1956-1957 ANNUAL REPORT

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THE ROCKEFELLER INSTITUTE BULLETIN

ANNUAL REPORT

1956-1957

NEW YORK 21, NEW YORK
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The strength of a university is determined by the quality of its faculty. It is the members of the faculty who fulfill the mission of a true university as they study and teach, research and diffuse knowledge. It is they who attract the students, justify the selfless efforts of trustees and deserve the support society gives the university. As the faculty train and select their successors, they assure the continuity of the university which, with the church, is the most enduring of human institutions.

Selection of the senior members of our faculty requires especial care, for the Institute is devoted only to research and graduate study. Only those of the faculty who have high competence for scientific research and the preparation of future investigators have a significant role at the Institute. Because few scientists are thus endowed in unusual degree, our search must be exacting and unending. To those whom we select we have a heavy obligation to provide the best possible environment for a career of creative, scientific scholarship.

We have been fortunate in securing excellent new colleagues during the past three years. Frank Brink, Jr. and H. Keffer Hartline came to us from Professorships of Biophysics at The Johns Hopkins University, Paul Weiss from a Professorship of Zoology at the University of Chicago. George W. Corner joined us after retiring as Director of the Laboratory of Embryology of the Carnegie Institution of Washington and Vladimir Zworykin as Vice President of the Laboratories of the Radio Corporation of America. This year Edward Tatum joined our faculty from Stanford University where he had been Professor of Biochemistry and Chairman of the Department.
It is easy to recognize the quality of mature scholars such as those I have just mentioned. It is more difficult to select young scientists who can fulfill the mission of the Institute a decade or a quarter-century from now; we must do so if we are to build for the future. If we choose wisely, we shall leave a heritage of excellence; if we choose unwisely, we encumber the faculty for years to come with those who are not creative scientific scholars.

The choice and elimination of younger members of our faculty pose unique problems. Universities whose activities include undergraduate and professional education, extensive programs of athletics and entertainment for students and the public, many social services for the community of which the institution is a part, and extensive administrative duties, provide many varied opportunities for those who lack ability for research or lose interest in such creative work. The Institute does not provide those diverse opportunities. Accordingly, if we are to fulfill our responsibilities to the younger members of our faculty, we should continually evaluate their competence and promise. We must endeavor to distinguish between those who should be the nucleus of our future faculty and those who should be aided to find other opportunities for service. Because we are attracting an ever increasing number of able young scientists, selection becomes more difficult; more must be lost to us whom we would choose to keep because we have but a limited number of positions with permanent tenure. There is no limit to the efforts we should make to provide an ideal environment and exceptional facilities for the development of young scientists while they are with us. This is a primary objective of the Institute; it is a major contribution to the furtherance of science.

The vitality of even the largest university faculties is enhanced by prolonged visits of colleagues from sister institutions. The intellectual horizons of scholars are thus extended, and the scope of knowledge that can be encompassed in any one institution is widened; international amity is fostered.
Last year I reported the appointment of fifteen distinguished scholars from other universities as Institute Lecturers; five of them were from abroad. This past year we added four: Th. Dobzhansky, Professor of Zoology, Columbia University; K. Linderstrøm-Lang, Director, Department of Chemistry, Carlsberg Laboratory, Copenhagen; H. W. Magoun, Professor of Anatomy, University of California School of Medicine, Los Angeles; Homer W. Smith, Professor of Physiology, College of Medicine, New York University. From the Lecturers who spend a week or two in residence at the Institute, the faculty and students derive much benefit through seminars, lectures and friendly associations. We are also indebted to scores of others who come for but a day and lecture to us on their research.

These associations with our colleagues from other universities and other countries will be fostered by the Faculty and Visiting Scientists’ Center which is now approaching completion. This unique facility has been created to increase the amenities of life at the Institute and to promote the friendly relations of faculty with one another and their guests. Because it has such purposes, it is eminently appropriate that it should be named Abby Aldrich Rockefeller Hall for a gracious, friendly lady of great charm who did so much to enrich the life of New York. The building has been made possible by a munificent gift of approximately $2,500,000 from John D. Rockefeller, Jr. In expressing our appreciation to Mr. Rockefeller for the beautiful and richly meaningful addition to the Institute which he has thus provided, we gratefully recall his devotion and selfless service to the Institute throughout its entire history of more than fifty years.

