A REPORT FOR THE YEAR 1950-1951 ON THE RESEARCH AND OTHER ACTIVITIES OF THE DIVISION OF BIOLOGY
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The year 1950-1951 has been one of gratifying progress for the Division of Biology of the Institute. In collaboration with the Division of Chemistry, the joint Chemistry-Biology Program has been substantially advanced, particularly with respect to our understanding of the chemical and biological properties of proteins and related highly specific large molecules.

The range of activities within the Division of Biology has been great—involving, for example, the day-to-day chore of providing facilities for some four-score researchers; the preparation of requests for research grants; the reporting of progress from time to time to some two dozen research-sponsoring agencies; the building of equipment, such as an automatic machine for determining the radioactivity of a hundred samples; and the pursuit of varied research programs, such as a determination of the chemical nature of Los Angeles smog and its influence on plant growth and development, and studies on the mechanism of gene action.

It is the purpose of this report to give a somewhat systematic account of the activities of the Division of Biology and its staff members. It is hoped that this will be informative and interesting not only to the President, Trustees and other members of the Institute's Administrative Staff, but also to members of the Institute's academic staff inside and outside the Division and to the many friends outside the Institute who for one reason or another follow the activities of the Division.
Financial Support

The total budget of the Biology Division for 1950-1951 was roughly $500,000. Approximately half of this was from the Institute endowment income and half from special short-term grants or from government contracts. The funds used during the year came from the following sources:

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Riker Laboratories  Veratrum growth and alkaloid formation
Rockefeller Foundation Fund  Joint chemistry-biology program
Edward G. Robinson Fund  Electronarcosis
Leonard G. Strater Fund  Plant physiology
Elbridge and Mary Stuart Foundation  Milk protein research
University of California - Beet Sugar Fund  Sugar beet research
United States Atomic Energy Commission (Administered by Office of Naval Research, United States Navy)  Radiation genetics
United States Atomic Energy Commission (Administered by Office of Naval Research, United States Navy)  Protein synthesis
United States Navy, Office of Naval Research  Betaine-glycocyamine therapy
United States Public Health Service  Mammalian physiology
United States Public Health Service  Transmethylation
Williams-Waterman Fund for the Combat of Dietary Diseases (See Research Corporation)

Physical Plant

Earhart Laboratory  During 1950-1951 the Earhart Laboratory for Plant Research reached full-scale operation. The many research activities made possible by this unique laboratory are described elsewhere in this report. The very troublesome problem of smog damage to plants has been checked by the installation of a set of special filters
designed by the Building and Grounds Department and containing as an active smog removing agent a mixture of materials developed by Professor Haagen-Smit. Investigations are still continuing in an attempt to make the filtering agent still more effective.

**Proposed Plant Research Center** At the present time plant research in the Division is carried on in four physically separated sites, viz., the Earhart and Dolk laboratories, the Arcadia Farm, the Orlando Street Greenhouse, and the main Kerckhoff building. It is hoped that within the next several years a research center can be developed at the Earhart-Dolk site which will permit consolidation of much of the plant work. Such a center would clearly make for more effective and efficient use of both physical facilities and manpower. In addition it would greatly help staff members with interests at the various sites in various collaborative research efforts.

The development of such a plant research center would require that additional greenhouse facilities be made available at the Earhart-Dolk site to replace those at the Farm and at Orlando. Smog damage is a serious problem at the Orlando Street and Dolk Greenhouses and could easily become one at the Arcadia Farm if it were necessary to grow plants other than corn there. The proposed consolidated greenhouse group would make feasible the installation of a smog-removing filter system that would serve both the Dolk Greenhouse and the proposed additional greenhouse and laboratory.

In addition to greenhouse facilities, space for the out-of-door growing of experimental plants is essential for the proposed development. It is hoped that eventually a small area of land can be acquired for this purpose.

**Marine Station** The arrangement between Pomona College and the Institute whereby Pomona's marine biology instruction is given at the Corona del Mar laboratory during July and August has continued to work to the satisfaction of all parties concerned. The improved dormitory facilities provided last year have turned out to be highly worth while.

**Main Kerckhoff Laboratory** An active program of rehabilitation and modernization of laboratories and other facilities has continued. Several special stainless steel fume hoods have been installed and plans are under way for adding others. The necessity of this was emphasized by a fume hood fire during the late afternoon of April 20 which caused a good deal of excitement but fortunately did no serious damage. The fire was partly caused by a makeshift hood
constructed of flammable materials.

The Library  During the years 1948-1950 strenuous efforts were made and appreciable sums of money were spent to bring the journal collections of the Library to a condition of adequacy. Because of the success of these efforts, periodical acquisitions during 1950-51 were considerably fewer in number than in the two preceding years. Nevertheless, the troublesome and sometimes discouraging task of filling in gaps went briskly forward, and over 400 volumes of back issues were secured. Some of these, like volumes of the "Journal of Biological Chemistry" and the "Journal of Genetics," are items for which the Library had searched two and three years. Others were valuable sets or parts of sets which could not previously be obtained at favorable prices. In this category fall vols. 1-19 of the "Biologisches Zentralblatt," vols. 1-40 of the "Skandinavisches Archiv für Physiologie," vols. 1-36 of the "Journal of Biochemistry," and vols. 1-14 of the "Zoologischer Bericht."

Eight new bookcases installed in the main reading room provided over 500 feet of new shelving and made possible a complete rearrangement of the journal collection. At the current rate of growth, space is now adequate for a seven-year period.

The book collection was increased by over 350 volumes, representing an increase of nearly 10%. Most of the books of the Clokey Botanical Library were catalogued and added to the botanical section of the Library. Eight items from the Biology out-of-print book want list of 46 items were purchased during the year, in some instances completing searches of several years' duration. Cockayne's "Inherited Abnormalities of the Skin and its Appendages," an important monograph which has been out of print for several years, has been on the want list for some time and has been vigorously searched for by many staff members as well as by friends of the Division. During the year Lord Rothschild of Cambridge University generously and kindly presented the Library with a bound photostatic reproduction of this valuable work.

Eleanor Bjornerud, Biology Librarian for 1950-1951, left the Institute in June for studies in the field of Education, at U.C.L.A. and was replaced by Georgie Evans.
The Staff

The Division of Biology Staff for the year 1950-1951 was made up as follows:

G. W. Beadle, Chairman of the Division

Professors

Ernest G. Anderson, Ph.D.
G. W. Beadle, Ph.D., D.Sc.
James F. Bonner, Ph.D.
H. Borsook, Ph.D., M.D.
Max Delbrück, Ph.D.
Sterling Emerson, Ph.D.
A. J. Haagen-Smit, Ph.D.
A. H. Sturtevant, Ph.D., D.Sc.
Albert Tyler, Ph.D.
A. Van Harreveld, Ph.D., M.D.
Frits Went, Ph.D.
C. A. G. Wiersma, Ph.D.

Visiting Professors

Ernst Hadorn, Ph.D.
Felix Gustafson, Ph.D.
Arthur W. Martin, Ph.D.
University of Zurich
University of Michigan
University of Washington

Research Associates

Gordon A. Alles, Ph.D.
Charles E. Bradley, D.Sc.
Henry O. Eversole, M.D.
William M. Hiesey¹, Ph.D.
Albert E. Longley, Ph.D.
Lilian V. Morgan, M.A.
Lord Rothschild², Ph.D.
Esther B. Tietz, M.D., Ph.D.
Jean J. Weigle, Ph.D.

¹Carnegie Institution of Washington, Stanford, California

²University of Cambridge

Associate Professors

Arthur W. Galston*, Ph.D.
Norman H. Horowitz, Ph.D.
Edward B. Lewis, Ph.D.
George E. MacGinitie, M.A.
Herschel K. Mitchell, Ph.D.
Ray D. Owen, Ph.D.
Austin Phelps, Ph.D.

*On leave of absence
Senior Research Fellows

Bernard Axelrod, Ph.D.
Clara L. Deasy, Ph.D.
Jacob W. Dubroff, Ph.D.
Renato Dulbecco, M.D.
Geoffrey L. Keighley, Ph.D.
Edward Novitski, Ph.D.
Bruno Rosenfeld, Ph.D.
Samuel J. Singer, Ph.D.
S. J. Wildman, Ph.D.

Gosney Fellows

Joseph Lein, Ph.D.
Thomas C. Nelson, Ph.D.
Warren P. Spencer, Ph.D.
Tsuneo Y. Tanabe, Ph.D.
Marguerite M. Vogt, M.D.
Katerina I. Zarudnaya, Ph.D.

Research Fellows

Adolph Abrams, Ph.D.
William C. Ashby, Ph.D.
Daniel Atkinson, Ph.D.
Robert S. Bandurski, Ph.D.
Seymour Benzer, Ph.D.
Giuseppe Bertani, Ph.D.
Guy Ch. Camus, Ph.D.
Robert Casamajor, B.S.
E. E. Dale, Ph.D.
Ellis P. Darley, Ph.D.
Frank H. Dickey, Ph.D.
E. C. Dougherty, Ph.D.
Marjorie G. Douglass, Ph.D.
William Drell, Ph.D.
Marguerite Fling, Ph.D.
Helen R. Garman, Ph.D.
Harold Garner, Ph.D.
Edwin A. Goldsmith, Ph.D.
Felix L. Haas, Ph.D.
Charles E. Harrold, Ph.D.
Francis A. Haskins, Ph.D.
Henry H. Hammers, Ph.D.
James H. Henderson, Ph.D.
Herbert M. Hull, Ph.D.
Anton Lang, Ph.D.
George G. Laties, Ph.D.
Urs Leupold, Ph.D.
Margaret Lieb, Ph.D.
Fred Lorenz, Ph.D.
Peter H. Lowy, Doctorandum
Jean Mauron, Ph.D.
A. M. Michelson, Ph.D.
Mary H. Mitchell, M.A.
Alfred Novak, Ph.D.
Axel Nygren, Ph.D.
Daphne J. Osborne, Ph.D.
Edwin A. Phillips, Ph.D.
Derrick Rowley, Ph.D., M.B., B.S.
Pierre Schaeffer, Ph.D.
T. C. Sheng, Ph.D.
Alberto Soriano, Ph.D.
Harrison D. Stalker, Ph.D.
John S. Stamm, Ph.D.
Melvin L. Stehsel, Ph.D.
Gunther Stent, Ph.D.
A. G. R. Strickland, Ph.D.
Howard Teas, Ph.D.
Albert Ulrich, Ph.D.
James Watson, Ph.D.
Emanuel Windsor, Ph.D.
Elie Wollman, M.D.
1 United States Forest Service
2 Frasch Foundation Fellow
3 American Cancer Society Fellow
4 Union College, Schenectady
5 Eli Lilly Fellow
6 University of London Fellow
7 Lalor Foundation Fellow
8 United States Public Health Fellow
9 University of California, Davis
10 Commonwealth Fund Fellow
11 Rose Sidgewick Research Fellow
12 Rockefeller Foundation Fellow
13 Washington University, Saint Louis
14 Merck Fellow
15 University of California, Berkeley
16 Rockefeller Foundation Fellow

Graduate Fellows and Assistants

Howard J. Boroughs1, B.A.
George H. Bowen, Jr.2, B.S.
Roderick K. Clayton2*, B.S.
George R. Dubes, B.S.
Luther Eggman, B.S.
George L. Ellman3**, M.S.
Gerald D. Fasman**, B.S.
Robert J. Foster**, B.S.
Peter Goldacre4, B.Sc.
Norman Good, B.A.
Francis A. Haskins2, M.Sc.
Claude W. Hinton5, M.A.
David Hogness6**, B.S.
Bruce W. Holloway7, B.Sc.
Herbert M. Hull, B.S.
Lionel Jaffe5, B.S.

Leonard L. Jansen, M.S.
Armin Dale Kaiser, B.S.
Edwin B. Kurtz2, M.A.
Dan L. Lindsley2, M.A.
James L. Liverman8, B.S.
Dougal Harold McRae, M.A.
Jean P. Nitsch8, Dipl. Ing.
Joseph L. O'Brien**, B.S.
Earl B. Patterson, B.Sc.
Robert Phillips9**, B.S.
Irving Rappaport, B.Sc.
Jose Luis Reissig, B.S.
Sherman H. Ripley, M.S.
Donald S. Robertson, A.B.
Philip S. Thayer6, M.A.
David R. Viglierchio8, B.S.

*Physics Major
**Chemistry Major
1 Frasch Foundation Fellow
2 Atomic Energy Commission - National Research Council Fellow
3 McCallum Foundation Fellow
4 Commonwealth Scientific and Industrial Research Organization Fellow
5 Atomic Energy Commission Fellow
6 National Institutes of Health Fellow
7 Smith-Mundt Fellow
8 Lucy Mason Clark Fellow
9 Abbott Laboratories Fellow
Graduate Students

Bruce N. Ames, B.A.
James H. Berrian, M.A.
Glenn Fischer, B.A.
Henry Gershowitz, B.A.
Willoughby GoodSmith, M.A.

*Chemistry Major

Kenneth Johnson*
Paul D. Saltman, B.S.
Albert Siegel, B.A.
Fred Wolfgram, A.B.

Visiting Graduate Students

Andre Jagendorf, B.A.
Robert Manyik, B.A.

Research Assistants

Elsie Atkinson, M.S.
Lyle Bacon
Rosamond Scott Baker, B.A.
Elizabeth Ann Bartron, B.A.
Dorothy Benzer, R.N.
Elizabeth Bleecker, M.D.
Barbara Bostwick, B.A.
Jean M. Campbell, M.S.
Ruth C. Corbett, B.A.
Mary E. Durrell, M.S.
Guiseppina Dulbecco
Amelia A. Dvorsky, M.A.
Mary E. Emerson, M.S.
Phyllis Ellman, M.S.
Ruth E. Estey
Helen R. Fahy
Margaret M. Fox, B.S.
Emma Jane Griffith, B.S.
Cathryn S. Haas, B.A.
Frank N. Hirosawa, M.Sc.
Mary R. Hull, B.A.

Frances Huszarik, B.A.
Helen E. Jeffrey, R.N.
Nylan Jeung, B.A.
Thomas M. Jones, B.S.
Marcella C. Juhren, M.A.
May G. Keighley, B.A.
Anna Kilim, B.A.
Carol Lotz, M.A.
Alice R. McColloch, B.S.
Alison Adele Millerd, M.Sc.
John Julian Norris, B.A.
Kenneth Ohki, M.A.
Marilyn B. Rusler, A.B.
Roy Signer, M.S.
Ruth E. Singer, A.B.
Helen E. Surber, M.S.
Donald E. Sutton
Anna Newton Teas, M.S.
Selina Weinbaum, B.S.
John Wesley Yale, M.S.
Milton Zaitlin, B.S.

Laboratory Technician

Eleanor Kerr
Technical Assistants

Leora DuBerg
James Gay Helmuth
Jack Kelleher
Henry C. Kirk
Matt Meselson

Richard C. Myers
Karl J. Niedermann
Gilbert Peppin
Glenn A. Swinehart
Edith M. Wallace

Laboratory Assistants

Richard J. Andrews
Mitchell Bain
Richard Bates
David Beadle
Egon Bittner
Malcolm Paul Boghosian
Stuart Bowen
Sherwin A. Carlquist
Solomon D. Chase
Richard DuBridge
William H. Estabrook
Richard Harrison Fuller
Pauline Garand
Jack T. Gordon
Philip Green
Olga Gunn
Jan Haagen-Smit
Alan Haber
Bruno Herscovici
Jack Livesey

Philip Neher
Sudie B. Newman
Dan Pletsch
Marion Popenoe
Morris Robkin
Judith Rodekohr
James A. Ross
Elmer O. Slater
Henry Sturtevant
James Trew
Jerrold Turner
James S. Tyler
Thomas Tyson
Anthonie Van Harreveld, Jr.
Eva Von Huene
Cora Wells
Ruth Wendt
Jess Williams
John Seymour Winslow

Custodians

W. A. Bowness

Paul Pierrson

Gardeners

Charles Bates
Patrick A. Burke
James E. Cunningham
Andrew Drummond

Raymond J. Dunbar
Clarence Edward Lippa
Albert Miotto
Clifford G. Young
Librarians

Eleanor Bjornerud  Jirina Maria Evans

Office Staff

Andre Crehan, Secretary  Jeanne L. McCune, Secretary
Dorothy Egbert, Secretary  Elizabeth Morse, Secretary
Jeanne Fischer, Secretary  Hilda Rook, Chief Clerk
Gerald Fling, Administrative Assistant  Pamela Rowbotham, Secretary
Marjorie Krenz, Secretary  Donna P. Thorpe, Secretary
Elizabeth Land, Secretary  Elvaelsie Webber, Secretary
Helen Lilly, Secretary

Shops

Henry A. Garand, Machinist  William P. Thomson, Mechanic
Anthony Nunn, Maintenance Carpenter  Milo Webber, Carpenter's Assistant
Frank Ostrander, Machinist

Stockroom

L. E. Castle, Supervisor  Anna Teas, Supervisor
R. W. Gibbons, Assistant  George A. Zentmyer, Assistant

Superintendents

George G. Chalmers, Kerckhoff Marine Station
Charles W. Cochran, Biology Experimental Farm
John F. Dale, Biology Experimental Farm
G. P. Keyes, Earhart Laboratory
Charles M. Newman, Orlando Greenhouse

Assistant Superintendent

Robert G. Hoover, Biology Experimental Farm
Staff Promotions, Awards and Honors

Arthur W. Galston, promoted from Senior Research Fellow to Associate Professor.

Albert Tyler, awarded special Guggenheim Foundation Fellowship for two years.

Sterling Emerson, awarded Guggenheim Foundation Fellowship and Fulbright Travel Fellowship.

Alfred H. Sturtevant, named Thomas Hunt Morgan Professor of Biology at the Institute; awarded Sc.D. degree by Yale University, June, 1951.

Bernard Axelrod, given Superior Service Award by United States Department of Agriculture.

Philip S. Thayer, Thomas Hunt Morgan Award for 1951.

George W. Beadle, Lasker Award, 1951; R. E. Dyer Lectureship Award, National Institutes of Health.

James Bonner, elected Fellow of American Camellia Society.

The Morgan Memorial Plaque

During the year Professor Albert Stewart of Scripps College completed the Thomas Hunt Morgan Plaque. A simple dedication ceremony was held in the Kerckhoff Memorial Library on February 23. At the same time President DuBridge announced the appointment of Professor Sturtevant as Thomas Hunt Morgan Professor of Biology and also presented the 1951 Thomas Hunt Morgan Award to Philip S. Thayer. The Morgan Award is presented annually to a graduate student finishing work for the Ph.D. degree. A sum of $100 is a part of this award.

Visiting Lecturers

Thirteen visitors gave lectures or seminars in the Biology Division during the year. In many instances their presence at the Institute was made possible by the Eversole Lecture Fund.
Speakers and their subjects were as follows:

Doctor Boris Ephrussi, University of Paris, "Genetic Study of a Respiratory Deficiency in Yeast."

Doctor E. A. Adelberg, University of California, Berkeley, "Bio-synthesis of Leucine and Valine."

Lord Rothschild, Cambridge University, "The Kinetic Aspects of Fertilization."

Doctor Perry Stout, University of California, Berkeley, "Plant-Soil Interrelations with Special Reference to the Micro-nutrient Element Nutrition of Plants."

Professor Fred Lorenz, University of California, Davis, "Gametogenesis Studied by Means of Isotopic Phosphorus."

Doctor Carl F. A. Pantin, Cambridge University, "Spontaneous Activity and the Elementary Nervous System."


Professor Robert L. Crocker, University of California, Berkeley, "The Australian Sand-Ridge Deserts."


Professor Clyde Stormont, School of Veterinary Medicine, University of California, Davis, "The Immunogenetics of the Blood-Group Systems of Cattle."

Professor Ernst Hadorn, University of Zurich, Switzerland, "Developmental Physiology of Lethal Mutations in Drosophila."

Doctor Derrick Rowley, St. Mary's Hospital, London, "Some Effects of Penicillin on Bacteria."

Professor Mogens Westergaard, University of Copenhagen, "Induced Reversions in Neurospora with Reactive Chemicals."
Hixon Lectures

A Hixon Fund Symposium on "Cerebral Mechanisms in Behavior" was held at the Institute September 24 to September 25, 1948. Professor Lloyd Jeffress of the University of Texas undertook to collect the papers presented and organize them and the accompanying discussion for publication. The resulting monograph should be off the press of John Wiley & Sons shortly.

During March, April and May of 1950 a series of five lectures on "The Scientific Basis of Psychoanalysis" was sponsored by the Hixon Fund. It is hoped that these lectures will be published in book form soon.

In March Doctor George Marmont of the University of Chicago gave a series of four lectures at the Institute on modern concepts of nerve impulse transmission. This series was sponsored by the Hixon Fund. It is hoped that these lectures will soon be ready for publication.

On April 3 and 5, 1951, Professor Stephen W. Kuffler of Johns Hopkins University presented two lectures under the sponsorship of the Hixon Fund. These were as follows:

The Physiology of the Mammalian Small-nerve System; Nervous Control of Muscle Spindle Discharge.

Nerve-muscle Transmission Problems in Crustacean and Frog Muscle.

Teaching

Plans have been completed to revise the Biology Option curriculum beginning in the fall of 1951. This revision consists mainly of combining histology, histological technique and mammalian anatomy in a single course to be taught by Professor Van Harreveld and Doctor Keighley with the assistance of Mrs. Estey.

For the past two years the lectures in Biology 1, the first course in the Biology Option and a general course required of all students in Science Options, have been given by Professor Owen. Professor Beadle has had charge of laboratory instruction. Much effort has been spent by the professors in charge of this course and by the Graduate Assistants assigned to it to improve it. Student
evaluation of the course has been encouraged. Short laboratory demonstration talks given by various staff members in the Division on their special fields of research have done a great deal to acquaint students with the field of biology and to arouse interest. During the past year half a dozen "guest lecturers" took part in the course, each giving a one-hour lecture on his special field. The responsibility for this course is a large one and the burden should not be carried by a single person for a long period of time. It has therefore been arranged that Professor Horowitz will assume responsibility for the lectures next year.

One of the last courses taken by students in the Biology Option is Biochemistry (Biology 207). This comes in the last two terms of the senior year and consists of lecture-discussion meetings led by Professor Borsook and laboratory work. For the past two years Professor Mitchell has taken the responsibility for seeing that the laboratory work is properly organized and well taught. In this he has done an especially noteworthy job. For two years the last term has consisted of special research assignments to the students. In carrying these out they work in pairs in the various research laboratories of the Division in which some phase of plant or animal biochemistry is being studied. The students are given some special task, often involving doing original experiments, the results of which are expected to be of definite scientific value. The interest on the part of the students in this type of laboratory work is remarkable. In a number of instances the work done has led to results valuable enough to justify their publication in standard scientific journals.

Graduates

Over the twenty-year period since the Division of Biology was established at the Institute, some eighty men have earned the B.S. degree in the Biology Option. Almost eighty percent of these have gone on for doctors' degrees, of which 40% have earned Ph.D. degrees while 40% have taken work leading to an M.D. degree.

Eight men were awarded B.S. degrees in Biology in June 1951. Their names and their plans for future work are as follows:

Charles Bates
Thorne J. Butler

Graduate work
Stanford Medical School
James A. Enslow  No definite plans
Carl Hildebrand  Fuller Theological Institute
Carl Hirsch  Biological Laboratories,
Edwin Matzner  United States Army
Harry Rappaport  Chemical Corps, Frederick, Maryland
Raymond A. Sjodin  University of Chicago Medical School

Seven men completed work for advanced degrees during the year under review. Glenn Fischer, M.S. in Genetics, is continuing his graduate studies at the Institute. Roderick K. Clayton, Ph.D. in Physics, who did his thesis work in the Division of Biology, will spend the next year at the Hopkins Marine Station as a Merck Fellow. Francis A. Haskins, Ph.D. in Genetics, and Herbert Hull, Ph.D. in Plant Physiology, have been appointed Research Fellows at the Institute. Jean P. Nitsch, Ph.D. in Plant Physiology, is spending a year at the Harvard Biological Laboratories. Donald Robertson, Ph.D. in Genetics, has joined the staff of the Bible Institute in Los Angeles as instructor of a general biology course. Albert Siegel, Ph.D. in Plant Physiology, has been awarded a United States Public Health Service Fellowship at the University of California, Los Angeles.

**Fellowships and Scholarships**

_**Gosney Fellowships**_ The following held Gosney Fellowship appointments during the year 1950-1951:

Joseph Lein, Syracuse University, Research on Chemical Genetics.

Thomas C. Nelson, Columbia University, Research on Bacterial Genetics.

Warren P. Spencer, College of Wooster, Research on Drosophila Genetics.

Tsuneo Y. Tanabe, Pennsylvania State College, Research on Physiology and Metabolism of Spermatozoa and Ova.
Marguerite M. Vogt, Institute for Brain Research, Neustadt, German, Research on Bacterial Virology.

Katerina Zarudnaya, University of Missouri, Research on Genetics of Maize Anthocyanins.

**McCallum Foundation - Nutrition Foundation Fellowships**

The first McCallum Predoctoral Fellowship, established by the McCallum Foundation through the Nutrition Foundation, was awarded to Mr. George L. Ellman, a graduate student working for a Ph.D. degree in chemistry with Professor Mitchell. During the past year a second McCallum Fellowship was established. This has been awarded to Mr. Bruce Holloway of the Waite Agricultural Research Institute, Adelaide, Australia, who is working for a Ph.D. degree in chemical genetics.

**McCallum Foundation Summer Scholarships**

For a long time the Division has felt a need for scholarship funds for the support of graduate students who desire to spend summers working on their thesis research programs. Institute policy encourages this by providing tuition-free registration privileges for graduate students during summer periods when there is no regular academic session. Mr. and Mrs. Arthur McCallum appreciated the need for such summer scholarship support and have generously established a fund for an experimental program for the summer of 1951. Some 15 graduate students were awarded McCallum Summer Scholarships under this program. The usual award is $100 per month for the three-month summer period. It is already clear to both faculty members and graduate students that these unique scholarships are meeting a real need.

**Lucy Mason Clark Fellowship in Plant Physiology**

This Fellowship, established in 1949, was held during 1950-1951 by Mr. James L. Liverman, who is working for a Ph.D. degree with Professor Went.
Pioneer Hi-Bred Fellowship

Established in 1947, this predoctoral fellowship was held for the fourth year by Mr. Earl Patterson.

DuPont Fellowship

For some time the E. I. DuPont de Nemours and Company has supported predoctoral fellowships in chemistry and physics in a number of leading American universities. For the first time, here or elsewhere, one of these highly desirable fellowships has been made available in the field of biology. Mr. Paul Saltman has been recommended to the DuPont Company for this fellowship. He is working in plant biochemistry with the Bonner group.

Seeley W. Mudd Scholarship

For several years, Doctor Seeley G. Mudd has made available funds for a tuition scholarship to be awarded to a third or fourth year undergraduate interested in some phase of medicine. During the past year this fellowship was held by a junior in the Chemistry Option, Mr. Milo Webber. Mr. Webber will be a member of the first class in the new medical school at U.C.L.A. and will begin there in the fall.

Other Fellowship Appointments

In addition to the fellowship appointments listed above there are a number of research workers who hold the title Senior Research Fellow or Research Fellow whose stipends come from grants made to the Institute in support of an area of research rather than specifically for a fellowship.

During the year 1950-1951, nine men held predoctoral fellowships awarded by an agency operating outside the Institute, usually on a national basis. These were distributed as follows:

- Atomic Energy Commission Fellowships: 2
- Atomic Energy Commission Fellowships: 4
  Administered by National Research Council
Commonwealth Scientific and Industrial Research Organization Fellowship 1
National Institutes of Health Fellowship 1
Smith-Mundt Fellowship 1

Twelve persons held nationally awarded postdoctoral fellowships during this period. These were distributed as follows:

- American Cancer Society Fellows 3
- Commonwealth Fund Fellows 2
- John Simon Guggenheim Memorial Foundation Fellow 1
- Lalor Foundation Fellow 1
- Rockefeller Foundation Fellow 1
- Rose Sibgwick Memorial Fellow 1
- United States Public Health Service Fellows 3

In connection with nationally awarded postdoctoral fellowships, Roderick K. Clayton and Philip S. Thayer were awarded Merck Postdoctoral Fellowships. Dr. Clayton, who completed his Ph.D. work in June, will spend a year at the Hopkins Marine Station, while Mr. Thayer, who will complete his Ph.D. work in September, 1951, will work with Doctor Michael Doudoroff at the University of California, Berkeley.

Research

In the pages which follow the investigators in the biological sciences at the California Institute of Technology have summarized their activities for the year ending June 30, 1951. Some 140 investigators, with 132 technical assistants, have applied themselves to a variety of research projects. We cannot hope to treat of their work and findings in detail here. The interested reader must of necessity be referred to the scientific journals in which the investigators make their reports. It is possible, however, to touch briefly on the major programs under way in the Division of Biology.

