. This year four more were visited and identified: Kanchunup near Sotuta, Tecoh near Izamal, Tuchicaan near Maxcanu, and Quizil near Uman. Hunacti and Kanchunup were subject to European influence for the shortest time, since they were left deserted prior to 1582, and very possibly as early as the 1560's. The churches of these two towns, as well as at Tecoh and Quizil, were of an early type. Only the chancel, sacristy, and priests' quarters were built of masonry. There was no nave, and the congregation evidently occupied an open structure of pole and thatch, called a *rarnada*, of the sort described in various sixteenth-century Spanish reports. It seems probable that all four sites were abandoned at an early date. Since there are cenotes at three of them and a pond of potable water at Tecoh, they would appear to be promising sources of late preconquest and early colonial pottery. Tuchicaan and Tecoh are of especial interest, since both played an important part during the Spanish invasion of Yucatan. There are still extensive Maya ruins at all seven sites mentioned above except Kanchunup and Cauich.

Indications exist that a very large number of the colonial churches in Yucatan were built partly of hewn blocks taken from ancient structures in the immediate vicinity, although such material apparently was sometimes supplemented, as at Kinchil and Kaua, by hewn stone from other sites as far as 6 km. distant. Two of the oldest convents, at Merida and Izamal, were

built on the summits of fairly high substructures, and in the northwestern and the northern parts of the state, as far east as the vicinity of Izamal, a great many churches are set in spacious patios which are raised from 1 to 2 m. above the level of the town. In such cases this platform appears to have been constructed from the rubble fill of a large Maya substructure. Possibly it consisted partly of the flattened base of a pyramid and was completed from other neighboring mounds. There is some evidence of this at Hunucma and still more at Chuburna. Raised platforms on which the churches stand are much rarer in the Sotuta and Valladolid areas farther east, and in the former Xiu Province of Mani to the south.

These raised church patios are frequently enormous structures, although they probably average only about 1.5 m. in height. Roughly paced off, those at Hunucma, Kinchil, Hocaba, and Humun have an average surface of about 8000 sq. m. The platform at Humun is 3 or 4 m. in height, but here it is partly formed of a low rocky knoll. Nowhere did there appear to be a depression from which this material might have been excavated. There are still many large Maya ruins in northern and northwestern Yucatan, but this use of the substructures for church platforms probably explains why they are not so numerous today as would appear from the sixteenth-century Spanish reports. In spite of the important fisheries and salt industry in former times, agricultural conditions in northwestern Yucatan are such that it is difficult to explain the great building activities which evidently existed. Apparently there was a highly developed social organization over a very long period of time, and in this connection it seems relevant to note that some sherds gathered at Cuzco, when submitted to Dr. G. W. Rainerd, were identified as corresponding

in age to the Chicanel phase at Uaxactun.

In a number of towns, where the site of the church is raised but little or not at all above the surrounding area, some of the old pyramids or other substructures were left standing in the immediate vicinity. This has been recorded of the cathedral at Merida and the church at Motul. It is still the case at Ucu and Cauce, both of which might be considered sites of some promise for recovering sherds of the conquest period. Other instances can be cited at Yaxkukul, Acanceh, Sotuta, Dzan, Chapab, and Teabo. At Sotuta a large substructure near the church is surmounted by a fortified colonial building. Similar remains are also to be seen at Chicxulub, Ticul, Oxkutzcab, and near Tekax, but, like many others reported from Yucatan towns, they lie at some distance from the church and the center of town.

Visits were made to the ruins at Ake, Acanceh, Izamal, Uxmal, Chichen Itza, and Mayapan, and to the town of Champoton, all of which figure in the historical legends of the Books of Chilam Balam. At the time of the Spanish conquest Champoton was a large and commercially important town with many stone buildings. Although the architectural remains are scanty, there are still a number of scattered drums of cylindrical columns. They are rather roughly hewn and are reminiscent of those at Mayapan. Surface sherds are abundant in the streets, and the gullies which intersect the town seem to offer some promise of stratified deposits.