In my report of last year I wrote of the need for a new laboratory building to provide working space for our larger faculty and student body; I told of how we could thus with more efficiency use the many central facilities and auxiliary services which support our research and educational activities. While we were in the process
of drawing plans for such a building, we received a generous gift of $600,000 from the United States Public Health Service of the Department of Health, Education and Welfare. These additional funds will enable us to build a larger building than we had thought possible.

The making of plans for this new laboratory, for a student residence hall, and for many changes in existing structures coupled with the completion of Abby Aldrich Rockefeller Hall, Caspary Hall and the President’s House, have made heavy demands upon members of the administrative staff, and especially upon our Superintendent of Buildings and Grounds who has valiantly carried these extra burdens.

The Institute has a campus that is almost unique among university campuses in this country, for although it is located in the heart of our great metropolis and close to scores of notable cultural institutions, it has a potentially beautiful setting. Above the ever changing panorama of the East River and at the foot of the magnificent building of the New York Hospital our fourteen hilly acres are a challenging opportunity to a landscape architect; our beautiful new buildings now increase that challenge. Dan Kiley has undertaken to make this a unique area of natural beauty that will enrich the lives of those who spend their days at the Institute and of the many who pass by. When this has been achieved, we shall be forever grateful to David Rockefeller, our Chairman, who has already contributed $125,000 to this lovely undertaking. It is fitting that the gardens he has thus enabled should surround the building named for his mother who created at Mount Desert one of the most beautiful gardens in New England.

During this past year we have begun a series of publications which are designed to inform our trustees, faculty, students, administration, and the academic and scientific world at large about the manifold activities of the Institute.

The Descriptive Pamphlet which had been issued for many
years was completely rewritten and published in a new format.  
An Annual Report on the work of the previous year was published for the first time. It contains the President's review of the Institute's program and new undertakings, reports of scientific research accomplished or in progress, a list of faculty publications, and a record of their professional activities.

A Calendar of Events is now issued fortnightly throughout the academic year in order to inform the faculty, staff and colleagues in sister institutions of our lectures, seminars and other intellectual and social activities.

The Rockefeller Institute is a vital organization of nearly 1,000 men and women with diverse abilities and interests who are engaged in many different activities. They further science and promote human welfare in many different ways through the Institute and with the Institute's support. A new Rockefeller Institute Quarterly tells of this vast and changing pattern of manifold activities so that all those associated with the Institute or who are interested in its activities may know of what we do.

For all of these publications we are indebted to Charles I. Campbell, formerly of the staff of the National Academy of Sciences, who came to the Institute this year to fill the newly created position of Administrative Associate for Information Services. In addition to these publication duties, Mr. Campbell will have general responsibilities for the Library and the Publications, Illustration and Duplicating Services.

The Institute is a member of the world-wide, informal association of universities; the members of our faculty are members of the great fraternity of academic scholars. There is frequent interchange of our faculty with the faculties of other institutions of higher learning. For these reasons it has become desirable to designate our faculty by titles which are consistent with titles used throughout the academic world. Accordingly, beginning with the next academic year, we will use the titles Professor, Visiting Pro-
Professor Frank Brink, Jr. has accepted appointment as Dean of Graduate Studies; he is the first to hold this newly created office. It is a timely appointment because the increasing number of students, the growing participation of more members of our faculty in our educational work as teachers and student advisors, and the development of courses of lectures and seminars in many specialized fields of science all require guidance and coordination. Dr. Brink will also serve as Secretary to the Trustees' Committee on Educational Policies.