Projects are arranged according to groups of workers classified under subject headings which correspond more or less closely to conventional academic departments. In a number of instances the procedure followed results in the inclusion of projects under headings not entirely appropriate to them. For
example, Clayton's work on phototaxis appears under Virology because he works with the Delbrück group which at the moment is mainly concerned with virology. For a similar reason virus work on higher plants appears under Plant Physiology and Biochemistry.

The Laurabelle Arms Robinson Fund and two specific grants from the American Cancer Society are used in support of work bearing on the cancer problem. Because this work is basic rather than clinical in nature, accounts of it will be found under various headings in the reports on research activities. It should be emphasized, as it has been in previous reports, that much of the work of the Division that bears on cancer could not be done were it not for the Laurabelle Arms Robinson Fund which has made possible the establishment and maintenance of faculty positions in such fields as biochemistry, genetics, embryology, physiology, bioorganic chemistry, biophysics and virology.

The James G. Boswell Foundation Fund for Virus Research has made possible a development at the Institute of especial interest in the field of animal virology. This involves an attempt by Dulbecco and associates to develop a new type of animal virus assay fashioned after the now classical plaque count assay technique widely used in bacterial virology. In order to obtain desired background and the best available advice on this project Dulbecco spent two months during the early part of 1951 on a trip to various animal virus and tissue culture laboratories. Since his return active work has been under way with the virus of equine encephalomyelitis in a newly equipped and modernized virus-tissue culture laboratory suite.

"Viruses, 1950," a book on the Virus Symposium supported by the Boswell Fund and held at the Institute during March, 1950, has been in much greater demand than was anticipated. The first printing of 1,000 copies is now exhausted and inroads already made on a second printing of 700. The Institute Bookstore has cooperated in the sale of this monograph.

Animal Biochemistry

Staff: Professors: Borsook, Haagen-Smit; Senior Research Fellows: Deasy, Dubnoff, G. Keighley; Research Fellows: Abrams, Lowy, Novak, Windsor; Research Assistants: Bartron, Dvorsky, Jeffrey, M. Keighley, Rusler.
Protein Synthesis

Investigators: Borsook, Haagen-Smit, Deasy, G. Keighley, Lowy.

Support: General Funds, Atomic Energy Commission operating through Office of Naval Research

Borsook and associates have continued a study of the biological synthesis of protein using C\textsuperscript{14}-labeled amino acids as tracers. Hitherto a mixture of all the proteins of a tissue was used. The development of the field called for work with a single, well-defined protein. Additional desiderata were that the protein be easily purified, the tissue in which it is contained readily obtainable in large quantities, and the labeled amino acids incorporated rapidly into it in vitro. These specifications are met by the hemoglobin in rabbit reticulocytes.

A reticulocyte is a circulating erythrocyte in the last stages of maturation before it becomes a normal red blood cell. In the healthy animal only about 0.5 per cent of the circulating red cells are reticulocytes. The latter can be made to constitute over 90% of the circulating red cells by injection of phenylhydrazine for seven days. The normal red cells are disrupted by the phenylhydrazine and are replaced, in part, by an outpouring of reticulocytes from the bone marrow.

Reticulocytes so obtained incorporate labeled amino acids in vitro faster than any tissue so far studied, whether in vivo or in vitro. The labeled amino acids are incorporated at about the same rate into the hemoglobin as into the other proteins of the reticulocyte. When the red cell picture changes from exclusively mature red cells to almost exclusively reticulocytes, the concentration of ribonucleic acid increases nearly twenty-fold and of desoxyribonucleic acid to twice. Their increase parallels the increase in reticulocytosis. The rate of incorporation of labeled amino acids also increase in proportion to the increase in nucleic acids and reticulocytes. The embryologists have pointed to the association of increase of ribonucleic acid and protein synthesis; this concomitance extends to incorporation of labeled amino acids into the proteins.

In most of the work with reticulocytes, so far, C\textsuperscript{14}-labeled L-leucine has been used. When it is incorporated into the hemoglobin 99 per cent has its amino group bound. It is probable that nearly all the incorporated amino acid is in the interior of the
molecule, indicating rupture and reconstitution of the molecule.

When reticulocytes, washed in Ringer's solution, are
incubated on the one hand in serum and on the other in Ringer's
solution, the rate of incorporation of the labeled amino acid is two
to four times faster in serum than in Ringer's solution. This
occurs with glycine, histidine, leucine, and lysine. The sera
of all animals so far tested, normal or anemic, have this accelerating
effect.

Studies of the nature of the accelerating factor in serum
are under way. It can be removed completely by boiling the
serum at pH 5.5. All the accelerating factor is in the colorless
filtrate. It is rapidly dialysable, can be adsorbed completely on
Lloyd's reagent at pH 2.0 and then removed with NaOH at
pH 10.0, and is heat stable at pH 5.5.

The serum factor consists of two kinds of components.
One consists of the amino acids histidine + phenylalanine +
valine, and possibly tyrosine; the other compound has not yet
been identified. The optimum concentration of each of the amino
acids is about 2 mg. per cent. In the presence of the second
component the four amino acids are three times as active as when
the second component is absent. When the four amino acids are
added to serum the latter becomes twice as active, indicating
that these four amino acids are not present in serum at their
optimal concentrations.

Two other substances have been found to have some
accelerating effect, namely, coenzyme I and ATP. It is interesting
and suggestive that it is precisely these two coenzymes that operate
to keep hemoglobin iron in the reduced state necessary to its
function as an oxygen carrier.

All the other known co-factors, amino acids, and metabolites
are inactive.

It has been reported in recent years by Sebrell that rats
rendered anemic by hemorrhage do not reconstitute well either
hemoglobin or red cells when their diet is deficient in either
histidine or valine, two of the four amino acids we have found to
constitute one part of the serum factor in our test system. Another
group of workers has found that amino acids (not analyzed in detail)
participate in the maturation of reticulocytes. It appears, therefore,
that the method of incorporation of labeled amino acids may be useful
in analyzing the factors involved in red cell and hemoglobin formation and that for this purpose it is more sensitive, convenient, and quicker than the nutritional methods used hitherto.

Even though the second component of the accelerating factor in serum has not yet been identified, two different practical applications of the above findings suggest themselves as deserving to be tested. One, the use of supplemental histidine + phenylalanine + tyrosine + valine in the treatment of secondary anemia; the other, the use of this amino acid supplement to raise the red cell count above normal. The second of these might help pilots operate at higher altitudes without oxygen.

When the second (as yet unidentified) factor becomes available in concentrated form it might be useful in combination with the above four amino acids. But as the second factor is present in serum, and the four amino acids are not present in optimal concentration in the blood, feeding them might be useful.

These findings, apart from any practical value, have revealed new phenomena concerning the process of amino acid incorporation into proteins.

**Therapeutic Action of Betaine plus Glycocyamine**

**Investigators:** Borsook, G. Keighley, Windsor.

**Support:** International Minerals and Chemical Corporation, Office of Naval Research.

During the summer or fall of 1951 four papers will be published on clinical trials of the usefulness of betaine and glycocyamine in cases of heart disease. The biochemical basis of this therapy was provided by work in the Kerckhoff Laboratories by Borsook and Dubnoff on the mechanisms of creatine formation and transmethylation. The clinical application was proposed by H. Borsook and Doctor M. E. Borsook of the Research Division, Leo N. Levi Memorial Hospital, Hot Springs, Arkansas.

It was assumed more of certain substances may be required for the recovery of a cell with impaired function than for the maintenance of the cell when it is healthy. Since phosphocreatine is the main reservoir of immediately available energy in muscle and nerve, it was hoped that the presentation to a damaged muscle or nerve of more creatine than is normally synthesized in
the body might facilitate its recovery. By providing extra creatine there would be more retention of creatine in the tissues and as a consequence more phosphocreatine in muscle and nerve.

Extra creatine can be formed in the body from betaine and glycocyamine, and accordingly these two compounds were fed together to approximately fifty patients with heart disease with failure. The majority of the cases were benefited significantly. The present indications are that betaine and glycocyamine will be a useful addition to cardiac therapy.

The clinical trials were carried out by M. E. Borsook, Captain Ashton Graybiel of the United States Naval School of Aviation Medicine, Pensacola, and Doctor V. Van Zandt of Los Angeles. The clinical studies have been in progress for more than a year and are being extended. Further biochemical and physiological studies on the effects of betaine and glycocyamine are being carried out at the Kerckhoff Laboratories by Borsook, Keighley, and Windsor.

**Action of Vitamin B₁₂**

**Investigator:** Dubnoff.

**Support:** United States Public Health Service.

It is known that directly or indirectly vitamin B₁₂ plays a part in the synthesis of methionine. Studies undertaken in this laboratory to elucidate the action of this vitamin have shown that in tissue slices and homogenates B₁₂ markedly catalyzes the reduction of disulfide to sulfhydryl groups. Methionine is synthesized by the addition of an methyl group to the sulfhydryl group of homocysteine. Normally this group is oxidized in air to the disulfide form, which is inactive in methionine synthesis. B₁₂ by directly or indirectly catalyzing the reverse reaction increases the amount of the sulfhydryl form available for methionine synthesis. Similarly B₁₂ catalyzes the reduction of oxidized glutathione. This compound is known to have a regulatory effect on a variety of enzyme systems, and its oxidation-reduction state is undoubtedly of importance in regulating alternate metabolic pathways.

This work has received striking confirmation from the Johns Hopkins Laboratories where it was found that the sulfhydryl groups of the blood of pernicious anemic patients were very low, but the administration of B₁₂ produced a rapid increase to normal values.
Further evidence for this role for B₁₂ has been obtained by the use of the E. coli mutant requiring methionine or B₁₂. This mutant is blocked between homocystine and methionine, and it has been assumed that the deficiency was in the inability to methylate the former compound in the absence of the vitamin. It has been found that this organism will grow on homocysteine but not on its oxidation product—homocystine. As in animal tissues it appears that in the absence of B₁₂ E. coli is unable to reduce the homocystine which is normally formed under aerobic conditions from homocysteine. Glutathione and cysteine were found to reduce homocystine to homocysteine and these also were found to support growth of the mutant.

Intermediates in Protein Synthesis

Investigator: Abrams.

Support: Eli Lilly and Company.

It has been shown by Borsook and coworkers that carbon-labeled radioactive amino acids injected into animals are rapidly incorporated into the tissue proteins. During the year under review Abrams and Borsook have investigated the mechanism of this conversion.

The criteria that have been set up for implicating a substance as an intermediate in protein synthesis are as follows:
1. It must contain a polypeptide with a molecular weight smaller than that of the tissue proteins.
2. It must contain the radioactive amino acid which has been injected.
3. In the initial period after the injection the specific radioactivity of the substance must be higher than that of the tissue protein.

The isolation technique used was as follows: The cells of the liver tissue of an animal injected intravenously with a radioactive amino acid were disintegrated and the protein removed by conventional methods. The solution which remained was concentrated to a small volume and subjected to chromatographic analysis in an ion exchange resin column. The effluent which emerged from the column was collected in suitable fractions by means of an automatic fraction collector. Each fraction was then analyzed both for radioactivity and free amino compounds. The analysis of hundreds of fractions for radioactivity has been made practicable by an automatic Geiger counter designed and built by Keighley and coworkers. When the results of the analysis were plotted on a graph with the abscissa representing volume of effluent
from the column and the ordinate the radioactivity and the amino nitrogen, a series of peaks were obtained. These peaks represented the "spectrum" of the radioactive compounds and those containing free amino groups.

Some of the many sharply resolved peaks contained radioactivity alone, some only free amino nitrogen, and some with both were exactly coincident. Since the amount of compound and its radioactivity are quantitatively determined, a calculation of the specific activity tells to what extent a radioactive amino acid has been converted into any other compound which has acquired radioactivity.

Through the use of paper partition chromatography and electrophoresis on paper, some of the peaks were identified with the originally injected amino acids, glycine, leucine, lysine, and histidine. Others, which were radioactive, were well known conversion products which arise directly or indirectly from the injected amino acids, such as serine, glutathione, aspartic acid, and glutamic acid. One peak was due to taurine (non-radioactive), which was isolated in crystalline form and identified by elementary analysis. Still other peaks are as yet unidentified. One of these, with a small amount of radioactivity, shows evidence of being a peptide and therefore may have some significance for protein synthesis. Another unidentified highly radioactive peak shows evidence of being a small histidine peptide.

Experiments along these same lines are being continued.

Effect of Hormones on Protein Synthesis

Investigator: Novak.


Experiments were carried out to determine the effects of male hormone (testosterone propionate) on protein metabolism. The incorporation of four different radioactive amino acids, namely glycine, leucine, histidine, and lysine, into visceral and muscle protein was studied in normal mice, castrate mice, and testosterone propionate-treated castrate mice during intervals of time ranging from 20 minutes to 28 days. The results showed greater incorporation of glycine, histidine, and leucine in proteins of castrate animals compared to normal animals. Thus the presence of testes and the hormone diminishes the uptake of these amino acids into tissue.
protein. The uptake of lysine apparently is not affected by the presence or absence of testes.

Three experiments with ACTH indicate that this hormone causes an increased incorporation of histidine into both visceral and muscle protein. This effect suggests that a greater amino acid turnover is occurring and the conclusion is in accord with the excessive amino acid excretion by the kidneys in ACTH-treated patients. The experiments on ACTH are still in progress.

Animal Physiology

Staff: Professors: Van Harreveld, Wiersma; Research Associate: Tietz; Research Fellow: Stamm; Graduate Students: Fasman (Major, chemistry), Foster (Major, chemistry), O'Brien (Major, chemistry), Ripley, Wolfgram; Research Assistants: Estey, Kerr.

Neurophysiological Studies on the Crayfish

Investigators: Wiersma, Ripley.

Support: General Funds.

Wiersma has continued experiments with the giant axon-peripheral nerve root system of the crayfish, Cambarus clarkii. Stimulation of the giant axons in the circumoesophageal commissures with a single impulse results in a repetitive discharge into the peripheral fibers of the second ganglionic root. In addition to this phenomenon these second root fibers exhibit a spontaneous discharge of 5 to 10 impulses per second. It was found that there was no correlation between the repetitive and the spontaneous discharges. Whereas the frequency of the latter can be increased by the application of such drugs as nicotine and pyrethrum, the number of repetitive discharges resulting from a single impulse in the giant axon can only be decreased. This decrease can be brought about by relatively short periods of stimulation at a low frequency and also by the application of those drugs which result in an increase of the rate of spontaneous discharge. This latter effect could be due to a decrease in the excitability of the root fibers as a result of the large number of spontaneous discharges induced in them by the drug.

Simultaneous records from two roots of the same ganglion or from one root from each of two separate ganglia have been made.
Whereas two roots from a single ganglion give discharges which differ only in minor points, those from separate ganglia exhibit considerably more variation. However, it is in general true that the level of activity is the same in all the roots which were available for study. The factor governing the rate of the spontaneous discharges thus appears to be active along the length of the central nervous cord. Four giant fibers run the length of the cord, making synapses with all the peripheral roots. Comparison of the effects of stimulation of different giant fibers show that in some instances a certain giant fiber gives a significantly longer repetitive discharge than another. The results have not been consistent, and in general it is the "central excitatory state" which controls the number of the repetitive discharges rather than the type of giant fiber stimulated.

It has been shown that with certain crustacean neuromuscular preparations the temporal sequence of stimuli has a profound influence on the nature of the muscular response. Previously these studies have been limited to the excitatory processes. Now Wiersma and Ripley have extended these studies to the peripheral system of contraction inhibition in the crayfish. If electrical impulses are applied in pairs to the excitatory axon, the resultant contraction is in general greater than if the stimuli are applied at regular intervals. Two methods of approaching the inhibitory problem were adopted. Inhibitory and excitatory axons were isolated and the muscular response recorded. In one case the frequency modulated stimuli were applied to the excitatory fiber and this was tested against a certain frequency of inhibitory stimulation. In the second case the procedure was reversed and the inhibitory fiber received the modulated stimuli. In both cases comparisons were made with normal non-modulated stimuli. As would be expected a contraction resulting from modulated stimuli is not as readily inhibited as is the smaller contraction resulting from regular stimulation. Similarly when the inhibitory stimulus is modulated a larger suppression of the contraction results. These studies were put on a quantitative basis for comparative purposes and because of the bearing they might have on the nature of the facilitatory processes.
Neuromuscular Mechanism of Flight in Insects

Investigator: Wiersma, Ripley.

Support: General Funds.

Much interest has recently centered around the neuromuscular mechanism of flight in the insects. Roeder and Pringle have unearthed some peculiar phenomena which have led to controversy about the muscular mechanism. A preliminary investigation by Wiersma and Ripley on a selection of insects has confirmed Roeder's and Pringle's observations. Suspended insects were made to fly and the action potentials of various muscles involved in the flight were recorded. The wing beat frequency was recorded by means of a microphone. In both the moth and the grasshopper there is an action potential for every wing beat, but in various wasps, including the tarantula hawk, _Pepsis_, there may be as few as one action potential for eight wing beats. The role of certain muscles in the moth _Celerio_ has been clarified and further experiments have been planned to elucidate the mechanism in the Hymenoptera, as the explanations given by workers in the field are unsatisfactory.

Other preliminary experiments by Ripley on the leg muscles of the locusts have shown that it is difficult but possible to obtain single nerve fiber-muscle preparations in these animals. Some evidence for the existence of discrete fast and slow axons has been obtained. For comparative purposes it is hoped that innervation maps like those for the crustacean leg can be made.

Studies on the Blood Circulating Mechanism

Investigator: Van Harreveld.

Support: Gordon Alles Fund

A preparation for the study of the blood circulation has been developed by Van Harreveld and coworkers which allows the simultaneous recording of systemic and pulmonary arterial pressures, left and right atrial pressures, and the total blood flow. The hemodynamic theory of this preparation has been well worked out. This preparation together with the theoretical considerations allows an extraordinary insight into the effect on the circulation of changes of its parameters.
During the past year this preparation was used by Van Harreveld working in collaboration with Doctor C. H. Ellis, of the Gordon Alles Laboratory, and Wolfgram to investigate the action of a number of drugs on circulation. The compounds investigated were histamine and 1-10-diamino decane, epinephrine, and nor-epinephrine. The first two named compounds lower the blood pressure. This action is established by the effect on two parameters of the circulation; by dilation of the small systemic arteries, which results in a drop of the peripheral resistance; and by a relaxation of the large arteries and veins, which reduces the capacitance of the system. The action on the smallest arteries tends to increase the blood flow, the effect on the larger vessels decreases this variable. Both effects on the flow were observed. Histamine in certain concentrations and in certain preparations seemed to cause a constriction of the vessels of the lung circulation, causing an increase in lung arterial pressure.

Epinephrine and nor-epinephrine, both naturally occurring compounds, raise the blood pressure by affecting three parameters of the circulation. Both compounds elicit a constriction of the smallest arteries and cause an increase in tone of the larger and largest vessels. Moreover, both have a complicated action on the heart. The constriction of the smallest arteries increases peripheral resistance and tends to increase the flow. Depending on which effect prevails both enhanced and diminished flow were observed. The effect of these drugs on the heart is of two kinds: the heart rate is increased and the heart contractions strengthened. This leads usually to a decrease of the heart volume and a drop in the atrial pressures, notwithstanding an increase in bloodflow which may be present simultaneously. There is little difference between the effects of epinephrine and nor-epinephrine. In some preparations the effect of epinephrine was more pronounced on the tone in the large vessels, whereas nor-epinephrine affected the smallest arteries more profoundly. In one preparation this difference could be demonstrated very clearly, since at certain concentrations epinephrine increased the blood flow, whereas nor-epinephrine at the same concentration decreased the flow.

Physiological Aspects of Electronarcosis

Investigator: Tietz.


In collaboration with Doctor Elly Jacobsen further investigations have been made on the physiological and
endocrinological effects of electronarcosis on experimental animals.

During electronarcosis, as well as electroshock and insulin shock, several common physiological effects are apparent. One of these is an interruption in the menstrual cycle. In the hope that further investigation of this phenomenon may reveal a common mechanism for the effects of these treatments, attempts have been made to investigate the means by which this interruption is brought about. The intra-ocular transplant technique is used. Ovarian and endometrial implants are made into the anterior chamber of the eye of castrate female Dutch rabbits. The animals are then given a series of electronarcoses. Observation of the implants can be made at any time, changes noted, and photographs made. Later, when the animals are sacrificed, the implants, adrenals, uterus, and pituitary are taken for histological studies. Although it is clear that some additional control material is needed, investigations thus far indicate that the endometrial implants are stimulated greatly in castrate animals having ovarian implants to the eye and receiving electronarcosis. The ovarian tissue in the eye is maintained also and growth of the follicles occurs. A hypertrophy of the adrenal cortex is apparent.

**Propagation of Nerve Impulses.**

**Investigator:** Wolfgram.

**Support:** General Funds.

The mode of propagation of the nerve impulse in myelinated (vertebrate) nerve fibers has been the subject of much interest in the last few years. Such nerve fibers show, at intervals of 1 to 2 mm., interruptions of the myelin sheath known as nodes of Ranvier. Japanese and English workers have suggested that the conduction in these fibers is saltatory, that is, jumping from node to node.

Wolfgram has been working on this subject and has mastered the difficult art of preparing and handling single nerve fibers which are not thicker than 1/2000 of an inch. Tasaki has claimed that there is a considerable safety factor in saltatory conduction and that the excitation can jump two nodes instead of one. He reached this conclusion from his observation that it is necessary to narcotize two nodes to stop conduction. Wolfgram has repeated these experiments with a number of narcotics in a variety of concentrations,
making sure by the use of stained narcotic solutions that one and only one node is narcotized. He found consistently that conduction is fully stopped by narcotizing a single node and thus that, if saltatory conduction exists at all, the safety factor is smaller than supposed by Tasaki.

Experiments are in progress now to isolate electrically one node from another. If conducting in myelinated nerve is saltatory, connecting the two nodes by an electrically conducting bridge should make impulse propagation possible, whereas breaking the connection should arrest it.

**Intracellular Transport of Chemicals in Nerves**

**Investigator:** Wolfgram.

**Support:** General Funds.

It has long been believed on theoretical grounds that highly specialized compounds like enzymes, nucleoproteins, etc., are formed in the nerve cell and transported down the axon. This concept has received support by observations of Doctor Paul Weiss of the University of Chicago that a constriction of a nerve produces an accumulation of axoplasma proximal to the constriction. Wolfgram is making an attempt to show that the enzyme cholinesterase is formed in the nerve cell and transported down the axon. The anticholinesterase dipropyl-fluorophosphate (DFP) was administered to rabbits. This compound inactivates the cholinesterase present in the body. If the above suppositions are correct one may expect an increase in the cholinesterase content, first in the proximal end of a peripheral nerve like the sciatic, and later in the more distal parts of such a nerve. The experiments performed so far seem to indicate that such an enzyme transport down the nerve fiber occurs.

**Mechanism of Action of Narcotics**

**Investigators:** Van Harreveld, Fasman, Foster.

**Support:** United States Public Health Service.

It was found earlier by Van Harreveld and coworkers that not all narcotics act in the same manner. Some like ether and alcohol act by depolarizing the nervous structure. When depolarization has passed certain limits, conduction and synaptic transmission stop.
Other narcotics, especially the barbiturates, produce only moderate depolarization. The narcotic effect of these compounds is produced by the inability of the post-synaptic membrane to build up a local potential into a conducted one. Eccles has called this action the membrane stabilizing action. A few compounds are known which scarcely affect synaptic conduction even though they produce a fair degree of depolarization. The anticonvulsants dilantin and mesantoin are compounds with such an action. It seemed of interest to investigate the administration of a compound like dilantin on the effects of the two groups of narcotics mentioned above. If the depolarizing effect of dilantin and that of ether are additive then it can be expected that the ether concentration necessary to produce a certain depth of narcosis can be diminished by dilantin administration. Since the barbiturates do not produce narcosis through the mechanism of depolarization, little effect of dilantin administration on pentobarbital narcosis would be expected.

Working with Foster and Fasman Van Harreveld has studied this problem. It was found that administration of dilantin in a dose of 50 mg./kg. bodyweight causes about 40% decrease in the blood ether concentration necessary to suppress the kneejerk. Dilantin in the same concentration administered to a preparation in which the kneejerk was all but suppressed by pentobarbital did not enhance the effect of the narcotic. On the contrary, it often produced an increase in the reflex contractions. This difference in the result of dilantin administration on ether and on pentobarbital narcosis emphasizes the different mechanism of action of these two narcotics.

Injury Potential and Loss of Potassium by Nerve Cells

Investigators: Van Harreveld, O'Brien.


There is evidence to show that the injury potential of a cut nerve produces ionic movements which result in a loss of potassium through the mantle of the nerve fiber. Van Harreveld and O'Brien have made an attempt to show more directly the movements of cations under the influence of the injury potential. Owing to the presence of the myelin sheath which is poorly permeable for ions, a nerve fiber placed in a silver nitrate solution will first be affected at the Ranvier node. It is there that the fiber is locally damaged, causing an injury
potential at each node. The direction of this potential is such that cations will move into the axon. When the nerves are removed from the silver nitrate solution, rinsed, cut on the freezing microtome, and exposed to light, the silver which migrated into the axon at the node can be observed as black granules. Under favorable circumstances the silver stains on a considerable length of the axon on both sides of the node. The effect of a number of conditions which are known to eliminate the injury potential is investigated. Cooling the nerve during the silver nitrate action, asphyxiation, and ether and alcohol narcosis all prevented the staining. This shows that the injury potential is the driving force of the migration of silver into the axon. Other cations are released through the mantle of the nerve thereby maintaining osmotic equilibrium. Since K is the most abundant cation in the axon it would seem that a loss of K after nerve injury occurs through the mantle of the fiber and that the driving force is the injury potential.

Spreading Depression of Cortical Activity

Investigators: Van Harreveld, Stamm.

Support: F. S. Markham Fund, United States Public Health Service.

The stimulation of the cerebral cortex in experimental animals may produce a curious phenomenon known as the spreading depression. It consists of a ringshaped field of depression of the normal cortical activity, which travels at a very low rate from the stimulated region.

Van Harreveld and Stamm have continued studies of this phenomenon. Recently some doubt was raised as to the existence of this phenomenon in normal cerebral cortex. According to Marshall the spreading depression can be produced only in dehydrated cortex. Marshall's contention could not be confirmed. It was for instance possible to stimulate the cortex through a hole in the frontal part of the skull and to pick up the depression through another hole in the occipital region of the skull. In order to reach the occipital lobe of the brain the depression had to pass under a wide bridge of bone, through cortex which was never uncovered.

The nature of the spreading depression has been the object of considerable speculation. A number of experiments were performed in an attempt to explain the underlying mechanism. The depression is invariably accompanied by a slow potential change.
which spreads at the same rate as the depression over the cortex. This slow potential change can be used as an indication of the spreading depression when the latter for one reason or another cannot be recorded. It was found that this slow potential change is remarkably little affected by even huge doses of pentobarbital, thereby indicating that the causative mechanism is not dependent on synaptic conduction. A small mirror placed on the cortex surface was found to be tilted by a front of vasoconstriction moving at the same rate as the depression. With an electrode built on the polarographic principle and placed in the cortex it was shown that the spreading depression is accompanied by a lowering of the cortical oxygen depression.

It thus appears that the primary process underlying the spreading is a wave of vasoconstriction which lowers the oxygen tension in the cortex to such a degree that asphyxiation of the neural elements ensues. This results in suspension of the normal cortical activity and so produces the depression. The slow potential change must then be regarded as an asphyxial depolarization potential. Such potentials have been studied especially in the spinal cord and are caused by the depolarization of the more sensitive parts of the neuron. As is well known a partial depolarization produces a negativity of the depolarized part with respect to the still more fully polarized parts.

Bioorganic Chemistry

Staff: Professor: Haagen-Smit; Research Associate: Bradley; Gosney Research Fellow: Zarudnaya; Research Fellows: Goldsmith, Lowy, Strickland; Microanalyst: Swinehart; Research Assistants: Fox, Hirosawa; Graduate Student: Viglierchio.

During most of the 1950-1951 year, Professor Haagen-Smit spent three-fourths time with the Los Angeles County Air Pollution Control District. Near the end of the year he returned to full-time duty at the Institute.

Microanalytical Laboratories

Support: General Funds.

For many years the Division has maintained under the direction of Haagen-Smit a special service laboratory for elementary
analyses of organic compounds on a micro scale. These laboratories serve not only investigators in the Division but also workers in other divisions of the Institute and investigators in other research laboratories. Analyses are made on a cost basis, with the result that the micro-analytical laboratory is to a large extent a self-supporting activity.

Mr. Glenn Swinehart, microanalyst, carried out some 600 analyses during the year under discussion. These were made for the following persons and institutions.