HISTORY OF SCIENCE

GEORGE SARTON

As my connection with Carnegie Institution ends August 31, 1949, it seems proper to devote this, my final, report not only to the last year **but** to the whole duration of my service, which began July 1, 1918.

Rereading my yearly reports, the first of which appeared in Year Book No. 18 (1918-1919), I find that my main work was not announced until the third report (Year Book No. 20) and that I began the writing of my *Introduction to the history of science* only on January 12, 1921. When I started that undertaking, I did not, and could not, realize its size, complexity, and difficulties; I thought that it would occupy only part of my time and that I would be able to complete two other projects, each of which was of lifetime size. These two projects were, first, a history of modern physics (physics in all its ramifications in the nineteenth and twentieth centuries); second, a full account of the life and achievements of Leonardo da Vinci.

As this second project is not unrelated to the main undertaking, I may be permitted to say a few words about it. In 1916, I had delivered six lectures on Leonardo da Vinci at the Lowell Institute in Boston. After the completion of that course of lectures, it dawned upon me that my knowledge of the subject was very insufficient. Leonardo, sometimes called the father of modern science, was the child of the Middle Ages. In order to appreciate his thoughts correctly it was necessary to have a deeper knowledge of medieval science than I could boast at that time. It was probably then that I resolved to make a systematic and thorough survey of the progress of science, century by century; I sincerely thought that I would be able to reach our time, or at least the **beginning** of this century, within ten or twenty years. In reality, so many were the obstacles that I did not even reach Leonardo's time, but had to stop my survey about the year 1400.

The main cause of delay was the necessity, unsuspected at first, of studying the Arabic language. This was a heavy task in itself. At first, I had **been** helped by my **kind** friend the Rev. Duncan Black

Macdonald (1863-1943), of Hartford, Connecticut, but in spite of his willingness he was not always able to help me, nor could I appeal to him as often as would have been necessary, and the obligation to study Arabic could not be eschewed any longer.

It is proved in great detail in my *Introduction* that for three centuries at least (the ninth to the eleventh), Arabic was the international language of science, and that in the following two centuries (the twelfth and thirteenth) the study of it remained the shortest cut to up-to-date knowledge. We often speak of the iron curtain separating eastern from western Europe; another curtain began to separate them (that is, to separate the Orthodox, Greek East from the Latin, Catholic West) as early as the fifth century, and three centuries later it had become almost impenetrable. The Latin doctors refused to read Greek; therefore, they were finally obliged to read Arabic, a language entirely unrelated to theirs, the language not of Christians but of Muslims. That is one of the paradoxes of history. It is because of it that medieval science and medieval culture cannot be understood without a sufficient knowledge of the Arabic writings.

While I was engaged in the survey of ancient and medieval science which would bring me back within a few years (so I thought) to Leonardo, the Carnegie Institution commissioned Professor J. Playfair McMurrich (1859-1939), of Toronto, to investigate Leonardo's anatomical drawings and notes. Indeed, it is clear that Leonardo's main source in this field could not be medieval knowledge, but only his own dissections, and the value of his anatomical drawings could not be appreciated except by a man with a long anatomical experience. The results of Professor McMurrich's investigations were published by the Institution in 1930 (J. Playfair McMurrich,

Leonardo da Vinci the anatomist (1452-75/9). Carnegie Inst. Wash. Pub. 411. xx+265 pp., frontispiece, 89 figs. *Isis*, vol. 15>PP- 342-344)-