Staff members of the Division of Biology
Staff members of the Division of Chemistry and Chemical Engineering
Staff members of the Guggenheim Aeronautics Laboratory
Staff members of the Jet Propulsion Laboratory
University of California at Los Angeles
University of California at Riverside
University of Southern California
University of Texas
United States Department of Agriculture
University of Calcutta
Los Angeles Air Pollution Control District

In connection with the smog program a combustion method was developed for the analysis of micro-quantities of hydrocarbons found in air.

**The Nature of Smog and its Biological Action**

Investigators: Haagen-Smit, Bradley, Fox, Hirosawa.

Support: Los Angeles County Air Pollution Control District and University of California.

The air pollution in the Los Angeles area is characterized by a decrease in visibility, crop damage, eye irritation, objectionable odor, and rubber deterioration. Haagen-Smit and coworkers have shown that these effects are closely related and are due to the oxidation of unsaturated hydrocarbons. The nitrogen oxides released in combustion processes function as oxidants in these reactions through release of atomic oxygen in a photochemical reaction. The oxidant formed closely resembles ozone in its action and its presence explains the accelerated rubber deterioration in this area. This action on rubber of sunlight and air containing nitrogen oxides was demonstrated in an experimental house of
plexiglas. Cracking of rubber under strain is a specific test for ozone and can be used to measure ozone quantitatively.

In the fumigation rooms at the Earhart Laboratory it has been shown that the same oxidant acting on higher olefins such as occur in cracked gasoline gives smog-type damage to each of five smog indicator plants. During the plant fumigations eye irritation was noticed, together with an odor typical of natural smog.

The oxidation of the hydrocarbons gives rise to aerosols which are especially pronounced in the oxidation of cyclic olefins also present in cracked gasoline. These aerosols account for the reduced visibility observed during smog periods.

Microanalyses of air samples have confirmed the presence of hydrocarbons and their degradation products, peroxides, aldehydes and organic acids.

Estimations, as well as actual measurements, have shown that ample quantities of hydrocarbons are released by evaporation losses and incomplete combustions. As a result of these studies it may be concluded that unsaturated hydrocarbons such as occur in gasoline should be considered as potential toxic materials.

The following persons at one time or another collaborated in various phases of the smog program:

For the National Bureau of Standards: Martin Shepherd;
for the United States Bureau of Mines: John Anderson,
Milton Orchin, Raphael Raymond, and Robin Van Meter;
and for the Air Pollution Control District of Los Angeles County: Julius Glater and Robert T. Lofberg.

**Essential Oils of Desert Plants**

**Investigator:** Bradley

**Support:** General Funds.

During 1950-1951 Bradley has continued his studies on essential oils of desert plants. The oil of *Bursera microphylla*, the elephant tree, was shown to consist mainly of phellandrene and sesquiterpenes. Under investigation are *Sapium bioculane*, which contains saponin-like compounds, and the cactus *Mammillaria MacDougallii*, which is unique for its latex content.
Milk Protease

Investigator: Goldsmith.

Support: Elbridge and Mary Stuart Foundation.

Goldsmith's research has been aimed at the isolation, characterization and analysis of the protease fraction of milk. This material is a complex mixture of fairly high molecular weight peptides. Little is known of its chemical composition or of its physiological action. It is believed that these studies may contribute to a better understanding of the foaming action of milk and the flavor changes which occur upon storage. Milk protease was isolated and subjected to complete amino acid analysis with the use of the techniques of starch chromatography and microbiological assay. Separation of the material into nine somewhat homogeneous fractions was accomplished through chromatographic techniques. A series of new solvents was developed for this purpose. Analysis of one of the fractions is almost completed, and that of another is in progress.

Anthocyanin Pigments in Corn

Investigator: Zarudnaya.

Support: Gosney Research Fund.

The biosynthesis of anthocyanin pigments---pigments responsible for the color of all blue and red flowers, fruits, and autumn leaves---is being studied in corn by Zarudnaya. The deep anthocyanin pigmentation that appears in certain genetic stocks of corn is known to be controlled by a number of genes. Substitution of a recessive allele at any one of these gene loci causes a reduction or interruption of anthocyanin formation. It is believed that a thorough chemical study of the genotypes in which the synthesis of the pigment has been interrupted will provide clues to the process by means of which the pigment is synthesized. The paper chromatography technique has been found to be particularly useful in this study. It was found that reduction of anthocyanin pigments is accompanied by appearance of a number (in some cases, as many as 15) substances detectable by their fluorescence in ultraviolet light. These substances are in most cases closely structurally related to anthocyanins. A detailed chemical investigation of these substances is in progress.
Synthesis of Carbon$^{14}$-Labeled Compounds

**Investigator:** Lowy.

**Support:** Atomic Energy Commission operating through Office of Naval Research.

In a cooperative program with Borsook and Haagen-Smit, Lowy has synthesized the amino acids leucine, glycine, aminoadipic acid, lysine and histidine in which one carbon atom is replaced by a radioactive carbon$^{14}$ atom. These are used in the protein synthesis work described under the section on Animal Biochemistry.

Lowy has found that quantitative recovery of the basic amino acid histidine from its salts was greatly simplified by an electrodialytic procedure. The new method was used in a synthesis of carboxyl C$^{14}$-L-histidine which has been worked out previously.

Radioactive betaine and glycocyamine were prepared by Lowy for studies of creatine metabolism. Both syntheses start with radioactive glycine and, depending on its label, either the $\alpha$- or $\beta$-carbon of the two compounds can be labeled.

The microscale synthesis of methylene labeled glycine from C$^{14}$ cyanide was investigated. The cyanide is converted to N,N-diphenylcyano formamidime and reduced to the methylene amino group of glycine with lithium aluminum hydride. The yield is not yet satisfactory.

Acetylcholine, an ester with one strong positive charge, is involved in the transmission of impulses by motor and parasympathetic nerves. Two specific enzymes govern its synthesis and decomposition. To study the pharmacodynamic action of analog compounds with two positive charges yet similar chain length, Lowy has synthesized the following compounds: trimethylglycylcholine dibromide, trimethylglycyl-homocholine dibromide, and trimethyl-$\beta$-alanyl choline dibromide.

**Grape and Wine Flavor Components.**

**Investigators:** Haagen-Smit, Strickland, Fox, Hirosawa.

**Support:** California Wine Advisory Board.

In an attempt to understand better the substances that give flavor to wines, Haagen-Smit and associates have continued
studies on the volatile flavor constituents of grapes and wines. The following compounds have been identified in wine made from Zinfandel grapes: Sulfur dioxide, acetaldehyde, n-butyl aldehyde, acetone, methyl ethyl ketone, diacetyl, acetyl methyl carbinol, methyl alcohol, n-propyl alcohol, iso-butyl alcohol, n-butylic alcohol, iso-amyl alcohol, acetic acid, propionic acid, n-butyric acid, n-caproic acid, succinic acid, glyoxylic acid, lactic acid, n-heptylic acid, oenanthic acid, ethyl acetate.

In an attempt to correlate the flavor of wines with the amino acids present in them, eight different varieties of grapes were analyzed for the content of individual amino acids. For these analyses Strickland has used a bioassay procedure in which the bacterium Leuconostoc mesenteroides is used. It was found that most of the amino acid content consists of the free acids. Arginine, alanine, and proline were the most plentiful of the amino acids found, while cysteine, tyrosine and methionine were least plentiful.

The Wound Hormone of Citrus Fruits

Investigator: Viglierchio.

Support: General Funds.

The concept of wound substances in plants were perhaps first formulated by Weisner some 65 years ago. Some 15 years ago English, Bonner and Haagen-Smit isolated and characterized the active agent in bean pod juice. Using Wehnelt's test (cell division and growth of the parenchymatous cells of the bean pod) for activity, they found that traumatic acid in conjunction with still unknown cofactors was responsible for the cell division and elongation. In the study of these factors Viglierchio has made an extensive survey of possible source materials. Isolation of the active materials from extracts of citrus fruit is now in progress.
Embryology

Studies on the Life Span of Spermatozoa

Investigators: Tyler, Rothschild, Lorenz, Tanabe.

Support: Rockefeller Foundation Fund, Guggenheim Fellowship.

The research of the past year has consisted largely in a follow-up of the previously reported discovery that addition of various amino acids and peptides prolongs very greatly the functional life span of spermatozoa of sea urchins. The experiments have been extended to other invertebrates and to vertebrates (F. W. Lorenz and T. Y. Tanabe), and some progress has been made in an analysis of the mode of action of these substances on the spermatozoa (Rothschild). Work has continued, too, on the specific interacting substances derived from eggs and spermatozoa. Along this line some special interest attaches to the results of chemical analysis of purified fertilizin (the substance comprising the surface coat of the egg), since there is indication of a type of structure not hitherto proposed for substances of this category. Other studies in this laboratory (Jaffe) deal with the manner in which the establishment of axes of symmetry and initial differentiation occur in eggs.

During a stay at the Marine Biological Laboratory at Woods Hole, Massachusetts, in the summer of 1950, Tyler was able to investigate the effect of amino acids on spermatozoa of other invertebrates among the annelids, mollusks and ascidians and found that the amino acids were effective in prolonging duration of motility and fertilizing capacity. Metabolic studies on sea urchin sperm were also initiated there and were continued in Pasadena in collaboration with Lord Rothschild, on leave from the University of Cambridge, England. The experiments showed that the added amino acid is not utilized metabolically by the spermatozoa. This was established by measurements of respiration and glycolysis under a variety of conditions and the conclusion was reinforced by the results of tracer experiments, conducted with the help of Professor Borsook, in which carboxyl C^{14}-labeled glycine was used. The utility of the antibiotic penicillin for avoiding bacterial interference in studies of sperm metabolism was also demonstrated. By this means it was shown that the rate
of respiration drops from an initial high rate to practically zero at the time the spermatozoa die. In the presence of amino acid a rapid drop still occurs, but the respiration remains at a low, but definitely measurable, level during the period of extended life. The results of these and related tests show that the initial high rate of respiration is not commensurate with the activity of the sperm since, in the presence of amino acid, activity and fertilizing capacity of the spermatozoa can be maintained near their original level for many hours after the rate of oxygen uptake has dropped to less than five per cent of the initial rate.

It is generally believed that the early death of spermatozoa or of other cells that are suspended in physiological salt solution is due to starvation, that is, to their having used up their limited food reserves as a result of their activity. The results of the present experiments contradict this view of the senescence of cells since the added amino acid does not serve as a food but enables the cell to utilize more fully its endogenous substrate. It has also been thought that bacteria which appear in large numbers in ordinary senescent suspensions of spermatozoa are responsible for the death of these cells. However, it has been shown that bacterial growth could be suppressed by means of penicillin without extending the life span of the sperm and that the presence of amino acid in the absence of an antibiotic delays the growth of the bacteria (ordinarily present in the initial non-sterile sperm suspensions) until the end of the extended life span of the spermatozoa. The situation is similar to that encountered in an earlier study on the life span of the unfertilized egg, wherein it was shown that the bacteria normally present in the medium do not initiate the death of the cell, but rather they begin to grow rapidly after disintegration has set in. The amino acids and other agents that extend the life span of spermatozoa evidently act by opposing the disintegration of certain constituents of the cell. Tests have shown that dissolution of a surface constituent is initially involved.

Another finding of some interest is the phenomenon of induced anaerobic motility. Sea urchin spermatozoa are ordinarily aerobic cells. They quickly become motionless and die in absence of oxygen. However, in the presence of added amino acid they remain active and retain fertilizing capacity for many hours anaerobically. This, too, is accomplished apparently without metabolic utilization of the amino acid.
At present the most probable explanation for the ability of various amino acids to extend the functional life span of the spermatozoa is that they act by virtue of their ability to bind certain heavy metals that are present in trace amounts in the suspending medium and that have a toxic action on these cells. Tests with other metal-chelating agents have provided support for this view.

Experiments on spermatozoa of fowl were performed mainly by Professor F. W. Lorenz while on leave from the Division of Poultry Husbandry of the University of California at Davis. With this material considerable extension of the duration of motile life was obtained by addition of glycine (0.003 to 0.133 molar) to the saline diluents. Certain egg-white proteins (conalbumin and a derived protein, hydroxylamidoovomucoid) had a similar effect.

Professor T. Y. Tanabe, on leave from the Department of Dairy Husbandry of Pennsylvania State College, has been conducting similar experiments on bull sperm in collaboration with Tyler. Material for this work is obtained from the Roger Jessup Farm in Glendale. When incubated at 37° C the bull sperm are found to survive longer generally in amino acid solutions than in the standard yolk-citrate diluent that is customarily employed, and survival is considerably longer than in physiological salt solutions. The optimum concentration of glycine ranges from 0.025 to 0.075 molar. At storage temperatures of 4° to 9° C the results are somewhat variable with the yolk-citrate being generally more effective. This temperature relationship is being investigated further with the use of certain synthetic high molecular weight polymers in order to check on possible influence of the viscosity of the medium.

Studies on the Chemical Composition of Fertilizin

Investigator: Tyler.

Support: Rockefeller Foundation Fund.

The use of high speed centrifugation has simplified further the purification of fertilizin (the egg-surface-coat substance which, when in solution, has specific agglutinating and other effects on the spermatozoa). Fertilizin of sea urchins belongs to the category of high molecular weight, sugar- and amino acid-containing, substances termed glycoproteins or mucopolysaccharides. In the species of sea urchins so far investigated the molecule is found to contain two different sugars and at least 12 different amino
acids. Certain differences in these are found in some species and this is being further investigated in relation to the problem of species specificity in fertilization.

The generally held view of the structure of substances that are designated as glycoproteins or mucopolysaccharides is that they possess distinct protein and polysaccharide moieties bound more or less intimately together. However, extensive attempts to dissociate fertilizin into such parts have been unsuccessful. This, together with recent chromatographic evidence of amino acid-sugar complexes obtained from fertilizin subjected to mild hydrolysis, has led to the suggestion of a type of structure that has not hitherto been proposed for such substances. This view is that protein and polysaccharide constituents do not exist as such in the molecule but that the individual amino acids and sugars are interlinked. Considerably more research will be required to determine whether or not this hypothesis is valid.

**Studies on Problems of Differentiation.**

**Investigator:** Jaffe.

**Support:** General Funds.

Jaffe is continuing his investigations of possible physico-chemical differences arising in correlation with differences in prospective developmental fate before visible differentiation occurs within developing eggs. For this purpose he is now investigating eggs of the marine alga Pelvetia in which the determination of axes and initial differentiation is known to be subject to control by means of unilateral irradiation and other agents. A mathematical analysis has been made of the possible consequences of a linearly varying metabolic rate and experiments have been designed in which, initially, the possible existence of a CO₂-gradient is to be examined by use of C¹⁴. In the course of this work it was found that a change in light intensity acts as a spawning stimulus. Further experiments are planned along this line.
Genetics: Corn

Staff: Professor: Anderson; Research Associate: Longley; Research Fellows: E. Dale, Stehsel, H. Teas; Research Assistants: Bostwick, A. Teas; Graduate Students: Patterson, Robertson; Superintendents: Cochran, J. Dale, Hoover.

Genetic Effects of Atomic Bomb Radiation

Investigators: Anderson, Longley, Teas.

Support: Atomic Energy Commission operating through Office of Naval Research; United States Department of Agriculture.

Beginning with the "Able" bomb test at Bikini in 1946, samples of dry maize seed have been exposed to high-energy radiations in dosages adequate to give clearly measurable effects. In the first test, duplicate samples were exposed to measured dosages of X-rays. By comparison, it was possible to measure the biological effect of an atom bomb exposure in terms of the X-ray dosage required to produce an equivalent effect. The comparisons were made on the early seedling leaves, each of which is made up of the cell progeny of 500 or more cells of a leaf primordium in the dormant seed. As each primordial cell is affected independently by the radiation, the first seedling leaves represent a large cell population, of which some cells are unable to develop normal chlorophyll. The result is a mottling or mosaic of normal green with abnormally pale, yellow, colorless, or even necrotic areas. On this basis, one Bikini sample, Lot A, showed an effect approximately equal to that produced by 15,000 r units X-ray. A further comparison was made by determining the percentage of plants with sectors of pollen semi-sterility in the central spike of the tassel. The Bikini Lot A and the 15,000 r-unit X-ray lot gave almost identical percentages 63.1 and 63.0 respectively.

The pollen semi-sterility is due to gross chromosome alterations (such as translocations, inversions, and deletion), which do not interfere with the growth of the diploid tissues, but lead to meiotic difficulties resulting in pollen and egg nuclei some of which do not have a normal chromosome complement and therefore abort. Each semi-sterile sector is made up of the cell progeny of a primordial cell in which chromosomal alteration has been induced. The frequency of semi-sterile pollen sectors
was determined by a tabulation of lateral tassel branches in the Bikini Lot A culture. A little over \(30\%\) of the lateral branches examined had semi-sterile pollen. This is a direct measure of the extent of gross chromosomal damage in the "germ plasm" or reproductive tissue. Thus \(30\%\) of the pollen mother cells, the potential germ cells, carried gross chromosome abnormalities induced by the radiation.

Some types of chromosome abnormality cannot be transmitted to the next generation. Others, such as translocations and inversions, are transmitted to one-half of the offspring. If transmissible and non-transmissible alterations were equally frequent, about \(4.7\%\) of semi-sterile plants would be expected among the offspring. About \(4\%\) were obtained, indicating that somewhat less than half of the chromosome alterations were transmissible.

Later tests on a smaller scale involving Eniwetok bomb radiation, gamma rays, and X-rays, have given data in fair agreement with the Bikini test.

A large number of translocations and inversions have been isolated and stocks built up for study. Of these, Doctor Longley has made prophase (pachytene) examination of about 700, determining the position of break in each chromosome. These stocks then become available for linkage studies, for chromosome mapping, as aids in locating genes, as means of control of genes for physiological or biochemical studies, and as tools in the genetic analysis of difficult traits of scientific or economic interest.

A comparable number of gene mutations have been induced by radiation from different sources, including the atomic bomb. Mutation frequencies could not be used for comparing dosages in the Bikini test on account of numerous recessive mutant genes in the commercial single cross seed used in this test. In all later work our own tested seed has been used. Data are being collected on the correlation of radiation-induced gene mutation and chromosome alteration.

Mutant genes, useful as chromosome markers or otherwise interesting, are being isolated and kept in stock. Special translocation techniques are being used to locate these genes on the chromosomes. Those which show interesting biochemical, physiological, or morphological effects are made the basis of
special studies by our staff and collaborators. For example, all "glossy" mutants are being tested for allelism with all previously known glossies and their gene locations determined. The glossy trait is due to a deficiency of wax coating on the seedling leaves. Wax measurements and studies on wax development on the different glossies are being made by Kurtz of the plant physiology and biochemistry group.

The Behavior of Su Abnormal Form of Chromosome 10 in Translocations

Investigator: Longley.

Support: United States Department of Agriculture.

The use of certain derivatives from crosses of TB-10a with plants carrying the abnormal form of chromosome 10 (ab 10) has made it possible to determine the amount of transmission of different types of hyperploid gametes when in competition with normal or euploid gametes. Transmission through the pollen is less than 10% for hyperploid gametes with chromosome 10, B¹⁰, ab¹⁰ B¹⁰, 10 Bab¹⁰, or ab¹⁰ Bab¹⁰ present in this chromosome complement. The ratio of euploid to hyperploid gametes increases in the order in which the gametes are listed. Transmission of hyperploid gametes through the egg is highest for 10 B¹⁰ and decreases in the same order as that noted for pollen with transmission of ab¹⁰ Bab¹⁰ gametes reduced to 10%. It is apparent from these data that gametes with the normal chromosome complement have a decided advantage over hyperploid gametes during the developmental and transmission period of both egg and pollen. This advantage is more pronounced when the chromosome Bab¹⁰ is substituted for the B¹⁰ chromosome in hyperploid gametes.

Heterozygous TB-10a plants produce by regular meioses two euploid types of gametes, but a certain amount of mis-divisions gives some hyperploid gametes. The percentage transmission of these has been determined.

A comparison of the transmission through the pollen of euploid gametes shows that ab¹⁰ gametes have a survival advantage over the 10⁵ B¹⁰ gametes. Through the egg, the 10 and 10B¹⁰ gametes are transmitted with equal frequency. The substitution of ab¹⁰ for normal 10 chromosome introduces the likelihood that preferential segregation at megasporogeneses produces more eggs with ab¹⁰ than with 10⁵ B¹⁰ chromosomes and so that data of the last two parent types do little more than suggest that 10⁵ B¹⁰
transmitted more frequently than $10^3 B^{ab10}$ gametes. The $34\%$ of $10^3 B^{10}$ gametes transmitted through the pollen show a 7 to 29 ratio of disjunction to non-disjunction of the $B^{10}$ chromosome. Most of this non-disjunction is thought to occur in the division of the generative nucleus. The ratio of deficient to hyperploid gametes that fertilize the egg is one to three, with this ratio reversed for the fertilization of the polar nuclei to produce the endosperm.

Some derivatives from TB-10a crosses give plants that produce from regular meioses an euploid and a hyperploid gamete, but mis-divisions give a certain amount of a second type of euploid gametes. The percentage transmission of these has been measured. These measurements suggest that in eggs the $10^3 B^{10}$ gametes are transmitted more frequently than the $10^3 B^{ab10}$ gametes. The $65\%$ of $10^3 B^{10}$ gametes transmitted through the pollen are in the ratio of 32 to 53 disjuncted to non-disjuncted $B^{10}$ chromosomes. Again the ratio of deficient to hyperploid nuclei effecting fertilization of the egg is one to three.

The studies of the $ab10$ chromosome when in translocations involving other A-type chromosomes have shown that the amount of preferential segregation at megasporogenesis is approximately 10\% less than the segregation of the $10$ and $ab10$ chromosomes in appropriate control tests. This study is still in the preliminary phase and is being continued during the present season.

Recovery of Mutants Induced by the Bikini Atom Bomb

Investigator: E. Dale.

Support: General Funds.

Maize seeds of the $F_1$ hybrid L289 X 9205 exposed to the atomic explosion at Bikini in 1946 and other lots of the same stock exposed to varying dosages of X-rays have been tested for induced seedling mutations. Unexpectedly, control cultures grown from the untreated seed stock segregated numerous recessive defects. These apparently came from spontaneous mutations in inbred lines L289 and 1205 while growing in isolation blocks on the breeding grounds of the seed company which supplied the seed stock.

Since a mutation induced in a single seed should affect only a fraction of the tassel or ear shoot of the resulting plant whereas a transmitted prior mutation should affect the entire
tassel and ear shoot, separation of the induced and prior mutations was possible only when the frequency of transmission departed significantly from 1:1. This excludes the bulk of the mutations.

On the above basis 60 Bikini-induced and 36 X-ray induced mutations have been demonstrated. Forty-six of the Bikini-induced mutations had their principal effect on chlorophyll. The remaining 14 included dwarfed, deformed, necrotic, leafy coleoptile, tall sheath, and a type called "orobanche" after the root parasite which its appearance suggests.

Thirty-three of the 36 X-ray induced mutations chiefly affected chlorophyll. The remaining three included dwarf and orobanche.

Prior mutations present in the controls included various chlorophyll types plus dwarf, abnormal, necrotic, leafy-coleoptile, glossy and ragged.

Biochemical and Genetic Studies on Blue Fluorescence

Investigator: Teas.

Support: Atomic Energy Commission operating through Office of Naval Research.

Several new radiation-induced genetic characters have been studied in which the mutants differ from normal only in that certain parts of the plants fluoresce in ultraviolet light. The first of these behaves as a recessive in the seedling and as a dominant in the tassel. Crosses involving this character, Blue fluorescent-1, have been found to give off-ratios in crosses in which heterozygous pollen is used on heterozygous or homozygous normal silks. It is expected that linkage studies will show one or more linked factors for gametic selection, since mutant ratios although usually deficient in certain crosses are occasionally found in excess. Paper strip chromatography has shown several blue fluorescent spots, one of which has been identified as anthranilic acid, and another, the major one, which is converted into anthranilic acid by acid or alkaline treatment. Three more blue fluorescent mutants of this first type have been obtained, all of which show similar chromatographic spots, one of which has already been shown by crosses to be allelic with Bf-1.
Three mutants representing another type of blue fluorescence have been found in which chromatograms show different fluorescent spots from the first type and in which blue fluorescence appears to bear no relationship to anthranilic acid. This last group is most fluorescent in the seedling stage.

Biochemical Studies on Niacin Content of Sugary vs Starchy Seeds

Investigator: Teas.

Support: Atomic Energy Commission operating through Office of Naval Research.

The well known higher niacin content of sugary corn (sweet corn) kernels has been studied further. Three new radiation-induced occurrences of sugary mutants allelic to sugary-1 have shown the same higher niacin content. This indicates that the sugary locus, not merely closely linked genes, is responsible for the niacin level. Embryos of normal and sugary kernels are not different in niacin. Tests with B translocations have shown that the embryo genotype plays no part in the determination of niacin level of endosperms. Sugary-normal mosaic kernels were assayed and shown to have significantly higher niacin level in the sugary portions than in the normal portions. It was conclusively shown that the niacin in corn is not found in the bound form in other cereal grains. Most of the "extra" niacin of sugary was found to be present as nicotinamide. The study of differences in form and amount of niacin between normal and sugary corn is being extended to the nicotinamide coenzymes triphosphopyridine nucleotide and diphosphopyridine nucleotide (TPN and DPN).

Chemical Studies on Auxin and Auxin-complex in the Maize Endosperm

Investigator: Stehsel.

Support: Atomic Energy Commission operating through Office of Naval Research.

It has been known for many years that animals fed a diet consisting primarily of corn develop symptoms characteristic of tryptophane and niacin deficiencies. Earlier work in this laboratory has demonstrated that tryptophane may be used at least three ways, viz., in niacin, indole-3-acetic acid (IAA), and protein synthesis. A high percentage of the tryptophane may be converted
to IAA and its derivatives in certain strains of corn, leaving less for production of substances which may be utilized by the animal body. Associated with these materials was found to be a substance which released large quantities of IAA upon alkaline hydrolysis. This "auxin complex" (AC), the function of which is unknown, is apparently capable of completely inactivating IAA for its usual role in the growth of the plant, for the AC itself has no physiological activity in the standard test plants. An attempt is being made to isolate it from one hundred pounds of corn. Dialysis and other procedures have indicated a molecular weight of about 500 units for AC. It has been possible to enrich the AC in terms of its IAA activity from 0.04% to about 10%.

Paper chromatography resolves AC into two substances both of which give a color reaction for IAA and develop growth activity in physiological tests on alkaline hydrolysis. Apparently, therefore, the AC is a mixture of two compounds both of which probably contain IAA. Recent alkaline hydrolytic procedures on the AC concentrate indicate the release of at least three substances--methyl anthranilate, IAA, and several sugars.

Work to determine the sites of accumulation and production of AC has been started, in which mutants are used with colored aleurone layers. Preliminary work indicates that the AC concentration decreases from the aleurone layer inward into the endosperm as well as from the base to the top of the endosperm.

Earlier work has shown the existence of an enzyme system capable of transforming tryptophane to IAA. A dialysis technique designed to reduce the IAA and AC background and separate the enzyme from all low molecular weight compounds has been perfected. Results confirm previous work in this laboratory that such an enzyme system exists in the corn kernel. It is of low activity. The presence of this enzyme system raises several questions, e.g., does AC serve as a reservoir for storage of IAA produced by the enzyme in an inactive form?

Genetic and Physiological Studies on Viviparous Mutants of Maize

Investigator: Robertson.

Support: General Funds.

Six mutations in maize which interfere with the dormancy mechanism of the seed have been studied. Seeds which are homozygous
for any of these mutant genes do not become dormant but germinate prematurely. Such mutants are called viviparous.

Indications are that the mutants viviparous\textsubscript{1} and viviparous\textsubscript{4} obtained from the Maize Genetics Cooperation, are allelic and are not located in the positions reported in the literature. Viviparous\textsubscript{2} shows 29.5\% crossing over with pr in chromosome five. Viviparous\textsubscript{9} is in the short arm of chromosome one. Viviparous\textsubscript{7}, pr and glossy\textsubscript{8} are linked in the order given. Viviparous\textsubscript{8} is located in the long arm of chromosome one. The following order and map distances were found: (br) T 32.4 vp\textsubscript{8} 13.3 bm\textsubscript{2} with no crossovers between br and the translocation in 175 plants. F\textsubscript{2} data indicate that viviparous\textsubscript{9} is in chromosome seven.

Physiological studies of normal and viviparous embryos suggest that a combination of low oxygen and high CO\textsubscript{2} tension is responsible for preventing germination of normal embryos during the development of the seed. Concentrations of approximately 1\% oxygen, 30\% CO\textsubscript{2} and 69\% N\textsubscript{2} will greatly retard the elongation of the plumule of excised normal embryos of immature seeds without inhibiting the enlargement of the scutellum, which will elongate from 0.5 mm. to 1.5 mm. in six days under these conditions. The plumules of viviparous\textsubscript{5} embryos, obtained from the same selfed ear as the normals mentioned above, are not inhibited by these gas concentrations. This would suggest that embryos of this mutant germinate prematurely because they are capable of plumule elongation under the conditions of low O\textsubscript{2} and high CO\textsubscript{2} in developing seeds.

**Studies on Crossing Over in Heterozygous and Homozygous Translocations.**

**Investigator:** Patterson.

**Support:** General Funds.

Studies are being continued in which an extensive collection of chromosome rearrangements (reciprocal translocations and inversions) is being used both as a means of correlating the cytological and genetic maps in maize and as a tool to yield information on the mechanism of crossing over.

The positions within chromosomes at which an interchange has occurred may be determined fairly accurately by cytological observation of the configurations assumed by the meiotic prophase
chromosomes in the microsporocytes of plants heterozygous for the interchange. A plant heterozygous for such a translocation typically produces normal-appearing and empty (aborted) pollen grains in approximately equal numbers. Thus in linkage tests a translocation may be followed by means of pollen semi-sterility, which behaves in outcrosses like a dominant gene.

By making use of the fact that such rearrangements may be followed both cytologically and genetically, information is being accumulated on the location of various genes in the visible chromosome threads. A detailed genetic placement is in progress in the case of rearrangements which were determined cytologically by Longley to have breaks at various positions in either the short arm of chromosome 2 or the short arm of chromosome 9. Data have been gathered on the genetic location of about 20 chromosome 2 rearrangements with reference to the genes \( l_{g1}, g_{l2}, B, sk, \) and \( v_4 \). About the same number of rearrangements are being placed with reference to the genes \( c, sh, \) and \( wx \) in chromosome 9. Placement in these two arms is especially important because of their content of useful genes.

In using heterozygous translocations to mark or control desired portions of chromosomes it is important to know something of the linkage relations which exist between genes carried in the two members of the translocation complex. For a study of such linkage relations, a series of translocations is being used which involves chromosomes carrying readily-classifiable combinations of endosperm or seedling genes. About a hundred reciprocal translocations representing 18 chromosome pairs are being followed in this manner.

In cases where it is possible to determine the cytological position of breakage in one chromosome with sufficient accuracy, it is sometimes possible, as a result of the linkage relations which result when the translocation is homozygous, to infer something of the position of breakage in the other chromosome with reference to its centromere and marker genes.

Rearrangements are also being used to give information on the mechanism of crossing over. The specific question being studied is whether the amount of crossing over within a certain region of a chromosome is an inherent property of that region or whether it is dependent upon the position of the region with respect to the rest of the chromosome. Such studies are being carried out with 12 rearrangements involving a shift of the \( c-wx \) region of
chromosome 9 and seven in which the \( g_1-g_2 \) region of chromosome 2 is shifted.

Genetics: Drosophila

Staff:  
Professor: Sturtevant; Visiting Professor: Hadorn;  
Research Associate: Morgan; Associate Professor: Lewis; Senior Research Fellow: Novitski; Gosney  
Research Fellow: Spencer; Research Fellow: Stalker;  
Graduate Students: Lindsley, GoodSmith, Hinton;  
Research Assistants: Bacon, Bleecker, Fahy, Griffith, Huszarik, Meselson.

Fourth Chromosome Map in Drosophila melanogaster

Investigator: Sturtevant.  
Support: General Funds.

The location of the genes in Drosophila melanogaster has long been carried out through the study of crossing over, or exchanges between homologous chromosomes. Much of modern genetical theory stems from this kind of work on this species. In this organism there are four pairs of chromosomes, and it is a curious fact that in the smallest one of these pairs, known as the fourth chromosome, crossing over is so rare that is has been impossible to use this method of mapping the positions of the genes. Other methods that are more cumbersome and more easily misleading are available and have been used; it now appears, however, that the map deduced by these methods and currently accepted is quite incorrect.

It has now been found by Sturtevant that (for reasons that are unknown) the fourth chromosomes undergo crossing over in about three per cent of the eggs of females that carry three sets of chromosomes instead of the usual two sets. It is, therefore, possible to apply the usual method of locating the genes. A map showing the sequence of the five most-used genes in the fourth chromosome is now available (in press).

This result, besides completing the mapping of the chromosomes of the species, furnishes a sound basis for the study of other problems - notably the peculiar non-random segregation found in individuals with three fourth chromosomes.
It is also of importance in the study of the "position effect" of genes, since some of the conclusions in this field have depended on the formerly accepted map, and are now seen to be without foundation.

**Diploid Intersexuality in Drosophila**

**Investigator:** L. V. Morgan.

**Support:** General Funds.

The genic balance concept of sex determination was developed many years ago by Bridges through studies of so-called triploid intersexes in *Drosophila melanogaster*. In other species diploid inter sexes, differentiated from normal flies by a single gene, are known. For some time Mrs. Morgan has been studying such a diploid mutant type of intersex in *D. melanogaster*. This is being compared with diploid intersexes in other species and with the triploid intersexes of melanogaster.

**Studies on Development and Differentiation in Drosophila by Means of Paper Chromatography**

**Investigators:** Hadorn, Mitchell.

**Support:** Rockefeller Foundation Fellowship, Rockefeller Foundation Fund.

During the three months he was at the Institute Hadorn began this work with Mitchell in the hope that by using the method of paper chromatography new information might be gained with respect to the biochemical properties of the fruit fly. A new and simple method of preparing the material to be chromatographed was developed. Whole eggs, larval stages, pupae and flies were mashed directly on the filter paper. By using this direct technique it was possible to obtain clear chromatograms of single organs dissected from larvae or from flies. Even such small body parts as single Malpighian tubes and single gonads from larvae produced chromatographic spots of reliable distinctness. Two main groups of substances have been studied: first, the amino acids and the peptides and, secondly, those substances which show characteristic fluorescence in ultraviolet light. In the second category quantitative data were obtained by cutting out fluorescent spots from the paper sheet and extracting them with an ammonium solution. The amount of fluorescent material of the solvent was then measured in a photo-fluorometer. The material from the organs of single larvae or flies produced enough fluorescence to be reliably measured.
By using this method it was found that striking changes take place in the amino acid and peptide content during development. For instance, a peptide "larvine" becomes accumulated during the growth of the larval stages. It suddenly disappears with the beginning of metamorphosis and a new peptide, "pupine," of a different composition becomes apparent. The most interesting properties with respect to the free amino acids and peptides were noted in the case of a lethal genotype, the development of which is interrupted at the beginning of the pupal period. These lethal individuals accumulate many times as much amino acid in their blood as the normal larvae.

In regard to the fluorescent material it was found that the different eye-color mutants each possess a specific pattern of substances. A total of eight different compounds have been studied. The fluorometric values show that certain mutants either do not form some of these substances, or form them in quantity far below the normal level. On the other hand the same mutant can exaggerate the production of another fluorescent substance.

By working with single organs it was possible to find out where specific compounds are located within the body. The gonads, for instance, were found to contain different fluorescent substances in the two sexes. The few intersexual flies that have been tested showed an intermediate pattern of fluorescent material.

The approach used by Hadorn and Mitchell promises to have wide application. Combined with other methods of investigation this technique should become valuable in the study of many problems involving developmental physiology and physiological and biochemical genetics.

**Pseudoallelism and Position Effect**

**Investigator:** Lewis.

**Support:** Atomic Energy Commission operating through Office of Naval Research.

In general the organization of the genes in the chromosome seems to be at random with respect to the function of the genes. The shifting of genes from one portion of a chromosome to another portion of the same chromosome or to a wholly different chromosome usually does not noticeably change the effects of those genes. There are however important exceptions to these rules and it is by a study of such exceptions that we hope to determine more about the nature
of the gene itself. It is becoming more and more evident that the chromosomes often contain small clusters of genes which are so close together and so closely related in their functioning that they mimic the behavior of a single gene. An attempt is being made by Lewis to learn as much as possible about one such cluster of genes in the third chromosome of _Drosophila melanogaster_. This cluster, which is technically called the bithorax pseudoallelic series, has been found to contain at least three separate genes, which we may represent here simply as a, b, and c. A new gene effect belonging to this series (found by Bacon) is at present under study and recent results show that the responsible factor may be a fourth gene (d) located between a and c, with its order with respect to the middle gene, b, yet to be determined.

Studies of the salivary gland chromosomes in combination with X-ray studies, which have provided the necessary chromosomal rearrangements, show the bithorax cluster is contained in a region of four heavily staining discs or bands, the first two of which are associated with the a and b genes of the series. A series of X-ray induced bithorax-like changes had previously been obtained in another species, _Drosophila funebris_, and recent work has been concerned with determining whether in this species also there is a cluster of such heavily staining bands associated with the corresponding genes. Ten such changes have been analyzed and the results indicate that a very similar pattern of banding may be present in the two cases, whereas the regions surrounding the bithorax cluster are quite different in their banding pattern in the two species. These results provide only a suggestion that the genes are somehow very closely associated with the banded structure of the chromosomes and that there is possibly a direct one gene–one band relation.

The question arises as to whether there may be other genes which must lie close to the known genes of the bithorax cluster before that cluster can carry out its normal activities in development. An indirect answer in the negative may now be made as the result of recent studies of X-ray induced rearrangements having breaks very close to the left and very close to the right of the bithorax cluster. These studies show that the bithorax cluster will function normally, as far as can be detected, when removed from the chromosomal region which immediately adjoins it on the left side, or when removed from the chromosomal region beginning two salivary gland chromosome bands beyond the bithorax cluster. Thus, at most, the complex seems to be restricted to a very narrow region of the chromosome, probably containing four visible bands, and, at most, six such bands.
Recently, a dominant partial suppressor of one of the bithorax mutants has been obtained as the result of X-ray treatment. This change (discovered by Dr. Bleecker) appears to be closely linked to the bithorax cluster of genes and is expected to be an important tool in understanding the way in which these genes affect development. Tests show that this change is not the same gene as the one which lies several units to the left of the bithorax cluster and which behaves as a recessive and a complete suppressor of certain of the mutant forms of the bithorax genes.

Studies on the Mechanism of Chromosome Segregation

Investigator: Novitski.

Support: Atomic Energy Commission operating through Office of Naval Research.

It has been possible, by the irradiation of two specially selected chromosomes, to manufacture a new kind of chromosome which is made up of two normal chromosomes hooked end to end, with the centromere at one of the ends, and which crosses over with itself with normal frequency to produce chromosomes of half the size of the original. Results from crosses involving this chromosome show clear evidence of the phenomenon of non-random segregation, where the new shorter chromosome competes with the longer for inclusion in the functional egg nucleus and is recovered in the progeny more frequently than the longer. Obtaining this chromosome is the first step of a multi-step process of synthesizing a double ring chromosome, which will consist of two chromosomes joined at both ends to form a ring. It is anticipated that the analysis of the products generated by crossing over of the double ring chromosome with itself will provide information on certain ambiguities in the interpretation of the results of crossing over in simple ring chromosomes, which have been discussed in a previous report.

Germ cells containing both a normal chromosome and a ring chromosome produce a type of chromosome with two centromeres, or a dicentric. When the centromeres proceed in opposite directions during cell division, the dicentric is stalled by the resulting tug of war and a genetically detectable egg lacking that chromosome is produced. It has been found, however, that from individuals of this constitution only half as many such eggs are found as expected. Simpler types of experiments, not involving ring chromosomes, confirm this result and indicate that this happens only when one of the chromosomes forming the dicentric carries
a segment of extraneous chromosome material at or near the centromere region. Furthermore, when both chromosomes involved in the formation of the dicentric have this characteristic, the frequency of recovered cells lacking the chromosome drops to a small fraction of the expectation. The cause of this effect is not known, but may be related in some way to the forces involved in the separation of chromosomes at anaphase of cell division. On this basis, the first case would be explained by the greater strength of one of the centromere regions over the other, so that the dicentric is pulled into one of the daughter nuclei, and a second, by the tearing of the dicentric into fragments when two of the stronger types of centromere regions pull at each end of the dicentric. It has been established that there are three different kinds of centromere regions, designated as strong, weak and neutral. They are distinguishable by the degree of force they apparently apply when pulling on the chromosome.

Two new kinds of compound chromosomes have been synthesized. The analysis of one, the double X, is of interest: it must bend back on itself in a simple arc to cross over with itself. Genetic results provide a measure of this turning radius of the chromosome and it appears to be somewhat greater than results from other types of experiments would indicate.

**Taxonomic Relations of Species of Drosophila related to D. cardini.**

**Investigator:** Stalker.

**Support:** General Funds.

During a four-month period spent at the Institute Stalker has studied the taxonomic relationships of a group of species related to *Drosophila cardini* Sturt., which are found in the tropics and subtropics. With the aid of stocks from various localities and by the use of Sturtevant's collection of pinned material, keys and illustrations for separating the six members of the species group were prepared, and descriptions of two new species drawn up. Studies of hybridization between members of the group were continued.

**Parthenogenesis in Drosophila**

**Investigator:** Stalker.

**Support:** General Funds.

A second project worked on by Stalker was a study of diploid parthenogenesis in *Drosophila parthenogenetica* n. sp.
A study of the frequency of development of unfertilized eggs showed that in eggs from females kept at 25°C about one in 950 eggs develops into a diploid female. By keeping females at 29°C and removing their eggs to 25°C soon after, this frequency of parthenogenetic development could be increased to one egg per 375. X-ray treatment of this species resulted in production of two mutant genes to be used in the study of chromosomal behavior during parthenogenesis. This study, which is just getting under way, has so far shown that segregation of chromosomes takes place during development of unfertilized eggs, since heterozygous origin females can produce homozygous daughters. Two other members of the species group, D. polymorpha and D. acutilabella n. sp., were also found to develop from unfertilized eggs, although in these two species the frequency of parthenogenesis was found to be much lower than in D. parthenogenetica and so far none of the larvae produced have survived to the pupal state. The work on parthenogenesis seems of particular interest for two reasons. First, prior to its discovery in the above mentioned species it was unknown in Drosophila, the organism in which most genetic work has been done, and had not been studied genetically in any fly. Secondly, the occurrence of parthenogenesis in this species group offers a tool for the study of factors which may increase its frequency, and so result in the development of a technique for producing it in one of the other species of Drosophila which is better understood genetically and in which it may be studied more critically.

**Multiple Alleles at the Brown Locus**

**Investigator:** GoodSmith.

**Support:** General Funds.

GoodSmith has been working on the brown locus to see if any crossing over could be obtained between the various bw alleles. Crosses have been set up to test crossing over between these alleles, but to date no crossing over has been observed. However, only about 20,000 offspring have been examined, so this result cannot be considered conclusive. The different bw alleles have also been tested with two stocks of Drosophila simulans, one of which carries the gene "plum," an eye color not allelic to bw in D. melanogaster, and another termed "brown," but which like plum is not allelic to bw in D. melanogaster.
to non-adaptive and that of ethyl alcohol in the opposite direction - from non-adaptive to adaptive. This quantity of dinitrophenol almost entirely prevents assimilation so that the exogenous substrates are almost completely oxidized.

A third group of substrates, including glyceraldehyde and dihydroxyacetone, resemble the endogenous reserves in that they are respired at rates proportional to their concentrations. The rates of respiration of other substrates mentioned are independent of concentration during the major portion of the rapid phase.

Although Neurospora cannot grow in the absence of oxygen, the conidia are capable of rapid fermentation under anaerobic conditions.

**Cysteine and Tyrosinase**

**Investigators:** Horowitz, M. Fling.

**Support:** General Funds, Atomic Energy Commission operating through Office of Naval Research, Nutrition Foundation, Rockefeller Foundation Fund.

The study of what appears to be an unusual case of pleiotropic gene action begun by Doctor S. C. Shen, a former graduate student, has been continued. The initial aim was to repeat and confirm the findings of Shen. This has, in the main, been accomplished, and some new information obtained.

The mutant requires cysteine (or cystine) and tyrosine for growth at 25° C, but only cysteine when cultured at 35° C. At the same time, it can be shown that crude extracts of the mutant contain tyrosinase when the culture has been grown at the lower temperature, but not at the higher temperature. Crude extracts of the wild type show little or no tyrosinase activity at either temperature. This suggests that the tyrosine requirement of the mutant results from the activity of the tyrosinase it contains.

Further investigation has shown that the difference between the mutant and normal strains, with respect to tyrosinase activity at 25° C, is caused by a dialysable inhibitor of tyrosinase present in the wild type but absent from the mutant. At 35° C, neither the mutant nor wild type appears to synthesize the enzyme.
It has been found that a very active tyrosinase appears in crude, undialysed extracts of wild type Neurospora when grown under conditions in which growth is limited by the available sulfur. Under these conditions, the inhibitor of tyrosinase which is found in wild type grown on regular minimal medium is apparently not formed, suggesting that it is a sulfur compound. This effect is abolished if the culture is grown at 35° C instead of at 25° C. The temperature effect on tyrosinase production in Neurospora bears a striking resemblance to the effect of temperature on the development of melanin pigmentation in the Himalayan rabbit, also apparently an effect on the tyrosinase system. It was therefore of interest to find, in a nutritionally wild-type strain isolated from a cross by M. Fling, that tyrosinase production on low-S medium proceeds both at 35° C and at 25° C. This strain corresponds to the normal rabbit in producing active tyrosinase even at high temperatures. A search is under way for a mutant in which tyrosinase production occurs at neither temperature, corresponding to the albino coat color in rabbits. It is expected that further studies on these strains will throw some light on the much-investigated problem of color inheritance in animals.

The Enzymatic Synthesis of Tryptophane

Investigator: H. Mitchell.


This problem is concerned with the fundamental question of the mechanism by which the units of inheritance control the biochemical capabilities of living cells. It is presumed that they do so by functioning in the formation of the cellular catalysts, the enzymes. Prior to the present work it was found that an enzyme preparation from Neurospora would catalyze the formation of the amino acid tryptophane from indole and the amino acid serine. Subsequently a mutant of Neurospora (Strain C83) was selected which did not appear to produce appreciable amounts of this enzyme. The mutant will grow on tryptophane but not on indole. Work by Gordon in this laboratory indicated that catalytic activity could be obtained from the mutant but that this enzyme had properties which were different from its normal counterpart from wild type Neurospora. A thorough investigation failed to confirm the above findings and none of the enzyme could be detected within the limits of the experimental methods that were used. It is not possible, however, to make the unqualified statement that the
mutation resulted in the loss of an enzyme. It can only be stated that the mutation resulted in an extreme reduction in the enzyme activity obtainable from the mutant C-83. Investigations of numerous wild type strains and mutants of Neurospora other than C-83 produce the enzyme activity in characteristic amounts varying over a three-fold range.

An attempt has been made by Owen to investigate the question of the possible loss of an enzyme in mutant C-83 by immunological means. Unfortunately the enzyme, in crude preparations, is not sufficiently antigenic to make this approach profitable. It has been found possible to stabilize the enzyme in question by heating crude preparations. This is presumably due to destruction of other enzymes, but in any case it makes possible the purification of the tryptophane enzyme for further studies such as those concerned with reaction mechanisms and immunology. This problem has now been turned over to Hogness.

Peroxides as Mutagenic Agents

**Investigators:** Dickey, Lotz.

**Support:** Merck and Company Fund.

During the summer of 1950, Dickey and Lotz continued investigations of the action of several organic peroxides in inducing gene mutation in Neurospora. Comparisons of the action of five compounds on three different genes were completed. It was found that different genes differ both in their spontaneous mutation rates and in relative sensitivities to different agents.

Temperature Mutants of *E. coli*.

**Investigators:** Leupold, Horowitz.

**Support:** American Cancer Society, Rockefeller Foundation Fund.

By irradiating a total of $1.7 \times 10^9$ cells of the bacterium *Escherichia coli* with UV, plating the irradiated cells on a solid "minimal" medium, incubating the plates for two days at $40^\circ C$ and for five days at $25^\circ C$, and isolating bacterial colonies which appear at the latter temperature only, it has been possible to isolate 161 so-called temperature mutants. These are biochemical mutants which have lost the ability of the normal or wild type to grow on minimal medium at $40^\circ C$, but which still are able to do
so at 25° C. Whereas the wild type cells form visible colonies during the first two days, the few temperature mutants which have been produced by the UV irradiation will not start growing until the temperature is changed to 25° C. They are therefore easily recognized by their delayed appearance as visible colonies.

These temperature mutants are of considerable theoretical interest because they allow the investigation of problems which cannot be solved by studies on general biochemical mutants (i.e., mutants which have lost the ability to synthesize certain essential growth factors over the whole temperature range, as opposed to temperature mutants in which this inability to synthesize one or another growth factor is limited to certain temperatures only). One of the most interesting problems in this connection is the validity of the one gene-one enzyme hypothesis which claims that each gene has but one function in the organism. This hypothesis is based on numerous results in the field of biochemical genetics of the mold Neurospora. It has been suggested, however, that the usual way of isolation of biochemical mutants on an enriched medium (the so-called complete medium) might exclude the recovery of mutations of multifunctional genes even if they existed.

This depends on how many biochemical mutants are lost during the isolation process because of their inability to grow on complete medium. It is clear that any mutant with a requirement that cannot be satisfied by complete medium, either because of the unavailability of the growth factor which is required or because of the presence of specific inhibitors in the medium, will not be recovered. The intensity of this selection would be unknowable if there did not exist certain classes of biochemical mutants which under certain conditions can be isolated on a minimal medium. Temperature mutants unable to grow on complete medium at their "mutant temperature" can be recovered by growing them on minimal medium at other temperatures. It thus becomes possible to determine their relative frequency, which may serve as a measure of the selective effect of the complete medium.

In the material studied, only 23% of the temperature mutants have requirements at 40° C which are not satisfied by the complete medium. From this, it is concluded that the intensity of the selection which opposes the quantitative recovery of biochemical mutants on complete medium is of a rather low order, and it is calculated that selection cannot be expected to prevent the recovery of mutations of multifunctional genes - if such genes exist - to any considerable degree. The fact that the majority
of biochemical mutants in Neurospora (all that have been investigated so far) are found to have lost but one essential gene function can thus not be considered the artificial product of an intense selection that excludes the recovery of genes with more than one function.

Neurospora Mutants Requiring Unknown Growth Factors

Investigators: Mauron, Mitchell.

Support: Merck and Company Fund.

The study of so-called unknown mutants of Neurospora has been continued. A mutant is arbitrarily classified unknown when it does not grow on amino acids, water-soluble vitamins, adenine, cytidine and succinic acid, but does grow on complete medium (all the substances mentioned plus an autolysate of Neurospora mycelium) or on other complex media like liver extract, yeast extract or peptone. It is evident that most of these mutants do not really require an unknown growth factor, but rather a known substance or a mixture of known substances that have not been tested yet. Other mutants may actually require substances that have been tested for but are inhibited in growth by another factor present in the medium. Preliminary tests with unknown mutants from the stock and with freshly isolated mutants led to the selection of seven mutants for further investigation. Two of these seem to require a peptide for growth. This is fairly well established for mutant 44601. Genetic analysis of this mutant by M. Mitchell indicates that a single gene is involved with a centromere distance of 0. No single substance will promote growth of the mutant, but growth is obtained with arginine plus traces of adenine. Additional amino acids (equimolar quantities) may cause further increase in growth, may inhibit, or have no effect at all. The growth-promoting amino acids in decreasing effectiveness are: threonine, phenylalanine, aspartic acid, tyrosine, valine and isoleucine. The inhibitory acids are: serine, tryptophane, lysine, meth onine, hydroxypoline and glycine. Growth with threonine in the presence of arginine and adenine reaches 50 mg. This is comparable to the growth obtained with adenine plus acid hydrolyzed casein (40 mg.).

All this is but one side of the picture. By repeated paper chromatography it is possible to isolate a substance from Bactopeptone which in combination with amino acids supports growth (70 mg.) of mutant 44601 in the absence of adenine. This substance has an Rf value of 0.55 - 0.65 with propanol-NH₃
as a solvent, gives a very faint ninhydrin test and a negative bismuth diiodide test for adenine. Its activity is destroyed by hydrolysis with 5 N HCl for 24 hours. Chromatographic analysis after hydrolysis shows that five or six amino acids have been liberated. This suggests that we are dealing with a peptide-like substance. Preparation of crystalline derivatives has failed so far, the amount of substance available by paper chromatography being very small. Recently another way of isolating an active substance from Bactopeptone was found by forming a silver salt. It should now be possible to get enough material for a chemical investigation.

The occurrence of peptide mutants in Neurospora, if confirmed, would indicate that not only the synthesis of the amino acids but also that of the peptides and probably proteins is controlled by genes. Peptide mutants should increase our knowledge of the biosynthesis of peptides and their interrelationship with amino acids. Furthermore they might represent a first step in the understanding of the biosynthesis of proteins.

### Histidine Requiring Mutants of Neurospora

**Investigators:** Haas, M. Mitchell, Ames, H. Mitchell.

**Support:** Eli Lilly and Company, Rockefeller Foundation Fund, Atomic Energy Commission operating through Office of Naval Research.

Two new histidineless mutants were isolated and these plus five previously obtained by M. and H. Mitchell (two genetic types) and one by Harrold were subjected to an extensive genetic analysis. Four genetic types were demonstrated. Mutants C-94 and C140 are in linkage group A, C84 in linkage group E, and C141 in linkage group D. Genetic evidence shows that T1710, which has not been observed to cross over with C140, carries a chromosomal aberration that gives rise to non-identical spore pairs.

Three of the genetic types of histidineless mutants accumulate three different imidazole-like substances, but these are not biologically active as intermediates. Investigations of substances accumulated by double mutants show that the five strains carry mutant genes that are related to a sequence of biochemical reactions as follows:
The substances 1, 2 and 3 are chromatographically different. No. 3 may be lustidinol.

All the mutants and wild type have been shown to contain the enzymes histidinase and urocanase. Urocanic acid is not a biologically active intermediate in histidine biosynthesis, and it seems likely that the above enzymes are involved in the degradation of histidine but not in its biosynthesis.

All the histidineless mutants are inhibited by a combination of a basic amino acid and another one of several amino acids. Some evidence was obtained to show that this phenomenon is at least in part due to the action of modifying genes.

This work is in manuscript form and will be submitted to Genetics.

**Pyrimidine Biosynthesis**

**Investigators:** Drell, H. Mitchell.

**Support:** Williams-Waterman Fund of the Research Corporation.

Drell has investigated further the mechanism and specificity of a pyrimidine test useful in detecting this type of compound on paper strips after chromatographic separation. Uracil and cytosine appear to undergo somewhat different reactions and can readily be distinguished from one another. New growth requirements have been found for two adenineless mutants. 2,6-Diaminopurine, which was found in other laboratories through tracer studies to be a possible precursor of liver nucleic acid in animals, possesses growth-promoting ability equivalent to or greater than adenine at very low concentrations in the presence of the latter purine. However, higher levels of the compound produce inhibition, apparently by blocking the conversion of adenine to adenosine. Another compound, 2-aminopurine, stimulates the growth of Neurospora in the presence of adenine without any subsequent inhibition.
No adequate explanation for this behavior can be advanced at present.

**Biosynthetic Relationship Between Methionine and Threonine**

**Investigators:** M. Fling, Horowitz.

**Support:** Nutrition Foundation, Rockefeller Foundation Fund, Atomic Energy Commission administered through Office of Naval Research.

The question of the identity of the biologically active substances accumulated by a methionineless mutant blocked at step A in the diagram below has been finally answered.

\[ \text{Threonine} \rightarrow \text{Homoserine} \]

\[ + \rightarrow \text{Cystathionine} \rightarrow \text{Homocysteine} \rightarrow \text{Methionine} \]

\[ \rightarrow \text{Cysteine} \]

The substances are L-threonine and L-homoserine. L-threonine was identified by isolation, and L-homoserine, which is present in much smaller amounts, on the basis of chromatography and biological activity. These results give strong support to the scheme of reactions shown in the diagram. The results are of further interest in that they show for the first time the presence of homoserine in cells.

This work has now been reported in the *Journal of Biological Chemistry* 190: 272-285, 1951.

**Biosynthesis of Orotidine**

**Investigators:** Michelson, Drell and H. Mitchell.

**Support:** Research Corporation, Atomic Energy Commission Operating through Office of Naval Research, Merck and Company Fund.

Work begun by Michelson (Commonwealth Fund Fellow) on the isolation of an unknown nucleoside from Neurospora was completed. The substance was obtained in a pure form and it has been shown to be a riboside of orotic acid. A good deal of work on the details of the chemical configuration remains to be
done. The assistance of Merck and Company has been enlisted for production of the material in large quantities. There is a possibility that the substance is an important intermediate in the biological synthesis of nucleic acids.

**Threonine Biosynthesis in Neurospora**

**Investigator:** Garner.

**Support:** American Cancer Society Fellowship.

The present information regarding threonineless strains of Neurospora suggests a biosynthetic pathway with homoserine as a precursor of threonine. By postulating alternate paths of synthesis of threonine via α-aminobutyric acid or isoleucine, a scheme of reactions can be set up to include mutants 51504, 44104 and 35423. However, strain 44105 will not fit into this scheme. An attempt to throw more light on this matter was made by the use of double mutants.