In the meanwhile, volume 1 of the *Introduction to the history of science*, dealing with the period from Homer to Omar Khayyam, a period of two thousand years, had appeared in 1927, and volume 2, in two parts, devoted to the twelfth and thirteenth centuries, appeared in 1931. The effort made to bring these volumes to relative perfection had been so long-drawn and intense that it left the author exhausted. Thanks to the wisdom and generosity of the Institution, I was permitted to spend a sabbatical year abroad. I resided half a year in Syria, where I was a guest of the American University of Beirut and was able to extend my knowledge of the Arabic language, the Arabic people, Eastern Christianity, and Islam. Shorter times were spent in other countries which were (or had been) parts of the Arabic or Islamic world:- Egypt, Palestine, Turkey, Rhodes, Cyprus, Tunis, Algeria, Morocco, Spain, and Sicily. After my return to Cambridge my work was resumed and centered upon the fourteenth century. This again took far more time than had been expected—volume 3 (in two parts) appeared only in 1948—partly because my standards of scholarship had become more severe as my experience increased, partly because the amount of accumulated materials was so much greater. Materials had been accumulating for the whole work from the beginning; the accumulation had lasted about 9 years for volume 1, 13 years for volume 2, 27 years for volume 3. Many of the documents had been published, or at least listed, in *Isis*. By the time of publication of volume 1, 27 numbers (almost 8 volumes) of *Isis* had appeared, including 18 critical bibliographies; by the time of publication of volume 2, 46 numbers (al-

most 15 volumes), including 30 bibliographies; by the time of publication of volume 3, 103 numbers (35 volumes), including 67 bibliographies, plus 7 volumes of *Osiris*. The materials contained in the *Introduction*, *Isis*, and *Osiris* are integrated by means of thousands of cross references. Thus we may say that volume 1 was built on a foundation of 8 volumes; volume 2, on a foundation of 15; volume 3, on a foundation of 42.

Reference to *Isis* suggests that the mass of information included in the three published volumes of the *Introduction* is much larger than appears at the surface. These three volumes include 4334 pages, but there is scarcely a page which does not refer to *Isis* or *Osiris*, where more information can be obtained immediately. Moreover, additions and corrections to the published volumes are included periodically in the critical bibliographies of *Isis*, the 75th of which is now in process of preparation, to appear in volume 41.

The author is keenly aware of the need of correction and amplification, but such as it is, the *Introduction* is the most elaborate work of its kind, and by far, in world literature. This statement can be made without falling under the suspicion of boasting, for it is objective, controllable, and obviously correct.

At the end of my thirty years of service, I wish to express my deep gratitude to the Institution which made it possible for me to do the work which I loved best and for which I was most fit. Thanks to its patronage, Rooms 185 and 189 of the Widener Library, Harvard University, became an international center and clearing house for the history of science. These rooms were never called an "institute," but they deserved the name far more than many of the "institutes" attached to European universities.

It is certain that every student of the

history of science, the world over, shares my gratefulness to the Carnegie Institution for having allowed this fundamental work to be undertaken and partly completed. The history of science is like any other discipline in the field of science or the humanities, in that the fundamental work is slow and difficult, and the results austere. It is also expensive, or at least seems to be. It requires the most expensive of all scientific instruments, far more expensive than the greatest telescopes or cyclotrons—a large library, the larger the better (try to evaluate the total cost of such libraries as the Library of Congress or the Harvard College Library). To this one may answer that though the historian of science needs such a library more than any other scholar, he is not by any means alone in using it. Each library is used simultaneously by many thousands of people. In the second place, genuine scientific work is always expensive, at any rate as compared with secondhand work which requires only enough literary ability to exploit the investigations of other people or rephrase their reports; scientific work is apparently expensive, but it alone has any chance of permanence. The popular books, however successful (and their success is often in inverse ratio to their scientific value), are ephemeral; one can never really depend upon them; it is hardly worth while to refer to them. The writing of such books is a Sisyphean labor without value, except perhaps a monetary one. It must be done over and over again. There is no cheapness in them, at least no financial cheapness. For a longer explanation of my views than there is space for here, the reader is referred to my article "The scientific basis of the history of science," published by the Institution in the volume dedicated to the late President Merriam, *Cooperation in Research* (Carnegie Inst. Wash. Pub. 501, pp. 465-471, 1938).