Cystathionineless mutants are known to accumulate threonine by conversion from homoserine, presumably because the homoserine which cannot be converted to methionine in these mutants is shunted off in that direction. A double mutant, cystathionineless-threonine-less, should be unable to accumulate threonine. This expected situation was found true for double mutants containing either 35423 or 51504. However, doubles containing 44104 or 44105 still accumulate material which stimulates growth of the threonineless strains. This would indicate that the genetic blocks in these strains do not directly concern the pathway of threonine synthesis.

With this in mind, further attempts have been made to define these two mutants. Isoleucine, α-ketobutyric acid and α-aminobutyric acid have been found to spare but not replace threonine for strain 51504. Isoleucine, α-ketobutyric acid, methionine and tyrosine partially spare the threonine requirement of 44104. These facts would indicate that there are alternate pathways of threonine synthesis, or that threonine is a precursor of other necessary metabolites. Strain 44105 has been found to give maximal growth when 20 ml. of Fries medium is supplemented with 10 μg of α-aminobutyric acid or with larger amounts of isoleucine, α-ketobutyric acid, homoserine or threonine. An enzyme preparation from 44105 was found to be normal (like wild type) with respect to the ability to form α-aminobutyric acid from threonine and/or α-ketobutyric acid. Experiments are in progress in an attempt to clear up this situation.
Formate- or Serine- Requiring Mutants of Neurospora

Investigators: Harrold, M. Fling.
Support: Rockefeller Foundation Fund, Nutrition Foundation.

Two mutant strains, T2269A and T3207A, having alternative requirements for L-serine or formate have been studied. It is presumed that formate provides a one-carbon fragment for the betacarbon of serine in these strains, indicating for the first time that the following scheme, based on the results of animal isotope studies, also holds true for Neurospora.

\[
\text{Formate} + \text{Glycine} \rightarrow \text{Serine}
\]

Formate is also active for the two strains, although as its concentration is increased it soon becomes inhibitory. Formate was found to have the same activity, on a molar basis, as formaldehyde at pHs 4.6 and 5.0. At higher pHs it was found that the responses to formate became drastically reduced while the responses to formaldehyde changed but little over the same range. Under optimal conditions for formate response there is no indication that formaldehyde is more effective as a growth factor than formate for the two strains. These relations also hold for the formateless mutant C24 which is genetically different from T2269 or T3207.

These findings may offer some explanation of the conflicting reports from animal isotope studies where certain workers using rat liver homogenate systems at pH 7.5 found formaldehyde to be a far more active source of the \(\beta\)-carbon of serine than formate.

The activity of other compounds implicated in the metabolism of one-carbon fragments has been studied. C24, T2269 and T3207 all give limited growth on adenine alone. C24 gives nearly normal growth on a mixture of adenine and methionine and maximum growth on formate alone. This is the first indication of the source of the methyl group of methionine in Neurospora and is in line with animal studies which indicate that two carbon atoms.
of the purine skeleton arise from formate. Methionine, however, has little or no effect on T2269 and T3207. Glycine and choline have stimulatory activity at sub-optimal concentrations of formate for all three mutants, but no activity alone. Histidine has this type of activity for C24 but not for the serine mutants.

Genetic studies have shown that the requirements in all three mutants are due to single gene mutations. It is not yet known whether the mutant genes in the two serineless strains are allelic. In all asci so far examined from crosses of T2269A to wild type, two mutant genes are segregating – one serineless, the other methionineless. T2269, however, does not require methionine in addition to serine or formate. T2269 is probably a natural heterocaryon which arose during subculture of the original serineless strain when some of the serineless nuclei also acquired a mutation causing a methionine requirement. This requirement is masked in T2269 by the presence of wild type alleles of the methionineless gene. However, no asci yet examined have only the serineless locus segregating, so that the double mutant nuclei must be in large excess if T2269 is a heterocaryon. There is a further interesting possibility. Since one-carbon fragments are involved in both serine and methionine metabolism, T2269 could conceivably be a double mutant in which formate or serine could also satisfy a block in methionine synthesis. Although neither the methionineless strains nor the double mutant strains (serineless-methionineless) derived from T2269 will grow on formate, this is also true for the reisolated serineless strains and therefore does not exclude the possibility of a common metabolic link between the formate-serine and methionine pathways in T2269.

Regardless of what conditions pertained during the crossing and isolation procedures all the serineless reisolates so far tested from crosses involving T2269A or T3207A show a different phenotype from their parents. Their responses to serine are lowered and they produce only traces of growth on formate. Double mutants from T3207AxC24a (both formateless strains) require serine + formate for growth and will not use serine or formate alone. The formate response conditioned by the C24 locus goes through crosses to wild type, T2269, or T3207 unimpaired. No explanation of this phenomenon of altered phenotype is known at present. There is no second gene governing formate requirement segregating in the asci analyzed. There is no evidence that maternal cytoplasmic factors are involved, nor does the presence or absence of formate during the crossing or isolation procedures influence the phenotype, and this is perhaps not surprising since at no time have the parent strains been cultured on media to which formate has been added as a separate component.
Fatty Acid Mutants in Neurospora

Investigator: Lein.

Support: Gosney Research Fund.

Work was directed along two channels. The first involved obtaining more fatty acid mutants so that a detailed study of fatty acid synthesis could be carried out. Secondly, a series of mutants was studied which had been obtained previously and which grew on minimal medium supplemented with acetate. For the detection of mutants, crosses of the temperature sensitive morphological strains isolated by Mrs. Mitchell were used. More than 3,100 probable mutants were picked and at least three of these have been shown to be fatty acid mutants. These will be studied in the future.

Acetate-Requiring Mutants of Neurospora

Investigator: Lein.

Support: Gosney Research Fund.

Growth studies on acetate-requiring mutants showed that they would also grow on pyruvic acid. It was indicated that an inhibition phenomenon is responsible for the metabolic block, the inhibitor being a normal product of glucose metabolism. When grown on acetate, the mutant was found to accumulate pyruvic acid.

Suppressor of Mutant Strain 37301 in Neurospora

Investigator: M. Mitchell.

Support: Rockefeller Foundation Fund.

As a result of efforts to learn by what mechanism this suppressor removes the pyrimidine requirement of strain 37301, interactions between the suppressor gene, the pyrimidineless gene, and genes which introduce requirements for arginine have been observed. That such interactions might occur was suggested by the fact that the pyrimidine requirement is not suppressed in the presence of added arginine, citrulline, ornithine, or proline. Appropriate crosses were made involving 37301, the suppressor, and two mutants which utilize proline, ornithine, citrulline or arginine; three which utilize ornithine, citrulline or arginine;
and two which use citrulline or arginine (all arginine-requiring mutants mentioned have been shown by Dr. Adrian Srb to be genetically different). As a result the following observations were made:

1. The requirement of the two proline mutants is completely suppressed by the suppressor of pyrimidineless.

2. The ornithine and citrulline mutants are not suppressed, but the two citrulline mutants and one of the ornithine mutants which produce a small amount of growth in the absence of a supplement do not do this when they are combined with the suppressor.

3. The pyrimidine requirement of 37301 is suppressed, to an extent which has not been precisely determined, by the ornithine mutants.

These same combinations involving a mutant which uses only proline and one which uses only arginine (also studied by Doctor Srb) were tested previously and no interactions were observed.

A possible mechanism of the suppressor action suggested by these observations is as follows: The pyrimidine and proline requirements do not arise as a result of completely blocked reactions, but rather from reduction of reaction rates below necessary levels. The suppressor increases these rates by decreasing the rate of a competing reaction, possibly one leading to the formation of ornithine. This scheme, of course, requires the assumption that a common precursor of proline and ornithine, postulated by Srb and others, contributes to the biosynthesis of pyrimidine, and this is by no means established by the data. It is suggested as an example of ways in which the observations can be explained without invoking dual functions for the four genes which act as suppressors of pyrimidineless.

Apparent Wild Types from Pyrimidineless Mutant 45502 of Neurospora

Investigator: M. Mitchell.

Support: Rockefeller Foundation Fund.

This mutant, which, as Doctor Barbara McClintock found, carries a translocation, may be an allele of 37301. There is no evidence to the contrary. About 5% of the progeny from crosses between these two mutants appear as wild type on minimal agar.
plates, and if they are kept on minimal medium, or minimal supplemented with cytidine, their growth in liquid minimal medium is like that of wild type. However, if they are kept on a complete medium and then tested in liquid culture they grow very slowly on minimal medium and are markedly stimulated by cytidine. Crosses of these strains to standard wild types give about 45% mutant progeny. The cross of 45502 to 37301 does not give complete asci; crosses of 45502 to wild type, however, give complete ascis, as do those of the apparent wild types to standard wild types, and ascis from the latter crosses show abortion patterns which are characteristic of the former. Hence, it appears that the strains which are phenotypically wild but genotypically mutant carry the translocation, and it seems possible that they arise as a result of the translocation. In a limited number of experiments it has been found that these strains occur among the progeny of crosses of 45502 to wild and of apparent wild types to wild, in about the same proportion that apparent wilds were observed from crosses of 45502 to 37301, that is, about 4 or 5%. Many more observations are needed, however. Ascis from the first two crosses are now being studied and, so far, the apparent wild types have been found in two ascis (from a cross of 45502 to wild), both of which had the same abortion pattern, namely, four small, colorless spores on one side of the ascus and four normal-appearing spores on the other. The latter four spores all gave rise to apparent wild types in each case. More work is considered necessary before an interpretation is undertaken.

Mechanism of Crossing Over in Neurospora

Investigator: M. Mitchell.

Support: Rockefeller Foundation Fund.

In connection with an attempt to repeat with Neurospora the work of Beadle and Emerson with Drosophila, which suggests that the various types of double crossovers occur at random, eight genes have been placed in linkage groups. No combination of markers which has been tried has been considered entirely suitable for the experiment on crossing over. However, a number of combinations, especially ones involving the recently located genes, remain to be tried.
Carotenoid Pigmentation in Neurospora

Investigator: Sheng.

Support: United States Public Health Fellowship.

Genetically it has been shown that the genotype of wild type Neurospora may be represented as AB and that of the albino (15300) strain as ab. The pigments of the four color types AB, Ab, aB, and ab from a cross of the wild type to the albino mutant have been investigated chromatographically. The difference in the quantity of pigments of the wild type and the ab type is of the order of thousands of times. The quantity of the colorless carotenoid phytofluorene parallels the colored carotenoids of the four types. When a wild type strain was grown in total darkness, the quantity of colored carotenoids was reduced to less than 4% of that present in cultures grown in light, while the phytofluorene was only slightly less. This suggests that phytofluorene is the precursor of colored carotenoids. The genetic blocks in the mutant strains studied therefore probably involve reactions prior to the formation of phytofluorene.

Mutants of E. coli with Altered Metabolism of Four Carbon Dicarboxylic Acids

Investigator: Nelson.

Support: Gosney Research Fund.

Genetic studies of a spontaneous biochemical mutant in the sexually fertile K 12 strain of the bacterium Escherichia coli have been undertaken. The wild type strain s- was incapable of growth or oxidation with four carbon dicarboxylic acids as the sole carbon source. The mutant s+ was capable of growth and oxidation of these acids. Manometric analysis placed the block at the oxidation of malic acid. In order to establish the dependence of the phenotypes of the substrains on a genetic locus it was necessary to perform crosses between the s- and s+ substrains. A diauxotroph of the s+ substrain, requiring histidine and phenylalanine, was detected following serial ultraviolet irradiation. This substrain was crossed by the method of prototroph selection with s- auxotrophs and the expected quantitative recovery of prototrophs, representing progeny of the cross, obtained. Standard tester stocks developed in other laboratories have been used to establish the position of the s locus. The s locus was found to belong to the maltose and xylose fermentation
streptomycin resistance linkage group and to be closely linked to the streptomycin resistance locus. The s locus was therefore independent of the lactose fermentation - coliphage T1 linkage group. The biochemical nature of the block is now being investigated, with the use of cell-free enzyme preparations from the substrains.

**Mutant Strains of E.coli with Decreased Penicillin Resistance**

**Investigator:** Rowley.

**Support:** Commonwealth Fund Fellowship.

In order to investigate the mechanism of the process by which bacteria become penicillin resistant, the reverse process was attempted. A naturally resistant organism, E. coli, was irradiated with UV light to increase its mutation rate and exposed to such a concentration of penicillin as would allow the original organisms to grow freely but would halt the division of any more sensitive mutants. After some hours the penicillin was destroyed by an enzyme, after which the few sensitive mutants were able to commence growth. They appeared as a very small second crop which could be separated from the overwhelming number of the primary crop by photographic devices. By this means five mutants were isolated which were from 10 to 50 times more sensitive than the original strain. This is strong evidence in support of the idea that resistance arises by spontaneous mutation and that the resistant forms are favored by the presence of the antibiotic.

**Enzyme Deficient Mutants of Neurospora**

**Investigator:** Schaeffer.

**Support:** Rockefeller Foundation Fellowship.

A study of the methods for detecting mutant strains of Neurospora deficient in activity of specific enzymes has been undertaken. Several methods using color reagents appear promising. For the detection of tyrosinase in strains made colonial with sorbose, no special reagent is required since the activity of the enzyme is indicated by the spontaneous darkening of the colonies when tyrosine is present in the medium and the active enzyme is in the mycelia. It is essential that the incubation time be sufficiently long enough (12 days at 25°C). UV-irradiated suspensions of microconidia plated on solid media
plus tyrosine gave colonies varying from colorless to black. The black mutant not only has a black mycelium but also gives a precipitate of melanin from tyrosine in liquid media. Extracts of this mutant, which are some 10 to 20 times more active than those of other strains so far studied, will be used to try to prepare tyrosinase-antibodies in the rabbit. Some white colonies have unexpectedly given active enzyme extracts. Further irradiation experiments are being made with the hope of obtaining strains which will remain permanently white; that is, are genetically unable to produce tyrosinase.

**Fluoro-Substituted Phenylalanines as Growth Inhibitors**

**Investigators:** P. Ellman, Surber, H. Mitchell.

**Support:** United States Public Health Service (Grant to Division of Chemistry).

In cooperation with Niemann of the Division of Chemistry mono-fluoro ring-substituted phenylalanines have been studied and shown to be ineffective for treatment of experimental infections. Although one of these compounds produces a slight regression in experimental tumors it is much too toxic to be of clinical value. The number of bacteriophage plaques is decreased by fluorophenylalanine by some unknown mechanism. The inhibition of wild-type Neurospora by the fluorophenylalanines is being studied and the characteristics of an adapted strain are being observed. An investigation of the behavior of the fluorophenylalanines with an enzyme system which metabolizes phenylalanine was begun. However, it was found necessary to develop a method for determining small amounts of organic fluoride in biological material before the investigation could be completed. This study is now in progress.

**Imidazoles of Neurospora**

**Investigator:** Ames.

**Support:** General Funds.

A method has been developed for the chromatographic separation and identification of imidazoles. The procedure has been applied to the problem of separation and identification of four different compounds of this class that are accumulated by histidineless mutants of Neurospora and Penicillium. One of the substances appears to be histidinol but the others are unknown.
substances. Imidazoles produced by the oxidation of histidine with peroxide and by the reaction of sugars, \( \text{NH}_4 \text{OH} \) and \( \text{Cu}^{++} \) are also being investigated. Several unknown imidazole derivatives have been shown by chromatographic methods to be produced by these reactions. Those produced from the reaction with sugar have properties in common with the substances accumulated by the mutants.

A manuscript on the chromatographic method has been prepared for publication.

**Peptide-Requiring Neurospora Mutants**

**Investigator:** Dubes.

**Support:** General Funds.

Experiments have been designed to clarify the biochemical requirements of five unknown mutants of *Neurospora crassa*. In general the procedure has been to examine six gross biological sources, malt extract, liver extract, yeast extract, Neurospora extract, enzymatically hydrolyzed casein, and peptone, for their ability to support the growth of these five mutants. The richest sources are then partitioned by adsorptive and extractive methods and the fractions bioassayed. Simple liquid partition, dry extraction, charcoal adsorption, and especially the techniques of chromatography including chromatostrips, chromatopiles, and resin columns have been employed. It now appears that two of these five mutants grow optimally only when supplied with some as yet unknown peptide or peptides. Since it is the purpose of this investigation to characterize chemically the growth factors for these mutants, some model experiments have been run on known peptides in order to evaluate the possibilities of serial degradation and concomitant characterization by splitting off the amino end residue as a heterocycle in the form of 4-substituted-2-thio-5-thiazolidone.

**Phospholipids in Neurospora**

**Investigator:** G. Ellman.

**Support:** McCallum Foundation Fellowship.

Procedures have been devised for the isolation of phospholipids from Neurospora. Analysis of the products indicates that the crude material contains at least six organic bases. Two of these have been identified as choline and ethanol amine. The others have
been purified as the dinitrophenyl derivatives. A large amount of material has been prepared for further analyses.

A general method has been devised for the synthesis of some peptide substrates to be used in kinetic studies with chymotrypsin. Three peptides of methylanthranilate phenylalanine have been prepared. The enzyme splits off methyl anthranilate, which can be determined fluorometrically in very small quantities.

**Lysine Biosynthesis in Neurospora.**

Investigator: Good.

Support: General Funds.

Investigations on the genetics and metabolism of the numerous lysine-requiring mutants of Neurospora show that apparently there are three distinct biochemical classes involving five different genes. Experiments with isotopes indicate that there is no exchange of nitrogen between lysine and the metabolic pool in any of the mutants. Several new compounds which have been postulated as intermediates in the biosynthesis of lysine are being prepared and tested. \( \epsilon \)-Hydroxy-norleucine and \( \alpha \)-amino adipamic acid have been shown to support the growth of some of the mutants.

**Tryptophane Metabolism in Neurospora**

Investigator: Haskins.

Support: General Funds.

Genetic studies have been made with strains 39401 and C86. These two strains are alike in that both utilize indole, tryptophane, kynurenine, 3-hydroxykynurenine, 3-hydroxyanthranilic acid, and nicotinic acid; but strain C86 can also use anthranilic acid, phenylalanine, tyrosine, and quinic acid while strain 39401 does not respond to these compounds. Thus, 39401 is apparently blocked between anthranilic acid and indole, and C86 seems to be blocked at some step in the biosynthetic pathway before quinic acid. It was surprising to find, therefore, that crosses between C86 and 39401 produced no wild types; that is, the mutations which prevent the growth of the two strains on minimal medium appear to be at the same locus. From crosses of 39401 by wild type and by C86, two new types were recovered, one of which appears to be blocked.
before anthranilic acid, and the other between tryptophane and kynurenine. A genetic analysis of the problem has furnished evidence which indicates clearly that modifying genes are effective in producing qualitative changes in the growth responses of the four strains. The results obtained in this work emphasize the inadvisability of attempting to assign the primary effect of any genetic mutation to a particular biosynthetic step on the basis of growth response data alone.

Heterocaryosis in Neurospora

Investigator: Holloway.

Support: Smith-Mundt Fellowship.

The sulfonamide-requiring mutant of Emerson undergoes mutation under certain conditions to produce a heterocaryon composed of sulfonamide-requiring nuclei and nuclei carrying a suppressor of the sulfonamide-requiring gene. Two such heterocaryons have been studied. The growth behavior of these suppressors both alone and with the sulfonamide gene has been investigated. Both heterocaryons were found to have a nuclear ratio which extensively favored one type of nucleus, in the first case the sulfonamide-requiring type nucleus and in the second the double mutant nucleus.

Biosynthesis of Cysteine

Investigator: G. Fischer.

Support: General Funds.

It has been found by Fischer that both elementary sulfur and hydrogen sulfide can replace cystine (or cysteine) for several cystineless mutants. These findings place the problem of cystine synthesis in an entirely new light. They indicate that the block in these mutants is not in the reactions by which the cysteine molecule is put together, but is in the ability to bring the sulfur atom to the lowest level of reduction. These mutants are thus brought into line with a series of mutants which are blocked at successive stages in the reduction of sulfate. On this basis it now seems possible to account for most of the known characteristics of the sulfate - cysteine series of mutants.
Methionine Biosynthesis in Neurospora

Investigator: Gershowitz.

Support: General Funds.

A suppressor gene has been studied which inhibits the action of certain methionineless mutants but not others. It apparently does not affect two choline mutants which are known to be deficient in their methylating mechanisms. The suppressor action is unaffected by temperature, pH variations, and supplements of vitamin mixtures or casein hydrolysate. Chromatographic analyses and cross feeding experiments of the mutants and suppressed mutants are planned.

The nine methionineless mutants studied fall roughly into three groups on the basis of their centromere distances: a) 5-8, b) 10-11 and c) 22-26. Tests are in progress to determine if the mutants within each group are linked, allelic or independent. Double mutants will be obtained and attempts made to determine the course of methylation of homocysteine in them by chromatographic analyses. It is hoped in this way to determine the relative order of the blocks in the mutants in the conversion of homocysteine to methionine.

Threonine Metabolism in Neurospora

Investigator: Reissig.

Support: General Funds.

The biosynthesis of threonine had been investigated with biochemical mutants of Neurospora unable to synthesize this amino acid. The metabolism of threonine and related substances was studied in the presence of extracts of wild type and different mutant strains in order to acquire evidence of a different nature concerning the biosynthesis of threonine. The following reactions were demonstrated to be catalyzed by Neurospora extracts: deamination of threonine and serine, decarboxylation of α-ketobutyric and pyruvic acids, and transamination between α-ketobutyric and glutamic acids.

Since these catalytic activities are present in strains unable to synthesize threonine as well as in wild type strains, it appears that the above reactions are not on the path of biosynthesis. Pyridoxal phosphate has been found to stimulate the deamination and transamination reactions, while cocarboxylase stimulates the decarboxylation
reactions. There are indications that the deamination reactions are catalyzed by different enzymes.

Mutant 44104, which in vivo responds much better to α-aminobutyric than to α-ketobutyric (the latter is inactive if added by itself, but has a powerful sparing action), is nevertheless able to catalyze the transamination reaction as well as wild type, thus affording an opportunity to study the relative significance of in vivo (feeding) and in vitro (enzymatic) methods in biochemical genetics.

L-Amino Acid Oxidase of Neurospora

**Investigator:** Thayer.

**Support:** United States Public Health Service Fellowship, Rockefeller Foundation Fund.

Thayer and Horowitz have completed a survey of the properties of L-amino acid oxidase and have examined the question of whether a strain difference exists with respect to the ability to produce it has been settled.

The enzyme catalyzes the oxidation of L-amino acids with the uptake of one atom of oxygen and the production of one molecule each of the corresponding α-keto acid and ammonia. The rate is linear with enzyme concentration, but with each increasing substrate concentration a concentration of optimal activity is observed. When two different substrates are simultaneously added to the enzyme a mutual interference, probably competitive in nature, is found. The enzymatic activity is strongly dependent on oxygen tension and is four to five times higher in oxygen than in air. The pH optimum of the activity varies with the substrate being attacked.

All wild strains tested produce the oxidase when grown with a marginal supply of biotin. Previously published failure to demonstrate the enzyme in certain strains was due to the fact that the enzyme preparations from them contain sufficient free amino acids to saturate the enzyme, so that no increase in oxygen uptake was observed on addition of substrate. It is probable, however, that minor differences in the quantity of enzyme produced exist among the strains.

It has been found that the enzyme is adaptive. Cultures grown with casein hydrolysate or synthetic amino acids as the sole nitrogen source show significantly increased enzyme production.
It has been found that the effect of marginal biotin can be imitated to some degree by culturing on limited nitrogen, but not on limited sulfur.

A report of this work is in press in the Journal of Biological Chemistry.

Neurospora Ascospore Activation

Investigator: M. Emerson.

Support: Rockefeller Foundation Fund.

Mary R. Emerson has continued studies of the chemical activation of ascospore germination and of the characteristics of an osmophobic mutant strain. In studying the effects of respiratory poisons on ascospore germination it was observed that sodium azide prevents heat activation and renders the spores insensitive to heat activation even after the removal of the azide. Such spores, however, are still sensitive to activation by furfural.

An Osmophobic Mutant in Neurospora

Investigator: M. Emerson.

Support: Rockefeller Foundation Fund.

In media of low osmotic concentration the osmophobic mutant resembles wild type in that it produces very long, occasionally branched hyphae. In solutions of high osmotic concentration, growth of the mutant is slow, resulting in extremely short and profusely branched hyphae. When transferred from high to low concentration, the tips of the branches begin to elongate in a very short time. When long hyphae are transferred from low to high concentration, growth in length ceases and numerous buds appear along the sides which soon develop into short lateral branches.
Two electrophoretically distinguishable strains of tobacco mosaic virus isolated by Wildman, Singer, and Bald have been shown to be related, but not identical, in terms of the antibodies they induce in rabbits and with which they react in test tubes. Antiserum to either pure strain cross-reacts with the other, indicating some degree of similarity between the two. The reactions, however, are quantitatively distinct. Antibody reagents specific for each strain have been prepared by straight-forward absorption techniques. Such specific reagents offer promise as tools for the study of mutations and possible recombination of serological characteristics of viruses - the sources of sudden changes in the character of pathogenic viruses.

Immunogenetics of Tyrosinase

In recent years the recognition that enzymes may serve as antigens, inducing an antibody response in individuals injected with them, has prompted several studies of the characteristics and possible utility of such enzyme-antienzyme systems. It is evident that these systems differ one from another, depending on the particular enzyme used, the particular antibody response evoked, and the techniques with which the reactions are studied. Among the most interesting of such studies is the largely unpublished work of C. L. Markert of the University of Michigan, formerly a Merck Fellow at the Institute, and Owen on the tyrosinase system of the fungus Glomerella.

Markert had found that the tyrosinase activity of a Glomerella extract was affected by both environmental and genetic factors in the
culture of this organism. The genetic aspects of the control of
tyrosinase activity in extracts are complex, since changes in any
of a number of independent genes are reflected in modified levels of
enzymatic activity in the extracts. Utilization of antisera produced
in rabbits by the injection of Markert's extracts suggests the following
conclusions:

a) Active tyrosinase in an extract serves as an effective
antigen, to a degree directly related to the level of the enzymatic
activity of the extract. No antibody capable of reacting with
tyrosinase in any detectable fashion is formed in a strain in which
either genetic or environmental factors have blocked the expression
of tyrosinase activity. There does not appear to be present under
such conditions, therefore, a significant quantity of antigenically
active material (as, for example, an "enzyme precursor") related
to the active enzyme.

b) No qualitative serological distinction has been possible
among the tyrosinases of different mutant forms of the fungus having
enzymatic activity. This suggests, although it does not prove, that
the genes that modify the quality or quantity of tyrosinase activity
in this fungus affect primarily the biochemical milieu of the enzyme
and its synthesis rather than the enzyme itself, directly.

c) Antibodies may have two quite different effects on the
enzyme. Over a period measured in minutes or hours, they
specifically precipitate the enzyme from solution. Over periods
measured in seconds, they may inactivate the enzyme. The latter
reaction is of particular interest, since its kinetic character
together with studies of the effects of changes in relative concentration
of enzyme, substrate, and antibody indicates that the inactivating
antibodies are directed against, and combine strongly with, the
particular part of the enzyme molecule involved in its biochemical
activity. Precipitation of the enzyme, on the other hand, may
involve less critical areas of the enzyme surface; and in fact some
antibody preparations precipitate the enzyme without inactivating it.

d) Enzyme preparations having similar specific biochemical
activities, but taken from different species may be entirely dis-
similar in their serological properties. This holds even in the case
of antibodies presumably directed against the active sites of the
enzyme molecule. Even when two enzymes from different species
are similar in their substrate - product aspects, the highly specific
antibodies distinguish clearly between them.
Antigenicity of a Synthetic Copolymer

Investigator: Owen.

Support: Rockefeller Foundation Fund.

A copolymer synthesized and under study by Singer and Kirkwood, which may have some eventual utility as a "plasma substitute," has been subjected to careful tests for possible antigenicity in rabbits and chickens. No immune response in these animals has been detected when either the copolymer or its "R-azo" derivative was injected with or without the use of adjuvants. It seems probable, therefore, that the use of this material as plasma substitute would not be accompanied or followed by undesirable "sensitization" effects. The failure of this synthetic material to induce an antibody response may have some pertinence to the immunochemical aspects of antigenicity.

Serology of Euglena.

Investigator: Owen.

Support: Rockefeller Foundation Fund.

Cultures tracing to different single-cell isolates from a mixed culture of the unicellular flagellate Euglena are being examined for their responses to different media and to antisera produced in rabbits. In several respects the organism responds to antibody in ways similar to the bovine sperm. Consistent phenotypic differences among cultures arise as a function of the particular medium used. No clear difference among such cultures, in terms of their induction of or response to specific antibodies, has as yet been established in this work. The investigation, however, is in its infancy.

Immunogenetics of Rats

Investigator: Owen.

Support: Rockefeller Foundation Fund.

Various aspects of the gene-controlled cellular antigens of rats have been explored and extended during the year. Among these are: the recognition of individual differences in rats by means of antisera produced in chickens; the unsuccessful search for a cellular difference associated with a chromosomal translocation
in rats; and the demonstration that the trypsin treatment of rat red cells affects them, with respect to a particular allelic pair of antigens, much as Rh-positive human cells are known, on the basis of recent research elsewhere, to be affected by the same treatment. In this work rabbits were also immunized with trypsin-treated rat cells to see whether qualitatively different antigenic sites on the cell surface were exposed by the enzyme. No new antigens, however, were detected by these antisera, although there was evidence that the cells became somewhat more effective as antibody-inducers, with respect to antigens previously known in untreated cells.

Organ Specificity in the Chicken

Investigator: Rappaport.

Support: General Funds.

Antisera to the saline soluble proteins of chicken livers, kidneys and brains have been prepared in rabbits. Whole chicken plasma is also used to incite antibody production.

Physical and chemical criteria in conjunction with immunological techniques are being applied to characterize specific fractions of the extracts. The information obtained may shed light upon the following:

a) What proteins are common to various organs?

b) Which proteins are specific for a particular organ?

c) What is the origin of the plasma proteins?

d) Are the enzymes which are concerned with the same reactions in the different organs identical by immunological standards?

Serology of Plant Enzymes

Investigator: Boroughs (Graduate Minor Problem).

Support: General Funds.

The nature of antibody action on enzymatic preparations from plant leaves is being studied with a view toward defining the degree of serological relationship, if any, among enzymes with distinct activities but of a common source. Boroughs' findings may
bear on the problem of whether there may be a definite reservoir of serologically recognizable "enzyme precursor" material available to the plant cell which may acquire distinct enzymatic specificities at some relatively late stage in enzyme synthesis.

### Individual Differences in Pigeons

**Investigator:** Gershowitz (Graduate Minor Problem).

**Support:** General Funds.

There has been developed a reagent recognizing a clear-cut individual difference in pigeon red-blood cells. This work is being extended to the identification of other individual characteristics. A later step will include a definition of the nature of the genetic control of such differences.

### Serology of Bovine Sperm

**Investigator:** GoodSmith (Graduate Minor Problem).

**Support:** General Funds.

This work has been done with bull semen generously provided by the Roger Jessup Farm in Glendale. It is hoped to identify individual differences among bulls in the inherent qualities of their sperm. Antisera against sperm and semen, produced in rabbits, have several effects on the sperm: they are immobilized, agglutinated in specific fashion, and their metabolism as measured by standard tests is seriously impaired. A skin sensitivity is observed in rabbits that have been injected with whole semen but not in those injected with washed sperm. This reaction is being further investigated. Problems of sterility in dairy cattle may possibly be clarified by the extension of this work.

Observations were also made on certain blood-cell characteristics of the bulls involved. These tentative observations appear to have some potential general interest in themselves, and arrangements are being made to confirm and extend them in collaboration with Professor Clyde Stormont, of the University of California at Davis. An extensive investigation of bovine blood groups is in progress there.
Marine Zoology

Staff: Associate Professors: MacGinitie, Phelps; Visiting Professor: Giese; Superintendent: Chalmers; Research Assistant: Sutton.

During the greater part of the year under review, Professor MacGinitie was on leave of absence from the Institute to serve as Scientific Director of the Arctic Research Laboratory at Point Barrow, Alaska. In addition to his duties as Director, MacGinitie carried on research involving oceanography, dredging and towing for biological specimens, identification of organisms, and studies of the ecology of Arctic animals. In August, 1950, it was necessary for MacGinitie to return to the States. Since then he and Mrs. MacGinitie have been active in working up the data obtained during a year and a half of work at Point Barrow. The final objective is to turn in to the Office of Naval Research a report of the ecology of the marine invertebrate animals in the vicinity of Point Barrow. Results of the work are already very gratifying as the investigation is yielding a fairly complete account of the marine animal life of the region and also will fill in a much needed link in the distribution of the marine life of the Arctic Ocean. Nineteen specialists in various invertebrate fields are working on the taxonomy of the material obtained.

Plant Physiology and Biochemistry--Earhart Plant Research Laboratory

Staff: Professor: Went; Visiting Professor: Gustafson; Research Associate: Hiesey; Research Fellows: Ashby, Casamajor, Darley, Garman, Hellmers, H. Hull, Osborne, Phillips, Soriano, Ulrich; Graduate Students: Kurtz,Liverman, Nitsch; Research Assistants: M. Bandurski, Corbett, Jones, Juhren, Ohki, Signer, Weinbaum, Zaitlin; Superintendent: Keyes.

During the year 1950-1951 the Earhart Laboratory under the leadership of Professor Went reached practically full scale operation, with about 80% of its growing space occupied. There were approximately 20 separate research projects in operation. In addition a number of investigators used the facilities of the laboratory in an incidental way.
A number of improvements in the physical set-up of the laboratory have been made. Most important was the installation of smog filters in the air intake of the laboratory, eliminating not only most of the smog damage to plants inside the buildings but also making the plant material more uniform in its growth.

A CO₂ analyzer has now been installed, making available a continuous record of the CO₂ content of the air in 12 localities.

The Photosynthetic Efficiency of Tomato Plants

Investigator: Went.  
Support: General Funds.

The efficiency with which light is used in the production of plant material by the tomato depends upon the temperature to which the plant is subjected. Maximum efficiency is reached during day temperatures of 17° or lower. Under those conditions and with light intensities below 1000 f.c., up to 20% of the total incident light energy is stored up in chemical form. When tomato plants which have been subjected to prolonged dark are again exposed to light, photosynthesis is reestablished only after an appreciable lag period. The lag period may, however, be shortened by application of sugar to the leaves in the form of a sugar spray. This may explain some of the effects of sugar sprays on plants.

Effects of Temperature on Tomato Growth

Investigators: Went, Weinbaum.  
Support: General Funds.

In a study of the effects of night temperature on the growth of tomato plants, the experimental procedure was to subject them to the same temperature during the whole dark period. Weinbaum is now investigating the effects of fluctuating temperatures. She found that even short exposure to either 30° or 30° C in darkness greatly reduces the growth which occurs at 17° C. Such temperature shocks will be investigated in greater detail.
Sexual and Vegetative Reproduction in the Strawberry

Investigator: WenL
Support: General Funds.

The flowering, fruiting and runner formation in strawberries has been investigated. The results of earlier investigators, such as Darrow, could be reproduced; at high temperatures (20° and above) and long photoperiods only runners are formed; at all temperatures and short photoperiods flowers develop, and also at low temperatures and long photoperiod. It is now found that only the temperature during the light period is of importance for flowering and fruit growth. Also it was found that the higher the temperature, the longer the short-day treatment has to be prolonged to cause flowering, or the lower the light intensity of the accessory light which will prevent flowering. These facts are of importance in a better understanding of photoperiodism.

Studies on Baeria

Investigator: WenL
Support: General Funds.

Baeria, a genus of small, rapidly growing desert annual composites sometimes known by the common name gold fields, has many advantages for experimental work on plant growth, reproduction and inheritance. Baeria chrysostoma is now being subjected to a more intense analysis as far as its photo- and thermoperiodic behavior is concerned. By using early flowering as a criterion, optimal conditions are as follows: During its first day of germination, best later development occurs in darkness at 20-23°, for the next three to six days the optimal temperature is 14°, in continuous light, and from then on the optimal temperature, still in continuous light, rises gradually through 17° and 20° to 23° about 15 days after sowing. Under these conditions the first flowers open 22 days after sowing. It is interesting that the longer the photoperiod to which Baeria is exposed, the earlier it flowers. Thus Baeria does not seem to have a critical day length, as does Xanthium.
A New Method of Studying Plant Roots
Investigator: Went.
Support: General Funds.

Fairly successful trials have been made to grow plants entirely in air, subjecting their roots to a fine mist of nutrient solution. This technique opens up new possibilities of studying root development.

Germination and Growth Requirements of Various Plants
Investigators: Went, Juhren.
Support: General Funds.

The temperature requirements of several plants other than those mentioned above are being investigated on a fairly small scale. The Joshua tree, in spite of the fact that it only occurs at higher altitudes in the Mohave desert, grows best at the highest temperature tried (30° during the day, 23° during night). This is also optimal for orange plants, which grow continuously near those conditions, but which hardly grow at all at 20° day and 10° night temperature.

Juhren has studied the effect of environmental conditions on the growth of certain grasses and their hybrids. She found that in the case of a Poa compressa hybrid, its range of growing conditions has widened to that of both its parents.

Some work has been initiated on the effects of environment on the growth of weeds. In an uncultivated field the majority of seeds present were found to germinate at low temperatures only (grasses, Stellaria). In an adjacent cultivated field the picture was very different; there most seeds are high-temperature germinators (Amaranthus). This seems to be due to the effects of cultivating per se.

Growth and Alkaloid Production of Veratrum
Investigators: Went, Corbett.
Support: Riker Laboratory.

Veratrum is a liliaceous plant that grows wild in the eastern United States and is of interest because it produces alkaloids
of therapeutic value in the treatment of hypertension. The culture of Veratrum is difficult and it is now clear why. When dug from the ground the plants are completely dormant as soon as the main shoot has developed early in the season. Corbett has been unable to find any chemical substitute for cold temperatures in breaking up the dormancy of the Veratrum corms. As the season progresses, the length of cold treatment required decreases. Only temperatures near freezing or slightly higher are effective. Corms collected in June require four to five months of cold treatment; those collected in September only two to three months. Applied vitamins increased root and top development. Bud initiation is now under study.

**Environmental Factors and Vitamin Production in Plants**

**Investigator:** Gustafson.

**Support:** General Funds.

Thiamine was found to be increased in the tomato plant with an increase in day temperature from $3^\circ$ to $30^\circ$ C and a night temperature of $23^\circ$ when the light intensity was above 500 f.c. In beans there was only a slight temperature effect. Peas had the highest thiamine content at a $30^\circ$ to $10^\circ$ C night temperature and $23^\circ$ C day temperature when they were of medium age. When very young $30^\circ$ was the best, but as the plants aged they deteriorated rapidly at $30^\circ$ and the vitamin content also decreased. Riboflavin showed very slight if any response to change in temperature.

In the tomato niacin was higher at $23^\circ$ and $30^\circ$ than at $3^\circ$, whereas in broccoli the reverse was true. In the black valentine bean the highest niacin content was obtained at $23^\circ$ C.

In later experiments the effect of photoperiod was studied at the temperatures $6^\circ$, $14^\circ$, $20^\circ$ and $26^\circ$. In general it can be stated that at the medium temperatures $14^\circ$ and $20^\circ$ riboflavin and niacin increased with photoperiod up to 24 hours, i.e., continuous light. Thiamine on the other hand decreased as the day was lengthened from eight to 24 hours. For this experiment pea, tomato and bean plants were used.
Growth and Sugar Formation in the Sugar Beet

Investigator: Ulrich.

Support: Beet Sugar Foundation through the University of California.

Experiments under way in the Earhart Laboratory have shown that a general relationship exists between night temperature, beet growth, and sugar production. As the night temperature increases from 36° F, the beet root weight increases rapidly. Maximal weights of beet roots are obtained between night temperatures of 57° F and 79°F. At 86° F the beet weights decrease sharply.

The sucrose concentrations of the beet roots grown at different night temperatures form an entirely different pattern from that of the beet weights. The highest sucrose concentration is found in beets grown at the lowest night temperature. As the night temperatures are increased, the sucrose concentrations of the beets decrease gradually from a high value of 12% sucrose at 36° F, to a low value of 7% at 86° F.

The total amount of sucrose produced per beet follows much the same pattern as the beet root weights. The maximum amount of sucrose, however, is produced at a lower night temperature than is observed for maximum beet root growth, a fact which is interesting, both commercially and scientifically.

The amount of light received by beet plants greatly influences the beet root weight. In an experiment comparing plants in a day period limited to eight hours of sunlight with plants in natural day length, the beet roots for normal day length doubled in size without changing the weight of the tops or increasing greatly the sucrose concentrations of the beet roots. Apparently, for reasons as yet unknown, the first photosynthetic products of beet plants are used for the development of tops and fibrous roots. If the sugar produced exceeds the amount required for development of tops and fibrous roots, the excess is diverted to storage root growth. Evidently the plants receiving more than eight hours of sunlight operate much as a modern factory does when the working day is extended from eight to 16 or 24 hours. Under the longer day lengths the beet roots and sucrose weights of the plants doubled.
In reviewing the effects produced by night and day temperature and of day length upon beet growth and sucrose production, it is of interest to note that in all of the experiments conducted thus far, the beets failed to reach a sucrose concentration generally found in commercially grown beets. Apparently, in the presence of an abundant supply of water and plant nutrients, the beet plants, both tops and roots, grew rapidly, utilizing the sucrose synthesized as it was produced by the plants. Under these conditions a high sucrose accumulation in the beet root is prevented, and in fact will be prevented as long as favorable conditions of growth prevail. It is only when the utilization of sucrose is decreased without curtailing sucrose synthesis, such as when beets become nitrogen deficient, that the beet root will accumulate sucrose to a high concentration. Other mechanisms for effecting sucrose accumulation, however, may also occur in the beet plant, and one of the objectives of future experimentation will be to find these.

Effects of Smog on Certain Physiological Processes in Plants

Investigators: Garman, Went.

Support: Los Angeles Air Pollution Control District.

The research of Haagen-Smit, Went et al. has established that certain plants are visibly damaged by natural smog and by synthetic smog produced by combining ozone with unsaturated hydrocarbons. Plants subjected to these synthetic fumes showed damage very similar to that produced by natural smog.

With these facts as a basis, studies were initiated on the effect on plants of synthetic smog produced by combining ozone with 1-hexene. Plants were grown in a completely smog-free atmosphere obtained by passing air through a series of three activated carbon filters into an air-conditioned, artificially lighted growing room kept at 20 to 22° C. Vermiculite was used as the growing medium and plants were watered with Hoagland's nutrient solution.

From early experiments on the five plants, alfalfa, oats, endive, spinach and sugar beet, it was found that changing the nutrient solution by doubling the calcium or the potassium did not alter the sensitivity of plants to smog damage. From stomatal observations made with an Ultra-Pak microscope, it was learned that if stomata are open they close rapidly when plants are fumigated. If the stomata are closed at the time of fumigation, plants are nevertheless seriously injured. Apparently smog is able to
penetrate and damage the leaf cells even when the stomata are closed.

With the use of a method of assay of smog damage involving weight measurements on tomato plants, plants smog-treated in the dark were not found to be significantly smaller than plants given similar dark periods but not smog. Sucrose spray did not overcome the inhibitory effect of smog in light. Plants which were given a minimum of water daily for six days and fumigated were the same size and weight as those not fumigated but also watered lightly. On the other hand plants which were watered heavily and fumigated grew less than the controls. Plants of different ages responded differently. In general the younger the plants at the time of the test, the greater the growth inhibition produced by smog. When whole tomato plants were placed in a potometer and fumigated, a rapid and significant drop in water uptake was observed. Repeated fumigations caused continued sudden drops in transpiration followed by return to slightly below the normal rate. This drop did not seem to be closely correlated with stomatal closing.

Etiolated pea internode sections placed in water and in 2,4-D solution subjected to smog showed no inhibition or stimulation of elongation. Even when whole etiolated plants were given two hours of smog just before the section test, the smog did not cause inhibition of elongation.

In other tests synthetic smog was found to injure the leaves of certain plants, asters, snapdragons, and petunias, without injuring the individual flowers or the total yield of flowers. Avocado fruits tested after harvest were likewise undamaged.

These studies show that one of the most pronounced effects of smog is an effect on the water relations of the plant. However, decrease in transpiration rate is apparently not directly related to stomatal closure. Another effect of smog is the decrease in growth which produces the so-called hidden damage before any visible signs of injury appear. The amount of this growth inhibition depends on light, on age of the plant, and on the amount of water given the plant. This hidden damage may be of great importance to agriculture.
Plant Control of Soil Erosion

Investigators: Hellmers, Ashby.

Support: United States Forest Service.

Hellmers and Ashby of the United States Forest Service are engaged in a research program of watershed improvement for the mountainous area of the Los Angeles River Watershed. The present research consists of studies of germination, growth, and rooting habits of plants suitable for the stabilization of steep slopes. Results of this study will be applied in the field to improve the vegetation on critical erosion areas. This program will tend toward reduction of flood damage in the populated areas and the rate of silting of reservoirs.

Ashby is currently working in the Earhart Laboratory on the germination of several grass species, the growth requirements of native chaparral grasses and shrubs, and inducing dormancy in native grasses. This work has shown that individual chaparral species have specific temperature requirements for germination, growth and development. All the grass species which have flowered have done so only under long-day photoperiods. These temperature and photoperiod characteristics of the native chaparral species will serve as criteria in the selection of exotic species for testing in Earhart and later field plantings in the mountainous chaparral areas.

Hellmers is carrying on several research projects. In transpiration experiments in Earhart he is correlating the moisture level in soil with transpiration rates of Coulter pine and several brush species. Soil moisture is determined through the use of Colman soil moisture units and by periodic weighing of the plant containers. Relative humidity over the leaf surface is determined by means of an electric hygrometer.

Research in progress at the Kerckhoff Laboratories includes the developing of methods to increase the germination of seeds of several brush species. Success has attended a method of scarifying Yerba Santa seed, a native shrub valuable for erosion control.

Hellmers' current work in the Orlando Greenhouse involves studies of the nutrient status of "native" soils, further work on the germination requirements of several brush species, and determinations of the possible occurrence of nitrogen fixation.
by "nodules" on the roots of Ceanothus (Mountain Lilac) species. A marked response has been found in the growth of brush species upon the addition of nitrogen and phosphorus fertilizers. Field tests of these fertilizers have furnished confirmatory results of the laboratory studies. Proper fertilization shows promise as a method of developing vegetative cover on critically eroding areas above reservoirs and above communities exposed to flood dangers.

Hormones and Other Substances Influencing Plant Growth

Investigator: Osborne.

Support: Rose Sidgewick Memorial Fellowship.

Osborne has been studying the effects of synthetic growth substances on fruit set of emasculated tomato flowers. At high temperatures they are not effective, but at low night temperatures, when there is a tendency towards natural parthenocarpy, they cause as good fruit set as natural pollination. This explains why spraying with synthetic growth substances to improve fruiting is particularly effective in the early season, when temperatures are still too low for natural fruit set.

In studies of the effects of temperature and light on the effectiveness of 2,4-D in injuring or killing tomato plants, it has been found that in general the direct response to 2,4-D (epinasty) is more pronounced in strong light, whereas the indirect killing effect is stronger in weak light.

The pea test was studied in greater detail and its accuracy and reproducibility have been greatly increased. This is probably partly owing to the improved constancy of the growing conditions available in the Earhart Laboratory. She has also discovered that a particular substance may cause growth in pea seedlings whereas it is completely inactive in other plants. This curious phenomenon leads to questioning the hypothesis on which most auxin work is based, namely, that all substances active as growth promoters act through the same master reaction.
Germination Studies on Desert Annuals

Investigator: Phillips.

Support: General Funds.

Field studies of the influence of amount, duration and time of precipitation on the germination, survival, and competition of annual desert plants have been continued by analysis of sample plots with rain gages in Death Valley National Monument and in Joshua Tree National Monument. These are being coordinated with work by other investigators in the Earhart Laboratory and a report for publication is being prepared on the first three years of these investigations.

Experiments on the Ecology of Desert Plants

Investigator: Soriano.

Support: Guggenheim Fellowship.

In January, 1951, some experiments on the effect of rain on the germination of these plants were initiated. Soil samples from Joshua Tree National Monument were used in these experiments, and different amounts of artificial rain in periods of different duration were given to them. For the most common annuals in the samples, it has been possible to determine that the amount of germination in relation to rainfall follows a curve with its optimum around 15 mm; when the same amount of rain was given in periods of different duration, the optimum of germination coincided with the longest rain.

The response to rain of the species which germinated in the soil samples has been further investigated, with the use of seeds. Among these species Amaranthus fimbriatus has shown some peculiar features. Accordingly, its requirements for germination as well as its response to early treatments are being investigated. The germination of this species is inhibited by exposure to continuous light, reaching 15% to 25% in continuous darkness, but is increased if before darkness a period of light is given. Both the duration of this exposure and the intensity of light influence the amount of germination.

In an experiment planned to find out the effect of rain on germination, on growth, and on development of Amaranthus fimbriatus,
it was found that not only does germination vary with different conditions of light and temperature during the rain period but also that the further growth and the rate of flowering of plants are influenced by these conditions.

The seeds of Amaranthus fimbriatus have shown a strong sensitivity to pretreatments of light and temperature and some remarkable effects of vernalization have been produced; the plants obtained differed in growth and rate of flowering.

An experiment has been made to ascertain the influence of wind on several plants. For tomato plants the plants kept in low wind showed a higher rate of transpiration, but neither the control nor the wind-treated plants showed significant changes of transpiration when taken from the quiet air into the wind, or vice-versa. In plants of Nicotiana glauca kept under a light intensity of 480 f. c. the transpiration jumped to much higher values when taken from the quiet air into the wind, but in the plants under 1000 f. c. the effect was exactly opposite. The only species which showed a real adaptation to wind condition was Larrea tridentata.

Carbohydrate Translocation in the Tomato

Investigators: H. Hull, Went.

Support: General Funds.

During the past year investigations on carbohydrate translocation in the tomato plant were continued, with particular reference to temperature effect. The majority of experiments previously reported which deal with translocation of carbohydrates in the higher plant have indicated a $Q_{10}$ of more than one, that is, a greater transport at higher temperature. However, it has been found, in the tomato plant and more recently in sugar beets, that under certain conditions a mechanism of translocation may be operative which acts almost independently of temperature, or even shows a $Q_{10}$ of less than unity.

In addition to different $Q_{10}$'s for carbohydrate translocation, vastly different rates of movement have been found, ranging from less than 15 minutes to almost 48 hours, for transport to take place from one leaf to the roots, to the growing point, or to other leaves. These experiments have been accomplished by utilizing radioactive tracers and a special bleeding technique. By the latter method,
two tomato leaves are submerged in a solution of the carbohydrate under investigation. There is an apparent transport through the phloem from leaves to roots, which results in increased metabolic activity of the latter. This increased activity causes a greater rate of bleeding from the tomato stem, which has been decapitated just above the two leaves that supplied the carbohydrate. The time lag between application of carbohydrate and bleeding increment over a control is considerably less when the stem is cooled to about 20°C, seemingly indicating a more rapid transport between leaf and roots at this low temperature. On the basis of these observations it has been suggested that in addition to a mass-flow type of movement in the sieve tubes there may simultaneously be present an extremely rapid translocation. This secondary type of transport may well be a molecular movement, mediated through some type of interfacial or particle adsorption phenomenon, and as such be highly dependent upon the physical and chemical nature of the substance being transported, as well as the media along which or through which it passes.

__Plantlet Development in Bryophyllum__

**Investigator:** Liverman.

**Support:** Lucy Mason Clark Fellowship.

It was shown some years ago that plantlets formed on the leaves of *Bryophyllum daigremontianum* under the influence of long day. During the past year experiments have confirmed this observation and have further shown that for this phenomenon to occur a daylight period of approximately 12 hours is needed. Optimal development occurs if eight hours of this period are high intensity light; however, light of 250 foot candles also permits somewhat slower development. A 16-hour day causes faster development but additional length appears to have little effect. Other experiments have shown that the fastest development in a constant temperature occurs at 20°C.

Incidental to the study of plantlet formation it was observed that temperature alone exerts a very pronounced influence on the general growth and leaf shape of both *B. daigremontianum* and *B. calycinum*. The optimum temperature both for general growth and for leaf development appears to be at a night temperature of about 20°C.
A spectacular phenomenon was observed to occur in B. daigremontianum at 10°C with either 16 or 24 hours of light. Chlorophyll failed to develop in all newly formed leaves and after the leaves produced at this temperature had expanded it was noted that an apparently unorganized mass of callus-like cells formed atop the midribs of the upper leaves. This proliferated tissue grew much faster than the remainder of the plant and eventually plantlets were formed around its margin, whereas plantlets never developed along the margins of the leaves. Plantlet development in this proliferated tissue apparently is quite similar to that found by workers from other laboratories who have recently reported that culture of crown-gall-induced callus tissue of Nicotiana and of Bryophyllum has resulted in the formation of plantlets. Thus in both instances apparently unorganized tissue has become capable of producing an organized plant body.

**Growth and Development of the Squash Plant**

**Investigator:** Nitsch.

**Support:** General Funds.

During the early months of this academic year Nitsch completed his work for a Ph.D. degree, studying fruit growth in vitro and the effects of temperature and photoperiod on sex in squash plants. At high temperatures most developing flowers were found to be male, whereas low temperatures favored the development of female flowers. Almost all ovaries tried by Nitsch developed into fruits when placed steriley on nutrient solutions.

**Plant Physiology and Biochemistry--Kerckhoff, Dolk and Orlando Laboratories**

**Staff:** Professor: Bonner; Associate Professor: Galston; Senior Research Fellows: Axelrod, Singer, Wildman (Associate Professor of Botany, U.C.L.A.); Research Fellows: Atkinson, Bandurski, Camus, Henderson, Lang, Laties, Stehsel; Graduate Students: Boroughs, Eggman, Goldacre, Jansen, Liverman, McRae, Saltman, Siegel; Research Assistants: Baker, Campbell, Durrell, McColloch, Millerd; Superintendent: C. Newman.
Catalase in Higher Plants

Investigator: Galston (work reported largely done while Galston was Guggenheim Fellow at Stockholm and Sheffield).

Support: Guggenheim Fellowship.

Reproductive activity in many plants is controlled by the relative length of day and night to which they are subjected. In such photoperiodically sensitive plants it is possible at will to stimulate or inhibit the production of flowers and fruits by the simple expedient of lengthening or shortening the daily light period. Despite the great practical and theoretical importance of this phenomenon, we know practically nothing of the internal mechanism by means of which light alters the growth habit of the plant. Work in plant physiology during the past year has centered about an attempt to understand this phenomenon.

Previous work had shown that red light is most effective in producing the photoperiodic effect. The complete "action spectrum," when plotted, indicates that the effective absorbing pigment is some non-chlorophyll compound with a tetapyrrole structure. One such compound known to exist in plants is the enzyme catalase. Because this enzyme has never been prepared in pure form from plants, its isolation was undertaken. Chemical fractionation of about 200 pounds of spinach leaves yielded plant catalase preparations of at least 50% purity. The chemical characteristics of this material were then determined.

The physiology of catalase in plants was also investigated by the use of plant tissue cultures. It is known from work with animals that the catalase content of malignant cells is much lower than that of normal cells. This same relation has been found to exist in plants. It has also been found that application of growth hormones to plant tissues results in a marked decrease in catalase synthesis which parallels the increase in growth. This indicates a possible relation between catalase and growth which has not before been appreciated.

Another research program has involved the mechanism of potassium uptake by cells. Results obtained with the aid of radioisotopes show that the enzyme carbonic anhydrase is involved in this process in certain kinds of tissues. This knowledge may prove important in understanding the general process of salt absorption.
Aerobic Respiratory Metabolism of Higher Plants

Investigator: Laties.

Support: Rockefeller Foundation Fund, Frasch Foundation.

Until relatively recently the study of the aerobic respiratory metabolism of the higher plants has been limited to physiological experiments of an indirect nature. Such experiments suggested the presence in higher plants of a coordinated complex of oxidative enzymes, operating serially and having as one of its functions the complete oxidation of pyruvic acid. This series of oxidations, presently known as the Krebs cycle or the tricarboxylic acid cycle, has been investigated by several groups of animal biochemists, and the component reactions have been unequivocally demonstrated in vitro. Attempts to isolate some of the component enzymes of the tricarboxylic acid cycle in the higher plants have by and large been unsuccessful, and the consequent lack of direct information relative to the aerobic metabolism of the higher plants has seriously impeded the pursuit of other related plant physiological problems.

An outstanding development in respiratory biochemistry in recent years has been the realization that the entire complex of enzymes previously mentioned is associated with the particulate components of the cytoplasm - the mitochondria. The activities of the mitochondrial enzymes are coordinated physically (by the spatial relationship of the enzymes within the mitochondrion) as well as chemically. It has been shown, with preparations from animal tissues, that the proper functioning of the complex of enzymes associated with the mitochondria is dependent upon the integrity of the latter. The mitochondria represent osmotic systems, and their integrity can be destroyed in many ways, one of which may be subjection of the particles to an unfavorable osmotic environment.