PUBLICATIONS

MARGARET W. HARRISON

Volume X of Contributions to American Anthropology and History (Publication 585), now in page proof, contains four papers: *The Maya Chronicles* (no. 48), by Alfredo Barrera Vasquez and Sylvanus G. Morley; *Guide to the Codex Perez* (no. 49), by Ralph L. Roys; *The Pendleton ruin, Hidalgo County, New Mexico* (no. 50), by A. V. Kidder and H. S. and C. B. Cosgrove; and *The prophecies for the Maya tuns or years in the Books of Chilam Balam of Tizimin and Mani* (no. 51), by Ralph L. Roys. This group completes the volume, which will be issued in the fall of 1949.

The Maya Chontal Indians of Acalan-Tixchel: a contribution to the history and ethnography of the Yucatan Peninsula (Publication 560), under the joint authorship of France V. Scholes, Vice-President of the University of New Mexico, and Ralph L. Roys, with the assistance of Robert S. Chamberlain and Eleanor B. Adams, was published early in the winter of 1949.

A. Ledyard Smith has completed his part of the general survey of the Division's work at Uaxactun. His monograph, entitled *Uaxactun, Guatemala: excavations of 1931-1937* (Publication 588), is now in press; it will be followed shortly by Robert

E. Smith's companion report on the pottery at this site. Dr. Kidder's introduction to the first book gives the archaeological background for both studies.

J. Eric S. Thompson's *Maya hieroglyphic writing: introduction* (Publication 589) is in galley proof. It forms the first volume of Mr. Thompson's projected series on this subject and will be published by the end of 1949.

Two compilations were mimeographed and issued by the Division this year: *Historical source material for the history of Mayapan*, by Ralph L. Roys; and *Selected references on the Maya area*, brought up to date as of December 1948, by Margaret W. Harrison.

The fourth volume of Notes on Middle American Archaeology and Ethnology was begun with three papers: the first two by Dr. Kidder, listed in the bibliography following this report; the third, *Some new discoveries at Coba* (no. 93), by William R. and Michael D. Coe.

Several manuscripts soon to be presented by the Division for publication await completion of editorial work: *The conquest of Honduras and Higuera*, by Robert S. Chamberlain; *Co pan ceramics: a study of southeastern Maya pottery*, by John M. Longyear III, of Colgate University; and *A study of Classic Maya sculpture*, by Tatiana Proskouriakoff.

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SPECIAL PROJECTS: HISTORICAL RESEARCH

E. A. LOWE, The Institute for Advanced Study, Princeton, New Jersey. *Collection and study of paleographical material.* (For previous reports see Year Books Nos. 9 to 35, 37 to 40, and 47-)

The four volumes of *Codices latini antiquiores* so far published have dealt with the oldest Latin manuscripts in the Vatican City, in Great Britain and Ireland, and in Italy outside the Vatican City. The next two volumes deal with the manuscripts preserved in France: volume V deals with the Paris manuscripts, volume VI with the manuscripts in French libraries outside of Paris. The Paris manuscripts are nearly all in the Bibliothèque Nationale; a few are in the Arsenal and Ste Geneviève libraries, and some papyri are in the Louvre. Together they comprise a round two hundred items, making volume V almost twice the size of volume I. The fifth volume is nearly all set up in type. The lay-

out of the facsimiles was completed last October, and work on the collotypes is to begin shortly. Barring unforeseen obstacles, the volume may see the light by the end of 1949. One hurdle, however, still remains to be cleared: the papyri in the Louvre Museum have so far been inaccessible. It is greatly to be hoped that it will soon be possible to photograph them.

Field work on volume VI has commenced. Preparations are now being made for taking the necessary photographs of items scattered in a rather large number of libraries. It is gratifying to report that the editor can count on the helpful co-operation of the French libraries.

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