The preparation of enzymes from plant materials is complicated by the fact that components of the cell normally separated from the cytoplasm in vivo (i.e. components contained in the vacuoles) are brought into contact with the cytoplasm when the tissue is macerated or homogenized. In view of the work carried out during the last year it may be said that such intermixing probably has much to do with the difficulties encountered in demonstrating certain enzymes in crude plant preparations. For example, the succinoxidase system (succinic dehydrogenase-intermediate carriers-cytochrome oxidase) of cauliflower buds
was investigated and was found to be associated with insoluble cytoplasmic particles, much as in animal tissues. Cauliflower buds, upon grinding, release into solution a strong competitive inhibitor of the succinic dehydrogenase enzyme. Thus, in crude preparations this enzyme appears to be lacking, whereas, when the particles are separated from the juice by centrifugation, appreciable activity may be shown.

A more serious difficulty, however, is the presence in the juice of yet another substance which causes irreversible destruction of the mitochondria. Whether or not this inactivation takes place appears to depend upon the osmotic state of the particles. Thus, when particles are made in strongly hypertonic solution (0.5 M sucrose) their activity is considerable. This inactivation has a large temperature coefficient, and thus is not purely an osmotic phenomenon. An aspect of this inactivation particularly pertinent to certain types of plant physiological studies is that inactivation may be effected, in vivo, simply by bathing the tissue pieces in hypotonic solution. Although hypertonicity exerts a protective influence for many hours in the cold, protection is shorter lived at room temperature.

When particles are first prepared in sucrose and then, after separation from the juice, transferred to distilled water or weak salt solution, inactivation does not occur, although the endogenous cytochrome C, an intermediate electron carrier, now becomes non-functional, and additional cytochrome C must be added.

When particles are properly prepared in hypertonic solution, they may be shown to carry out all the steps of the tricarboxylic acid cycle in vitro. Furthermore, such particles have no requirement for additional cytochrome C. It has thus become evident during the past year that the intermediate metabolism of the higher plants may in fact be quite similar to that found in animal tissues and that with proper precautions observed in the preparation of the respiratory enzymes plant metabolism may be studied more directly than heretofore.

Vernalization and Photoperiodism

Investigator: Lang

Support: Lalor Foundation Fellowship

Certain plants require an exposure to low temperatures before they become able to flower and produce fruit and seed.
Numerous plants flower and fruit only under a definite range of length of day - some only in long days, others only in short days. The study of these phenomena is of considerable interest in two respects: (1) It will help us to obtain an insight into the conditions which make plants flower; (2) it will help us to understand the general problem of how the growth of plants is controlled by climate. Both problems have important scientific and practical aspects. The onset of flowering means a dramatic change in the development of the plant, which is of general biological significance. The effect of the climatic environment on plant growth is of great importance for agronomy and plant breeding.

Most of Lang's studies were done using henbane, Hyoscyamus niger, for material, for this plant is sensitive both to low temperature and to day length, and permits the study of the two responses in the same material. In the work on vernalization the main problem studied was the exact temperature relation of the process. The following results have been obtained:

(1) The cold action has a quantitative effect, increasing with time until an optimum level is reached. This permits some general conclusions about the relation of the low temperature to flower formation. In the case of photoperiodism it was previously found (Lang and Melchers, 1943) that the effect is of an all-or-none type; flower formation is initiated once a definite number of suitable photoperiods has been given; an increase beyond this number has no additional effect. Flower formation is itself an all-or-none event; a flower either is formed or is not; half a flower cannot be formed. Therefore such a relationship is to be expected if the external factor has a rather direct effect on the process. Since in the case of cold action the relation is different, it must be concluded that the low temperature does not cause flower formation directly but that it controls some preparatory step, for example, the formation of an enzyme which in its turn controls the production of some substance determining flower formation.

(2) As long as the optimum effect has not yet been reached, the effect of different temperatures is different. The optimum, however, seems to be identical for all temperatures within the effective range. This again permits some conclusions to be drawn on the general mechanism of vernalization. During the low temperature treatment something is apparently accumulated in the plant and finally reaches the optimum level in any temperature which permits the accumulation at all. This is in agreement with an interpretation of vernalization suggested by Lang and
Melchers (1947) which can be formulated in the following way:

A → B → C → flowering

A substance (C) which is necessary for flowering to take place is formed by way of an intermediary (B); B, however, can be metabolically diverted along a different pathway (to D) and thus become unavailable for the reactions leading to flowering. The temperature quotients and/or maxima of the reactions are different in such a way that in high temperatures all of B goes to D whereas in low temperatures at least part of it can go to C. In this scheme, too, the material essential for flowering (C - which may be identical with the enzyme suggested above) will ultimately reach the optimal level in any temperature which will permit its formation.

(3) Whereas the effect of any temperature effective in vernalization increases with time, the temperature optimum shifts in the course of the treatment from higher to lower temperatures. The balance of the processes which cause the cold requirement of the plants is not fixed but changes in the course of the processes themselves.

It is thought that these findings help to form a general idea of the physiological bases of vernalization, an understanding of which is necessary before an attempt can be made to separate the individual processes involved. Some attempts to achieve such a separation have been made, by use of specific metabolic inhibitors, but this work is still under way, and it would be premature to state definite results.

In the field of photoperiodism, the response of several new long-day requiring plants (Crepis capillaris, Silene armeria, Arabis arenosa) have been studied. This work is carried out in cooperation with Liverman. Its purpose is to establish the detailed response of these plants as a necessary preliminary for studies on the mechanism of photoperiodism. So far, our knowledge of long-day plants is based almost solely on studies with henbane, and it is desirable to test the validity of the results with other species. As far as is now known, the behavior is basically the same.
Carbohydrate Metabolism in Plants

Investigators: Bandurski, Axelrod, Saltman.

Support: Frasch Foundation, United States Department of Agriculture.

Animals achieve the oxidative degradation of carbohydrate by a chain of reactions involving a number of enzymes and intermediates, most of which are phosphorylated. It has been assumed that the same glycolytic process is operative in higher plants and although many points of coincidence have been discovered two steps have remained undetected. It has now been shown that both these steps do occur in plants, the enzymes for each having been demonstrated. The first enzyme, phospho-fructokinase, which makes fructose 1, 6-diphosphate and adenosine diphosphate, has been partially purified and characterized in some detail. The second enzyme gives rise to adenosine triphosphate by phosphorylation of adenosine diphosphate by diphosphoglyceric acid.

The existence of these two enzymes was adumbrated by the employment of a chromatographic radioisotope procedure, devised here. This new procedure has made possible the separation and identification of the phosphate esters involved in glycolysis.

It is now reasonably certain that in pea seeds the conventional scheme of sugar degradation is operative. Nevertheless, some unknown phosphate compounds have been detected, and this suggests the possibility of alternative pathways which are as yet unknown.

In connection with the previously demonstrated ability of plant phosphatase to transfer phosphate from nitrophenyl phosphate to alkyl hydroxy compounds, the "apparent" free energy of hydrolysis of p-nitrophenyl phosphate was determined. This value, moderately high at high pH values, leveled out to only slightly higher than that shown by the "low energy" alkyl phosphates at pH values of about pH 7 and lower.

In view of the fact that leaves of many plants are known to continue many of their metabolic activities long after they have been detached from the plant, although they simultaneously undergo a considerable loss of protein, it was interesting to ascertain the fate of some leaf enzymes, which are also protein, during this period. In conjunction with A. Jagendorf it was shown that tobacco leaves stored in the dark for periods up to seven days, while losing
45% of their cytoplasmic protein, lost practically none of their invertase, phosphatase and peroxidase.

**Higher Plant Protein Metabolism**

Investigator: Boroughs.

Support: General Funds, Frasch Foundation, National Foundation for Infantile Paralysis.

The properties of green leaf proteins have been investigated in this laboratory for several years. It has been found that a large part of the cytoplasmic proteins is a single component electrophoretically and that this Fraction I, as it is called, has phosphatase but no other enzymatic. In an attempt to purify this protein, various aspects of its enzymatic nature have been studied. Fraction I phosphatase from tobacco leaves was shown to have a pH optimum of about 5.5 and a Michaelis constant of $1.9 \times 10^{-3}$ M. with disodium p-nitrophenylphosphate as substrate. The enzyme attacks many phosphates, including ATP. The temperature optimum is about 47°. Neither manganese nor magnesium was found to be an activator, but fluoride and zinc were completely inhibitory.

Studies of this protein in the ultracentrifuge with the use of a separation cell, a device which reduces the mixing of components after separation, indicated that the phosphatase is not necessarily an integral part of the molecule. It has not yet been possible to prove this rigorously, since all treatments for the separation of phosphatase from Fraction I result in appreciable denaturation of Fraction I. Thus there is a dissimilar response of Fraction I and the phosphatase to heat and acid treatment. The former is extremely labile even at room temperature, while the enzyme is purified by heating up to 60°, a treatment which precipitates up to 90% of the total proteins and leaves 90% of the enzyme activity in the supernatant. Electrophoretic analysis had to be carried out near pH 7, owing to denaturation in more acid regions, yet the phosphatase remaining from the heat treatment was further purified by bringing it to pH 4 or even 3.

Finally, of the total phosphatase present in the cytoplasm only about 13% is found associated with Fraction I as prepared by ammonium sulfate precipitation, while 61% was recovered from the remaining fraction.
Further studies on the relation of auxin to the cytoplasmic proteins showed that the bound auxin was of approximately equal magnitude in Fraction I and the remaining fraction. This work indicates that the role of Fraction I is still obscure, and is not clearly associated either with the phosphatase or with auxin.

Wildman, Cheo, and Bonner have pointed out evidence for the formation of tobacco mosaic virus at the expense of Fraction I. It was known that no methionine exists in TMV, and it was found that Fraction I has about 2%. This suggests that the pathway of TMV synthesis might be followed by studying the relation of methionine and TMV in an infected plant.

The second major problem investigated during the year involved the role of auxin in cell elongation with respect to protein metabolism. Corn and Avena coleoptile sections were floated on sucrose solutions with amino acids labeled with Cl4 in the carboxyl group. The elongation and the wet and dry weights were measured, and the tissue homogenized. The total proteins were precipitated and thoroughly washed with trichloracetic acid, and then hydrolyzed. An aliquot was removed to determine amino nitrogen and the remainder decarboxylated. It was found that the Cl4 which had been incorporated into the proteins using glycine and leucine increased with time, but this incorporation was not affected by auxin. There was also little or no change in the total protein during the 24-hour period. These results indicate that, although amino acids can be incorporated into tissue proteins during cell elongation and can increase in dry weight, auxin is not directly concerned with protein metabolism.

**Cytoplasmic Proteins of Higher Plants**

**Investigator:** Eggman.

**Support:** General Funds, National Foundation for Infantile Paralysis.

The work of the past year has been concerned with a study of some of the proteins found in green leaves; more specifically, with a particular fraction of the soluble cytoplasmic proteins which contains phosphorus more or less firmly bound to a high molecular weight protein moiety.

The attack on the problem involved perfection of analytical techniques and extraction procedures; a study of the stability of cytoplasmic proteins to freezing, heat and dialysis; and the
physical and chemical characterization of cytoplasmic proteins.

Modifications were made in standard procedures for determination of nitrogen, so that the Kjeldahl digestion method was combined with Nesselerization for the final determination of ammonia. Methods for the determination of phosphorus in semi-micro or micro quantities were also adapted to the material.

Lability studies have shown that the cytoplasmic proteins as a whole are stable only in the range from pH 6.75 to 8.5 and when kept as near 0°C as possible. Under mildly acid conditions (pH 5.5 - 6.3) the cytoplasmic proteins as a whole are precipitated, but a phosphorus-containing protein is precipitated much more rapidly than other proteins. This decrease in protein-bound phosphorus is correlated with a corresponding decrease in one of the components seen in ultracentrifuge scanning patterns of the preparations. Warming to moderate temperatures for short periods of time causes release of most of the protein-bound phosphorus without a concomitant precipitation or degradation of the protein.

From analytical ultracentrifuge studies of the P-containing protein preparations it has been found that this protein fraction is apparently homogeneous, that the molecules are approximately spherical in shape, and that the molecular weight is probably in the range 540,000 to 610,000.

The protein-bound phosphorus content of the fraction prepared by preparative ultracentrifugation ranges from 0.2 to 0.6 per cent. The purity of the preparations was not verified by analytical ultracentrifugation in each instance, but probably this variation in bound phosphorus is due to variations in the method of preparation.

The nature of the phosphorus bound to the protein has been investigated. Semi-quantitative experiments indicate that in preparations made by preparative ultracentrifugation the phosphorus can be accounted for in the components of ribonucleic acid which may be recovered from hydrolysates by chromatography, and that the increase in nucleic acid recovered from such preparations as compared to similar hydrolysates from whole cytoplasm parallels the increase in protein-bound phosphorus. Thus it appears that the labile, phosphorus-containing protein is a nucleoprotein. Further quantitative work to identify it with a particular component in the ultracentrifuge scanning patterns is still in progress.
Quantitative studies in the analytical ultracentrifuge indicate that the component which is thought to be a nucleoprotein comprises from 25 to 50% of the total soluble proteins. Since the soluble proteins constitute about 10% of the dry weight of the cell, including the cellulose material, this nucleoprotein constitutes 3% to 5% of the dry weight of the cell.

The Destruction of Indoleacetic Acid in Plant Tissues.

Investigator: Goldacre.

Support: General Funds.

An enzyme system, IAA oxidase, which oxidizes and destroys the biological activity of the plant growth hormone indole-3-acetic acid (IAA) has been studied and characterized, and a good deal is now known about the factors that influence its activity. One of its most interesting properties is that its activity is increased when irradiated with blue (or white) light. This has led to the belief that this system is important in the growth changes observed in plants in response to light. Accompanying the enzyme system in plant tissues is another factor, non-protein and of dialyzable dimensions, which catalyzes the photolytic destruction of IAA. Its activity, in vitro, amounts to 60% to 70% of the IAA-destroying power of whole tissue extract in the light. In the dark it has no activity, thus showing a greater differential activity owing to light than does the enzyme system. It does not appear to be a coenzyme for the IAA oxidase system. The identity of the active compound is not yet known, but the kinetics of its action and many of its properties have been studied. When added to IAA solutions in which pea stem growth rates are measured, it specifically inhibits growth in the light but not in the dark. This effect is not obtained when IAA is replaced by 2,4-D. Thus the effect is due to the photoinactivation of IAA. Other experiments indicate that it is probably active in situ in pea stems.

Fruit Growth

Investigator: Jansen.

Support: General Funds.

During the past year work on the study of fruit growth has centered mainly around the control and analysis of variables and
refinement of techniques involved in the in vitro growth of excised flowers of the red currant tomato. One of the biggest stumbling blocks, the selection of uniform research material, was brought under control with the installation of the smog filtering system in the Earhart Laboratory. Since this change, the amount of flower drop in tagged-flower experiments on the plants has been decreased from 22% to 10% and the failure of flowers to develop from 12.5% to 5%. Although there has been no change (no effect of smog) in the variability of sizes of fruits developed on the plant, the implication of pollination can be shown by the correlation between fruit diameters and number of developing seeds. One group of tagged flowers measured on the 14th day after full opening of the flowers, when the developing fruits had reached about 0.7 of their final size, gave a correlation coefficient of 0.89, which is highly significant. Calculations from the corresponding regression equation give the theoretical diameter of 1.75 mm for a 14-day ovary with no developing seeds. The initial diameter of the ovaries averages 1.07 mm.

The collection of flowers for culture from the smog-free plants has resulted in a far more significant development in cultures. In most of the recent experiments 80% to 100% of flowers develop as compared with 15% to 30% in previous determinations. Growth on the basal (mineral salts plus sucrose) medium has been increased 67% over the previous values obtained on the same medium. The average diameter of 14-day cultured ovaries has been found to be 3.14 mm, almost double the value for natural parthenocarpic development as it might be expected to occur on the plant. The present uniform growth on basal medium is the maximum yet achieved under culture conditions. None of the variously supplemented media (containing B-vitamins, amino acids, purines, 5- and 6-carbon sugars, and leaf extract fractions) has yielded any significant increase in growth over the sucrose-salt values nor supported the development of ovules into seeds. The optimal sucrose concentration has been shown to be 4% and the best temperature of incubation yet tried is 26°C constant. The pH of the medium has no effect over the range 4.0 - 6.0. The present technique of collecting fully opened flowers, sterilizing, and permitting corolla dehiscence (after one day in most cases) to occur in culture has resulted in fruits having a 34-day mean diameter of 4.15 mm. Pigmentation commences in 34 to 35 days both on the plant and in culture.

Unsuccessful attempts have been made to culture flowers and fruits of various other species. Those tried have been Malvaviscus,
lupine, mung bean, citrus, olive, and avocado. In most instances at least part of the lack of success has been due to ineffective sterilization and severe contamination. Equally unsuccessful have been trials to grow floral primordia of Xanthium, red currant tomato, and mung bean. Flower buds of the tomato have been grown through the stages of opening (anthesis), corolla dehiscence, and some fruit development, but the youngest which has so far yielded to treatment has been the third or fourth pre-anthesis bud and this again on just the basal medium.

**Plant Fats and Waxes**

**Investigator:** Kurtz.

**Support:** National Research Council Fellowship, General Funds.

The lipids, comprising fats, waxes, and phospholipids, which occur in plants and animals are well understood chemically, but relatively little is known of the function, synthesis, or metabolism of these compounds in living organisms. The work summarized here involves a study of the environmental and genetic effects on higher plant lipids and the mechanism of fat biosynthesis.

To study the effects of environment, two species of tobacco, corn, and the common desert shrub creosote bush were grown under a wide range of climatic conditions in the Earhart Laboratory. Extraction and analysis of the lipids of these plants have shown what the effect of day and night temperature, water stress, and photoperiod are on lipids, particularly the fats and waxes. Leaf fats, which occur as vital components of the cytoplasm, do not show very great changes in regard to temperature, but the trend is generally toward less amounts at higher temperatures. The type of fat, however, is rather greatly affected by temperature changes. Plants also respond similarly to water stress by producing more fat of a different kind. Waxes, which occur in the outer layer of the leaves, the cuticle, also are affected by environment. Just as was the case for the fats, high temperatures and high available soil moisture favor reduced amounts of cuticular wax.

A study of the genetic control of wax formation has shown that this is a much more potent factor in changing the amount and kind of wax than is the environment. In a series of corn mutants segregating in the seedling stage for glossiness of the leaves it has been found that glossiness is associated with the amount of wax.
in the cuticle of the seedling leaves. The amount of wax in the leaf of each type of glossy corn follows rather closely the degree of glossiness ascribed to it by external appearance and wetability of the leaves.

In addition to the genetic control of the yield of wax in glossy corn, analysis of the waxes shows that the kind of wax is also altered by the genes controlling glossiness. The problem of how the plant synthesizes lipids has been attacked by means of the fruit culture technique. If flax fruits, which when mature contain a large amount of fat, are grown in solutions containing various organic substrates, such as sucrose, acetate, glycerol, etc., the fruits may continue to grow and produce fat, the amount of fat depending upon how readily the fruits can utilize in fat synthesis the substrate fed to them. Although the results are as yet preliminary, it appears that acetate, when fed along with minute amounts of certain growth factors, is utilized by developing flax fruits to synthesize fat.

**Flowering in Xanthium**

**Investigator:** Liverman.

**Support:** Lucy Mason Clark Fellowship.

Experiments have shown that the first partial process of flowering in xanthium is directly concerned with photosynthesis since the high light intensity can be replaced by a number of respiratory substrates. From data available it may be concluded that the second partial process of flowering is concerned in some way with auxin since its effectiveness is inhibited by applied auxin and the otherwise inhibitory nature of the light flash can be reversed by applied auxin antagonists.

**Vitamins in Higher Plants**

**Investigator:** McRae.

**Support:** General Funds.

Plant vitamins act as hormones in that they are synthesized in certain parts of the plant and transported to particular sites of utilization. For example, in tomato plants, thiamine is synthesized in the leaves and is transported to the roots where it is required for root growth. Entire plants normally make all the vitamins
necessary for growth. However, suppose a plant were deficient in the synthesis of a vitamin or any other endogenously synthesized substances. How would one recognize the deficiency and what would be the symptoms?

One approach to this problem is through the use of competitive analog-metabolite antagonists. Many organic compounds have been found which inhibit growth of bacteria, fungi, and such animals as mice and rats. In the main, these organic compounds are vitamin, amino acid, purine and pyrimidine analogs. In many instances the analogs competitively inhibit the utilization of the corresponding vitamin, amino acid, purine, or pyrimidine by the organism. For instance, it has been shown that benzimidazole inhibits the growth of the bacteria, Escherichia coli. The inhibition can be reversed by adenine.

In an experiment in which tomato seedlings, grown under aseptic conditions, were treated with benzimidazole, it was found that benzimidazole, at a concentration of 10 mg per plant, almost completely inhibited growth of the tomato plants. There was slight inhibition of roots and stems at a concentration of 1 mg per plant. Many competitive analog-metabolite antagonists cause an acceleration of growth at concentrations just lower than inhibitory concentrations. It is also known that adenine is a leaf growth factor. Therefore, it was not surprising to see the reflection of these facts in the increased dry weight of leaves at a concentration of 1 mg per plant. Lower concentrations of benzimidazole had no apparent influence on growth.

An entirely different situation occurred when tomato seedlings were treated with pyridine-3-sulfonic acid, a nicotinic acid antagonist. At a concentration of 0.1 mg per plant, there was a dry weight increase of approximately 70%. At higher concentrations no observable inhibition of growth occurred.

Similar experiments are being carried out with isolated tomato and flax roots. In such work, it is desirable to use root clones. Heretofore, flax root clones have not been obtained by normal culture techniques because excised secondary roots will not grow. A culturing technique has been found which results in the growth of many, but not all, secondary roots. Experiments are in progress to attempt to find the growth requirements of flax secondary roots.
Further Studies of Aerobic Respiratory Metabolism in Higher Plants

Investigator: Millerd.

Support: Frasch Foundation.

The participation of a tricarboxylic acid cycle, similar to that of animal tissues, in the aerobic metabolism of plants would imply the participation of succinic dehydrogenase, an enzyme which in animal tissues is coupled with the cytochrome system, the whole forming the complex known as succinoxidase. Investigations carried out with potato (Solanum tuberosum, L.) tuber have shown that this tissue contains an active succinoxidase system, that the system is associated with particulate components of the cytoplasm and that succinic dehydrogenase is coupled to the cytochrome system in the plant as in the succinoxidase of animal tissues. Also associated with these particles are the enzymes, fumarase and malic dehydrogenase.

These investigations have been continued with seedlings of Phaseolus aureus Roxb., and cytoplasmic particles which resemble and may be identical with the mitochondria have been isolated. These particles have associated with them the constituent enzymes of the Krebs cycle, and in the presence of small amounts of one of the acids of the cycle are capable of oxidation of pyruvic acid.

Viruses of Higher Plants

(Work in this field has been carried on by staff members of the Plant Physiology and Plant Biochemistry Group.)

During the year 1950-1951 material progress has been made in the study of plant viruses by electrophoretic and ultracentrifugal methods. Earlier reports have described the general utility of these methods in two kinds of investigations, namely, for the detection of virus components in living tissue and for the study of the changes which occur in normal tissues as they are invaded and attacked by viruses. It has been shown that virus protein may be detected in plant tissue by the above-mentioned physical methods even before symptoms of the virus disease itself become apparent. This approach has been extended and it has been found that the peach mosaic virus may be detected at an early stage by a combined electrophoresis and ultracentrifuge method. A
combination of physical and chemical methods has been applied
to the study of curly top viruses. This virus, which had previously
resisted all attempts at isolation, has been prepared in what appears
to be a crystalline highly infective condition. These same methods
have also been applied to the detection of one virus in the presence
of a second virus, and in the case of tobacco mosaic virus (TMV)
two strains have been quantitatively separated from one another -
from plant tissue carrying a double infection. Finally, the electrophoretic method has been applied to a plant disease, crown gall,
which has not heretofore been recognized as a virus disease, and
it has been shown that in all probability this disease is due to a
virus which is in some way transmitted from plant to plant by a
bacterium.

Studies of virus reproduction detailed in earlier reports
have shown that the multiplication of TMV in the leaf is associated
with a corresponding decrease of a particular component of the
normal leaf cytoplasmic protein. This component has been further
studied and shown to behave as a typical ribonucleoprotein. This
protein is a remarkably labile one, and rapidly loses its nucleic
acid under mildly acid conditions, a fact which accounts for the
failure of earlier workers to detect its presence in either healthy
or virus infected leaves. Although this protein disappears during
TMV multiplication in the leaf, the nature of the relationship is
still unclear.

Studies of nucleic acid and nucleoprotein changes in virus-
infected bacteria have shown that as in leaves particular components
of the normal bacterium disappear as virus reproduction proceeds.
The component principally concerned appears to be bacterial
desoxyribonucleic acid (DNA), which has also been prepared in
essentially pure form.

Possibility of Virus Associated with Crown Gall

Investigators: Camus, Wildman.

Support: American Cancer Society.

The plant disease commonly referred to as crown gall
has been studied since the beginning of the twentieth century.
Its etiology was discovered in 1907 by E. Smith, who found that
a bacterium, Phytoponas tumefaciens, is responsible for the
appearance of galls on the stem, petiole and crown of many
plants. Although early investigators had already pointed out
analogies between animal cancer and crown gall (in some plants, secondary tumors or metastases that are very frequently bacteria free appear at a great distance from the point of inoculation), more definite proofs of the cancerous nature of the latter were obtained only in recent years. Through investigations carried out with the plant tissue culture techniques, it was found that:

1) Crown gall tissue, after elimination of the inducing bacteria, is able to grow indefinitely on very simple synthetic media, whereas normal tissue (callus induced by indole-acetic acid) has more elaborate nutritional requirements. Similar behavior is well known for animal cancer cells.

2) Crown gall tissue, over long periods of culture in vitro, retains its power of disorganized proliferation. When grafted on to a healthy plant, it gives rise to a voluminous tumor which escapes completely the correlation mechanism of the host. On the contrary, normal tissue used as a scion does not proliferate at all.

3) When grafted back on a plant, crown gall tissue not only develops into a tumor, but also induces the appearance at a distance of new cancers. This is not so in the case of normal callus.

The fundamental question then arises: What is the tumor-inducing factor produced by the Phytomonas, a factor maintained in the tissue even after long periods of cultivation in vitro and transmissible through a graft union? Among the several hypotheses proposed to explain the nature of this tumefaction principle, that of a virus carried, elaborated, or originated in the host tissue by the bacteria appears to be the more likely. So far no proof has been obtained.

Electrophoretic analysis, which was successfully used in this laboratory to detect the presence of virus in plant materials, seemed a logical approach to this problem, and comparison of the electrophoretic scanning patterns of the protein components of crown gall and normal tissues was undertaken.

The technique was the same in every instance. The tissues were ground in a colloid mill with a neutral buffer, and cell walls and unbroken cells removed by centrifugation in a basket centrifuge lined with filter paper. The whole protoplasm obtained was then centrifuged at low speed to throw down the particulate matter as a pellet. The supernatant (whole cytoplasm) was concentrated,
dialyzed against a buffer of low ionic strength and examined in the Tiselius electrophoresis apparatus. The study was carried out on both whole plants and tissues cultivated in vitro.

Normal stems of the sunflower plant showed very important differences in their protein patterns from stems bearing numerous well developed primary galls. Not only do the contaminated stems exhibit one more component than the normal ones, but there is also a complete redistribution of the electrophoretic mobilities of the individual components. Studies are now under way to discriminate between the modifications owing to the fact that the stem tissues undergo active proliferation and the specific alterations owing to the tumor tissue itself.

Extensive propagation of the tissue culture strains which had been isolated in the spring of 1950 was required in order to obtain sufficient material for the analyses. So far, more than 10,000 cultures from normal callus and crown gall of sunflower and Jerusalem artichokes, carried out in exactly the same conditions, have been utilized.

Many technical difficulties encountered during the analyses have been overcome to a certain extent. Although it has not yet been possible to obtain good scanning patterns from the cytoplasm of the artichoke strains, the results obtained with sunflower proved to be most interesting. It was possible to ascertain in this material clear cut differences between the protein constituents of the normal and tumor tissues. Whereas the normal callus pattern shows only three components, the crown gall tissue possesses, besides the same three components, an extra one of high molecular weight which makes up about 20% of the total protein content of the tissue. The patterns obtained are highly reproducible and it is believed that the extra component found in crown gall represents the tumefacient factor. The fact that some protein denaturation takes place during the preparation of the samples does not invalidate this conclusion since all the samples are prepared in the same manner and the percentage of denatured material is exactly the same for normal callus and crown gall.

Attempts have been made to concentrate this extra component of the crown gall by ultracentrifugation and partial success has been obtained. The conditions utilized so far (100,000 g for 90 min.) proved, however, to be too drastic, and careful timing studies under various ultracentrifugal forces are under way in view of its isolation in pure form.
Detection of Peach Mosaic Virus by Physical Methods

Investigators: Camus, Wildman.


The detection of several viruses has been successfully achieved by electrophoretical analysis. It was thought of interest to apply the same technique to the detection of another virus of great economic importance, the peach mosaic virus (PMV). The symptoms of PMV are characterized by a more or less accentuated mottling of leaves, flowers and fruits; the trees die within a few years after contamination. The virus is not transmissible by mechanical means, its insect vector, if any, is not known. The disease is usually carried by graft, and very little information has been obtained on it so far.

Leaves from healthy peach trees and from trees infected with the strain "San Saba severe" were ground and their soluble proteins extracted and analyzed in the Tiselius electrophoresis apparatus. It was found that samples from infected leaves presented an extra component of high molecular weight which could be removed easily by high speed centrifugation. The patterns obtained were highly reproducible and provide a good means of early detection of the disease. Similar analyses will be carried out with other strains of the same virus in the near future.

Isolation of Curly Top Virus

Investigators: Camus, Wildman.


The curly top virus (CT), which attacks particularly the sugar beet, is another virus of great economic importance, and much work has been devoted to the study of its properties. It is known to be transmitted only by insect or by graft and to be strictly localized in the phloem tissue. Several strains have been characterized and deeply studied from the morphological standpoint, but despite numerous attempts the virus has not yet been isolated.

In collaboration with Doctor C. W. Burnett of the University of California at Riverside, new attempts to isolate CT have been carried out. The blades of leaves from healthy and diseased (strain B) sugar beets were trimmed off, the petioles
ground in a colloid mill with a neutral buffer and the soluble protein extracted. Electrophoresis analysis of the whole cytoplasm failed to reveal any clear-cut difference between the virus-containing solutions and the healthy ones, indicating that the virus concentration in normal protein is probably extremely low.

The virus activity was tested by feeding the insect vectors small aliquots of the various extracts and by keeping the insects for two hours on healthy beets. The concentration of virus was assumed to be proportional to the number of beets coming down with the disease.

By high speed centrifugations (100,000 g for 1 hour) it was possible to concentrate all the virus activity in a pellet containing only 60% of the total protein. However, further centrifugation of the resuspended pellet gave no significant concentration. Analytical ultracentrifugation of solutions "recycled" three times proved to be very heterogeneous and identical to similar extracts from normal beets.

Another means of virus separation proved to be much more satisfactory. By a series of alcohol precipitations and medium speed centrifugations it was possible to obtain about 100 mgs of crystalline protein material from 12 pounds of leaves. The crystals, which were not obtained upon parallel treatment of healthy leaves, were lozenge shaped plates and present faint birefringence. They gave positive reaction to several protein tests, and, upon redissolution, yielded solutions which turned out to be infective. This work is now being repeated so that the exact conditions of crystallization and the chemical nature of the crystals can be ascertained.

Isolation of Strains of Tobacco Mosaic Virus by Electrophoresis

Investigators: Singer, Wildman.


In the course of a study of tobacco mosaic virus (TMV) synthesis, several components were observed during the electrophoresis preparations. Two of these components were isolated from the electrophoresis cell, and were shown to be physically and chemically similar, except for their electrophoretic properties, and to produce mosaic infections in tobacco plants. The symptoms, however, are markedly different, one strain producing mild, the
other severe, mosaic symptoms on Turkish tobacco. These studies were performed in collaboration with Doctor J. G. Bald of the University of California at Los Angeles. Owen has shown that the two strains are serologically related but distinguishable. A study of the comparative chemical properties of these strains should be of great interest in problems of the chemical nature of mutations, and of altered symptom patterns in mosaic infections, as well as in the study of the immunochemical specificity of antigens and antibodies.

Further Studies of the Proteins of Healthy Leaves

Investigators: Singer, Campbell.


It had previously been discovered that a variety of green leaf whole cytoplasmic extracts contain a particular protein (Fraction I) constituting from 25% to 50% of the total protein in the extract. Further progress has been made in the investigation of the preparation and properties of this protein. The analytical ultracentrifuge has proved itself indispensable in these studies, providing the only means of analyzing various preparations for their Fraction I content and studying the size and shape of the Fraction I molecules. By high-speed quantity centrifugation in the Spinco centrifuge, Fraction I can be separated from the whole cytoplasm extracts in as pure and least altered condition as it has so far been possible to obtain.

Preparation and Properties of the Soluble High Molecular Weight Components of Normal and Bacteriophage-Infected Bacteria

Investigators: Singer, Siegel.


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about by the infection. These preliminary studies have indicated that the free desoxypentose nucleic acid (DNA) of the bacteria becomes bound up, after the bacteriophage infection has occurred, with as yet unknown matter, probably protein, to form some of the precursor substances for the synthesis of the active bacteriophage. Further studies along these lines are strongly suggested on the basis of these results, and it would appear that this type of investigation is fundamental to the eventual understanding of virus synthesis.

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It has been shown that T₂ bacteriophage can be disrupted in the colloid mill, resulting in the release of various bacteriophage components. As yet the only one which we have examined is DNA. By the simple method of high-speed quantity centrifugation of the suspension of ground bacteriophage in the Spinco centrifuge, one can prepare supernatant solutions containing essentially pure DNA in good yield and in considerable quantity. This DNA has been shown to be ultracentrifugally remarkably similar to DNA's prepared from entirely unrelated sources. Subsequent studies of this system should be of considerable significance in problems of the structure of bacteriophage and animal viruses, and the structure, properties, and functions of DNA, the substance intimately concerned with reproduction.

Virology, Bacterial and Animal

Staff: Professor: Delbrück; Research Associate: Weigle; Senior Research Fellow: Dulbecco; Gosney Research Fellow: Vogt; Research Fellows: Benzer, Bertani, Lieb, Stent, Wollman; Graduate Students: Bowen, Clayton, Kaiser; Research Assistants: D. Benzer, G. Dulbecco, Hudson.
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Delbrück began studies involving phage T2 labeled with radioactive sulfur. Each particle of T2 contains about 30,000 sulfur atoms and several hundred radioactive sulfur atoms can be incorporated into each phage particle. Studies on the mortality of T2 as a result of the radioactive decay of these atoms and on the transmission of sulfur atoms from parent phage to progeny phage are in progress.

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The exclusion mechanism studied by Dulbecco is to be contrasted with another exclusion mechanism, to be referred to as exclusion by incompatibility. This type of exclusion is not time dependent, and is characteristic for dissimilar pairs of phages. The interactions between a carried phage and an infecting phage were studied. The carried phage, phage lambda of the lysogenic coli strain K12, is normally inside the bacterium in an immature form, called prophage. By irradiation of K12 with small doses of ultraviolet light, the prophage can be caused to mature in all of the bacteria of a culture of K12. About 60 minutes elapse between the application of the UV shock and the bursting of the bacteria. When the bacteria burst, they liberate on the average 150 mature lambda particles. If the bacteria are opened up at various times during the maturation period, the first mature lambda particles are found around 40 minutes after the stimulating UV shock. The bacteria were infected with phage T5 at various times during the maturation period. Complete mutual exclusion between T5 and lambda was found. Infections with T5 performed around 40 minutes after the UV shock, i.e., just prior to the appearance of mature lambda particles within the cell, resulted in pure T5 yields. This result shows conclusively that we are not dealing with an exclusion mechanism based on the formation of a barrier to penetration, set up by the excluding phage.
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Study of the Bacteriophage "Lambda"

Investigator: Lieb.


Strain K12 of the bacterium E. coli is lysogenic, i.e., a bacteriophage which has been named lambda is carried intracellularly and rarely released into the medium during normal growth of the culture. Treatment with ultraviolet light causes K12 to produce and release large numbers of phage particles, and stocks
of lambda are obtained in this way. A strain derived from Kl2, strain S, is sensitive to lambda and characteristic plaques are formed when lambda is plated on S. When S cells are plated with an excess of lambda, a large proportion of the cells, from 10% to 100%, give rise to colonies and thus appear to be resistant to the phage. However, many of these colonies contain chiefly lambda-sensitive cells. To determine whether this rapid shift in phage sensitivity is due to high mutation rates, cultures grown from single cells were analyzed for the proportion of lambda sensitive (S\textsuperscript{s}) and lambda resistant (S\textsuperscript{r}) cells. All cultures of approximately the same cell density were found to contain similar proportions of S\textsuperscript{s} and S\textsuperscript{r} cells; among cultures containing 10\textsuperscript{8} - 5 \times 10\textsuperscript{9} cells/cc no "pure" S\textsuperscript{s} or S\textsuperscript{r} culture was obtained. However, small cultures (200 cells or less) were invariably 100% S\textsuperscript{r}, whether the parental cell had been S\textsuperscript{s} or S\textsuperscript{r}. Studies of the proportions of the two types of S in growing cultures revealed that cultures growing in broth are almost 100% S\textsuperscript{r} until the cell density reaches about 10\textsuperscript{8} per cc. Then the culture becomes progressively sensitive, reaching a maximum of about 85% to 90% S\textsuperscript{s} at the time that growth becomes negligible. The factors responsible for this shift in sensitivity are not yet understood.

A study was made of the characteristics of lambda when grown on S. At 37°C the latent period is 38 to 40 minutes, the rise period is about 20 minutes, and the burst size 50 to 150. Lambda will not adsorb on S in M/15 phosphate buffer or in "F" synthetic medium. In broth, lambda adsorbs on both S\textsuperscript{s} and S\textsuperscript{r} cells, but the phage adsorbed to S\textsuperscript{r} is still sensitive to anti-lambda serum. When S\textsuperscript{r} cells and adsorbed lambda are plated on S\textsuperscript{s}, the phage forms plaques, so it can apparently desorb from S\textsuperscript{r} cells and be readsorbed on S\textsuperscript{s} bacteria. It is also possible that the phage remains adsorbed and finally enters the cell when conditions on the plate have caused most cells to become lambda-sensitive.

Colonies derived from S cells surviving treatment with lambda are often composed of lysogenic cells. Experiments have shown that S cells can become lambda carriers after an exposure as short as five minutes. They give rise to cultures which are not easily distinguishable from the original Kl2 strain. Potentially lysogenic cells (S\textsuperscript{rl}) form a part of the S\textsuperscript{r} component of S cultures, but the proportion of S\textsuperscript{rl} among the S\textsuperscript{r} is much higher in predominantly S\textsuperscript{s} than in chiefly S\textsuperscript{r} cultures. This suggests that the S\textsuperscript{rl} state is very close to S\textsuperscript{s}, and that it might be possible to raise the proportion of S\textsuperscript{s} cells in a culture by converting S\textsuperscript{rl} cells to S\textsuperscript{s}. It was found that ultraviolet treatment indeed caused an expected
increase in the number of $S^S$ cells in $S$ cultures; in predominantly $S^S$ cultures, most of the $S^F$ cells, of which over 50% are $S^F_1$, were converted to $S^S$. In predominantly $S^F$ cultures, where there are few $S^F_1$ cells, there was only a slight increase in $S^S$ cells.

Recent experiments indicate that strain K12 carries at least two types of lambda, which can be distinguished on the basis of plaque size. The ultraviolet sensitivities, adsorption rates, and latent periods of the large and the small plaque strains are very similar, but the large plaque strain appears to have a larger burst size.

Cofactor Activation of Bacterial Viruses

Investigators: Stent, Wollman.

Support: Merck Fellowship, Rockefeller Foundation Fellowship.

The studies of Stent and Wollman on the activation of phage T4 by cofactors will appear in forthcoming issues of "Biochimica et Biophysica Acta." The basic phenomenon consists in the fact that phage T4 is adsorbed to its host bacterium only if it has been brought into an "active" state by interaction with cofactor (principally L-tryptophan). Studies of the kinetics of the activation reaction indicate that activation involves a pre-equilibrium of adsorption of cofactor on the phage, followed by a kind of crystallization of cluster of a few cofactor molecules on the surface of the phage. It is this latter process which gives to the active state a relatively high stability and which also results in a very steep dependence of the activity on the concentration of cofactor. The activation process thus assumes practically the character of a threshold phenomenon. While the studies of Stent and Wollman clarified what goes on between cofactor and phage, these studies did not touch the question of why the deposition of cofactor on the surface of the phage lends the phage such radically altered physiological properties, enabling it to attack the host bacterium. It is at this point that the observations of T. T. Puck of the University of Colorado Medical School have thrown new light on the matter. Puck showed that activation by cofactor not only enables phage to attach to bacteria but also to the surface of glass. This finding rules out any interpretation of the effect in terms of enzymatic reactions or in terms of an alteration of the phage surface to give a specific steric fit between phage and bacterium. It shows, rather, that the result of the binding of cofactor must lie in an
alteration of the electrical properties of the phage surface, such as to permit phage and bacterium (or phage and glass) to get into sufficiently close contact for other forces to come into play. The process of activation is thus specific in the sense that the interaction between phage and cofactor presupposes a specific binding of the cofactor to the phage. It is unspecific in the sense that the binding of cofactor affects, and indeed affects quite radically, a very general property of the phage, namely its electro-kinetic potential. It is possible that this situation may be at the bottom of many well known and little understood physiological drug actions, like those of acetyl choline on synapses, of auxins on plant growth, of hormones on their effector organ. The possibility that many of these substances exert their effects through a physico-chemical mechanism of action has previously been discussed by Veldstra ("Biochem. Biophys. Acta," 1, 364-378, and 3, 378-412). Veldstra's conclusion should now be reexamined in the light of the special mechanism of interaction between cofactor and phage disclosed by the researches of Stent and Wollman.

**Bacterial Recombination in Escherichia coli.**

**Investigator:** Vogt.

**Support:** Gosney Research Fund.

Vogt has investigated the conditions controlling the formation of genetic recombinants of different mutants of the K12 strain of E. coli. Very few recombinants are formed in a very rich nutritional environment and in the complete absence of nutrients. More recombinants are formed if a period of limited nutrition is introduced. Presumably there are several phases involved in the formation of recombinants, namely, formation of zygotes, formation of segregants, and germination of the segregants. Each of these phases may have different requirements. The best conditions for each of these phases has not yet been determined, but it has been possible, by establishment of a suitable nutritional regime, to raise the overall yield of recombinants by a factor of about 100, as compared to yields obtained in previous experiments.
The Kinetics of Photoreactivation of Phage T2

Investigator: Bowen.

Support: General Funds.

Bowen has been studying the kinetics of the process of photoreactivation of bacteriophage which have been inactivated by ultraviolet light. Dulbecco found that the number of active phage particles increases with time, $t$, of exposure to reactivating light as $(1 - e^{-kt})$ - that is, as a "one-hit" curve - and this suggests that the process is a simple direct photochemical reaction. However, the rate of the reaction, $k_I(I, T)$, does not increase linearly with light intensity and has a strong temperature dependence. Dulbecco inferred that the process of photoreactivation includes a temperature-dependent, rate-limiting dark reaction in addition to the immediate act of light absorption and its consequences.

It has been possible to demonstrate this dark reaction explicitly at high light intensities, where it is the rate-limiting step. In this case a series of short flashes separated by long dark intervals is very much more effective than is the same total exposure at the same intensity when given as a single continuous flash. This could not be so unless some reaction necessary for photoreactivation is proceeding during the dark intervals.

A hypothetical model for the reaction sequence was then set up which included both a thermochemical and a photochemical step, and which gave the observed dependence of rate on light intensity. In addition it suggested a number of new experiments for which expected results could be deduced from the model. Most of these experiments have now been performed and have confirmed the predictions in great detail.

It is assumed that in the dark an equilibrium is established between a reactant $A$ and a product $B$. Light acts upon $B$ activating it to $B^*$ and disturbing the equilibrium between $A$ and $B$. The light-activated product $B^*$ may react, with a very small probability, to produce active phage, or, with a large probability, back to $A$. Thus at high light intensities the amount of activable material $B$ available is limited by the temperature-dependent dark reactions from $A$ to $B$ and reverse. In the dark following a high-intensity flash, the amount of $B$ increases slowly to the former equilibrium value.
This model describes the behavior of ultraviolet inactivated phage T2 adsorbed on E. coli in phosphate buffer. It has been tested using one flash or a series of flashes of variable intensity, length, and separation. The return to equilibrium during the dark intervals between flashes can be followed particularly clearly. Its progress with time is described very accurately by the function \(1 - e^{-k_2t}\), as predicted by the model, and good measurements of the rate constant \(k_2\) are possible. It has been measured over the temperature range 0° to 45° C and gives two rather well-defined activation energies of about 16 and nine kilocalories for the ranges of 0° to 23° C and 23° to 45° C respectively. Typical times for half completion of the reaction are about 24 seconds at 37° C, 6 1/2 minutes at 0° C. It is possible to obtain formal agreement with these observations by assuming that the dark reaction step is enzymatic and that the enzyme becomes saturated at higher temperatures (with suitable constants chosen to fit the data). At present there is no other evidence for this assumption except that it agrees with the data and is not an unreasonable mechanism. Experiments on the behavior of the system following temperature changes indicate that the dark reaction does, in fact, precede the photochemical step, as has been assumed.

Phototaxis in Rhodospirillum.

Investigator: Clayton.

Support: General Funds.

The purple bacterium Rhodospirillum rubrum is a photosynthetic organism which responds to a decrease of illumination by reversing its direction of swimming (phototaxis). Studies of its metabolism, a variant of plant photosynthesis, have thrown considerable light on the latter process; it is hoped that studies of its phototactic response will be fruitful in elucidating the mechanism of biological irritability (response to environmental stimulus).

Investigations of the past year have been aimed at increasing our knowledge of the relationships between the phototactic response, the photosynthetic metabolism, and the vital pigments of the organism. The pigments of R. rubrum consist of a chlorophyllous substance ("bacteriochlorophyll"), a red carotenoid pigment ("spirilloxanthin"), and some other carotenoids which are present in relatively small amounts. An investigation of the sensitivity of the phototactic response as a function of the wave length of the light employed revealed that the spirilloxanthin absorbs light
which is effective in evoking a response; previous investigations
had indicated that only the bacteriochlorophyll and some of the
scarcer carotenoids are phototactically active. Possible sources
of error, such as sensitization or inactivation of some pigments
under illumination, were examined and found to be inappreciable.

It has been hypothesized that the phototactic response to
a decrease in illumination is associated intimately and invariably
with a decrease in the rate of photosynthesis. In connection with
this hypothesis, the responses of *R. rubrum* to changes in oxygen
concentration (chemotaxis) were investigated. This was done with
cultures which were grown photosynthetically and with cultures
which were grown in the dark (in the absence of light the bacteria
can grow by an oxidative metabolism); conditions were found in
which an observed tactic response could not have been associated
with a decrease in metabolic rate.

Finally, the adherence of the phototactic response to the
Weber law was reexamined. This so-called law, which requires
that the least perceptible change in an environmental factor (such
as illumination) be a constant fraction of the initial intensity of
the factor, has received strong support from the remarkable
adherence reported for bacterial phototaxis. The Weber law
adherence observed was much less convincing and extensive than
had been reported earlier, apparently due to an early onset of
saturating effects at the higher intensities of illumination. The
possibility was considered that in many cases an early onset of
saturation might mask a potentially close Weber law adherence.
Detection of Peach Mosaic Virus by Physical Methods

Investigators: Camus, Wildman.


The detection of several viruses has been successfully achieved by electrophoretical analysis. It was thought of interest to apply the same technique to the detection of another virus of great economic importance, the peach mosaic virus (PMV). The symptoms of PMV are characterized by a more or less accentuated mottling of leaves, flowers and fruits; the trees die within a few years after contamination. The virus is not transmissible by mechanical means, its insect vector, if any, is not known. The disease is usually carried by graft, and very little information has been obtained on it so far.

Leaves from healthy peach trees and from trees infected with the strain "San Saba severe" were ground and their soluble proteins extracted and analyzed in the Tiselius electrophoresis apparatus. It was found that samples from infected leaves presented an extra component of high molecular weight which could be removed easily by high speed centrifugation. The patterns obtained were highly reproducible and provide a good means of early detection of the disease. Similar analyses will be carried out with other strains of the same virus in the near future.

Isolation of Curly Top Virus

Investigators: Camus, Wildman.


The curly top virus (CT), which attacks particularly the sugar beet, is another virus of great economic importance, and much work has been devoted to the study of its properties. It is known to be transmitted only by insect or by graft and to be strictly localized in the phloem tissue. Several strains have been characterized and deeply studied from the morphological standpoint, but despite numerous attempts the virus has not yet been isolated.

In collaboration with Doctor C. W. Burnett of the University of California at Riverside, new attempts to isolate CT have been carried out. The blades of leaves from healthy and diseased (strain B) sugar beets were trimmed off, the petioles
dialyzed against a buffer of low ionic strength and examined in the Tiselius electrophoresis apparatus. The study was carried out on both whole plants and tissues cultivated in vitro.

Normal stems of the sunflower plant showed very important differences in their protein patterns from stems bearing numerous well developed primary galls. Not only do the contaminated stems exhibit one more component than the normal ones, but there is also a complete redistribution of the electrophoretic mobilities of the individual components. Studies are now under way to discriminate between the modifications owing to the fact that the stem tissues undergo active proliferation and the specific alterations owing to the tumor tissue itself.

Extensive propagation of the tissue culture strains which had been isolated in the spring of 1950 was required in order to obtain sufficient material for the analyses. So far, more than 10,000 cultures from normal callus and crown gall of sunflower and Jerusalem artichokes, carried out in exactly the same conditions, have been utilized.

Many technical difficulties encountered during the analyses have been overcome to a certain extent. Although it has not yet been possible to obtain good scanning patterns from the cytoplasm of the artichoke strains, the results obtained with sunflower proved to be most interesting. It was possible to ascertain in this material clear cut differences between the protein constituents of the normal and tumor tissues. Whereas the normal callus pattern shows only three components, the crown gall tissue possesses, besides the same three components, an extra one of high molecular weight which makes up about 20% of the total protein content of the tissue. The patterns obtained are highly reproducible and it is believed that the extra component found in crown gall represents the tumefacient factor. The fact that some protein denaturation takes place during the preparation of the samples does not invalidate this conclusion since all the samples are prepared in the same manner and the percentage of denatured material is exactly the same for normal callus and crown gall.

Attempts have been made to concentrate this extra component of the crown gall by ultracentrifugation and partial success has been obtained. The conditions utilized so far (100,000 g for 90 min.) proved, however, to be too drastic, and careful timing studies under various ultracentrifugal forces are under way in view of its isolation in pure form.
ground in a colloid mill with a neutral buffer and the soluble protein extracted. Electrophoresis analysis of the whole cytoplasm failed to reveal any clear-cut difference between the virus-containing solutions and the healthy ones, indicating that the virus concentration in normal protein is probably extremely low.

The virus activity was tested by feeding the insect vectors small aliquots of the various extracts and by keeping the insects for two hours on healthy beets. The concentration of virus was assumed to be proportional to the number of beets coming down with the disease.

By high speed centrifugations (100,000 g for 1 hour) it was possible to concentrate all the virus activity in a pellet containing only 60% of the total protein. However, further centrifugation of the resuspended pellet gave no significant concentration. Analytical ultracentrifugation of solutions "recycled" three times proved to be very heterogeneous and identical to similar extracts from normal beets.

Another means of virus separation proved to be much more satisfactory. By a series of alcohol precipitations and medium speed centrifugations it was possible to obtain about 100 mgs of crystalline protein material from 12 pounds of leaves. The crystals, which were not obtained upon parallel treatment of healthy leaves, were lozenge shaped plates and present faint birefringence. They gave positive reaction to several protein tests, and, upon redissolution, yielded solutions which turned out to be infective. This work is now being repeated so that the exact conditions of crystallization and the chemical nature of the crystals can be ascertained.

Isolation of Strains of Tobacco Mosaic Virus by Electrophoresis

Investigators: Singer, Wildman.


In the course of a study of tobacco mosaic virus (TMV) synthesis, several components were observed during the electrophoresis preparations. Two of these components were isolated from the electrophoresis cell, and were shown to be physically and chemically similar, except for their electrophoretic properties, and to produce mosaic infections in tobacco plants. The symptoms, however, are markedly different, one strain producing mild, the
other severe, mosaic symptoms on Turkish tobacco. These studies were performed in collaboration with Doctor J. G. Bald of the University of California at Los Angeles. Owen has shown that the two strains are serologically related but distinguishable. A study of the comparative chemical properties of these strains should be of great interest in problems of the chemical nature of mutations, and of altered symptom patterns in mosaic infections, as well as in the study of the immunochemical specificity of antigens and antibodies.

**Further Studies of the Proteins of Healthy Leaves**

**Investigators:** Singer, Campbell.

**Support:** National Foundation for Infantile Paralysis.

It had previously been discovered that a variety of green leaf whole cytoplasmic extracts contain a particular protein (Fraction I) constituting from 25% to 50% of the total protein in the extract. Further progress has been made in the investigation of the preparation and properties of this protein. The analytical ultracentrifuge has proved itself indispensable in these studies, providing the only means of analyzing various preparations for their Fraction I content and studying the size and shape of the Fraction I molecules. By high-speed quantity centrifugation in the Spinco centrifuge, Fraction I can be separated from the whole cytoplasm extracts in as pure and least altered condition as it has so far been possible to obtain.

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Investigator: Lieb.


Strain K12 of the bacterium E. coli is lysogenic, i.e., a bacteriophage which has been named lambda is carried intra-cellularly and rarely released into the medium during normal growth of the culture. Treatment with ultraviolet light causes K12 to produce and release large numbers of phage particles, and stocks
of lambda are obtained in this way. A strain derived from K12, strain S, is sensitive to lambda and characteristic plaques are formed when lambda is plated on S. When S cells are plated with an excess of lambda, a large proportion of the cells, from 10% to 100%, give rise to colonies and thus appear to be resistant to the phage. However, many of these colonies contain chiefly lambda-sensitive cells. To determine whether this rapid shift in phage sensitivity is due to high mutation rates, cultures grown from single cells were analyzed for the proportion of lambda sensitive (S^s) and lambda resistant (S^r) cells. All cultures of approximately the same cell density were found to contain similar proportions of S^s and S^r cells; among cultures containing 10^8 - 5 x 10^9 cells/cc no "pure" S^s or S^r culture was obtained. However, small cultures (200 cells or less) were invariably 100% S^r, whether the parental cell had been S^s or S^r. Studies of the proportions of the two types of S in growing cultures revealed that cultures growing in broth are almost 100% S^r until the cell density reaches about 10^8 per cc. Then the culture becomes progressively sensitive, reaching a maximum of about 85% to 90% S^s at the time that growth becomes negligible. The factors responsible for this shift in sensitivity are not yet understood.

A study was made of the characteristics of lambda when grown on S. At 37° C the latent period is 38 to 40 minutes, the rise period is about 20 minutes, and the burst size 50 to 150. Lambda will not adsorb on S in M/15 phosphate buffer or in "F" synthetic medium. In broth, lambda adsorbs on both S^s and S^r cells, but the phage adsorbed to S^r is still sensitive to anti-lambda serum. When S^r cells and adsorbed lambda are plated on S^s, the phage forms plaques, so it can apparently desorb from S^r cells and be desorbed on S^s bacteria. It is also possible that the phage remains adsorbed and finally enters the cell when conditions on the plate have caused most cells to become lambda-sensitive.

Colonies derived from S cells surviving treatment with lambda are often composed of lysogenic cells. Experiments have shown that S cells can become lambda carriers after an exposure as short as five minutes. They give rise to cultures which are not easily distinguishable from the original K12 strain. Potentially lysogenic cells (S^rl) form a part of the S^r component of S cultures, but the proportion of S^rl among the S^r is much higher in predominantly S^s than in chiefly S^r cultures. This suggests that the S^rl state is very close to S^s, and that it might be possible to raise the proportion of S^s cells in a culture by converting S^rl cells to S^s. It was found that ultraviolet treatment indeed caused an expected
which is effective in evoking a response; previous investigations had indicated that only the bacteriochlorophyll and some of the scarcer carotenoids are phototactically active. Possible sources of error, such as sensitization or inactivation of some pigments under illumination, were examined and found to be inappreciable.

It has been hypothesized that the phototactic response to a decrease in illumination is associated intimately and invariably with a decrease in the rate of photosynthesis. In connection with this hypothesis, the responses of R. rubrum to changes in oxygen concentration (chemotaxis) were investigated. This was done with cultures which were grown photosynthetically and with cultures which were grown in the dark (in the absence of light the bacteria can grow by an oxidative metabolism); conditions were found in which an observed tactic response could not have been associated with a decrease in metabolic rate.

Finally, the adherence of the phototactic response to the Weber law was reexamined. This so-called law, which requires that the least perceptible change in an environmental factor (such as illumination) be a constant fraction of the initial intensity of the factor, has received strong support from the remarkable adherence reported for bacterial phototaxis. The Weber law adherence observed was much less convincing and extensive than had been reported earlier, apparently due to an early onset of saturating effects at the higher intensities of illumination. The possibility was considered that in many cases an early onset of saturation might mask a potentially close Weber law adherence.