The President and the Dean of Graduate Studies will be aided in the further development of our educational program by a Faculty Committee on Educational Policies under the Chairmanship of Professor Alfred Mirsky. The first members of the committee are Alexander Bearn, Lyman Craig, René Dubos and Edward Tatum. The appointment of this committee is in keeping with our intent to invite the faculty to participate in conducting the affairs of the Institute and in shaping its policies.

Somewhat more than a year ago we created the position of Trustee Emeritus. Ross Granville Harrison has accepted election as the first to be so honored following his resignation as a Trustee at the age of 86. Dr. Harrison had served as a member of the Board of Scientific Directors and then as a Trustee of the Institute for 18 years.
His was an unusually distinguished scientific career. He graduated from The Johns Hopkins University in 1889, received the degree of Doctor of Philosophy from the same university in 1894, and his doctorate in medicine from the University of Bonn five years later. After some years of teaching and research in the Department of Anatomy at The Johns Hopkins, he was for 30 years Professor of Comparative Anatomy and then Sterling Professor of Biology at Yale University. For eight years he was Chairman of the National Research Council. We are glad that this devoted servant of science and of the Institute will continue to be associated with us after being freed from the heavy responsibilities of an active Trustee.
Hog cholera. Study of the natural history of hog cholera is being continued and the role that the swine lungworm plays as reservoir and intermediate host for the hog cholera virus is being further elucidated. An attempt is being made to learn why the phenomenon of provocation of masked virus in the lungworms is so strictly seasonal and occurs largely only during the first 5 months of the year. A search for more effective provocative stimuli is being intensively pursued.

Methods for the unmasking of hog cholera virus in lungworms in vitro are being sought, thus far unsuccessfully.

Epizootic hemorrhagic disease of deer. Studies with this virus disease are being continued. No new outbreaks occurred in New Jersey last year but virus was obtained from an outbreak in South Dakota. This virus has been compared with the New Jersey virus by immunological means, and it has been found that, though it causes an identical clinical disease in deer, it is serologically distinct from the New Jersey strain of the epizootic hemorrhagic disease virus.

Deer fibroma. A study of the epidemiology of fibroma of deer has entailed attempts to transmit the causative virus by various biting insects. Thus far none have transmitted. An interesting aspect of the disease is that though most deer eventually recover, their sera do not contain antibodies capable of neutralizing the fibroma virus.

Eastern equine encephalomyelitis in pheasants. Work with this
Laboratory of John B. Nelson

The emergence of murine hepatitis virus in mice injected with an Ascites tumor and the interaction of the two agents. The appearance of an ascites tumor during the passage of Eperythrozoon coccoides in Princeton mice was reported from this laboratory in 1956. Separation from the parasitic organism was accomplished and the tumor maintained independently by the intraperitoneal injection of ascitic fluid in weanlings at intervals of 7 days. Growth in the peritoneal cavity was rapid and accompanied by the secretion of a creamy white fluid in which the neoplastic cells were suspended. The outcome was invariably fatal. Death usually occurred between the 7th and the 10th day as the result of massive hemorrhage. Intramuscular injection was followed by a local solid growth which was highly malignant but not inwardly invasive.

No significant departure from this course of events was observed during 131 passages in some 600 mice. In the 132nd passage the pooled ascitic fluid had a yellowish cast and the livers showed surface lesions suggestive of mouse hepatitis. This diagnosis was verified histologically and the causal agent identified as a virus of the hepatoencephalitis group. Several attempts to free the tumor cells of the virus by means of successive washings were unsuccessful. The tumor had also been maintained, meanwhile, by intramuscular passage. A second ascites series was then started with minced tissue from localized solid tumors and has been maintained to date in the absence of hepatitis.

Intraperitoneal injection of the virus-contaminated fluid was continued in both Princeton and Swiss weanlings. The latter were known to be resistant to other types of hepatitis virus from Prince-
Animal Pathology 3

mice but equally susceptible to the tumor. In the presence of the virus, however, there was a marked difference in the reaction of the two mouse strains. Princeton mice showed marked hepatic involvement accompanied by a progressive decline in the volume of ascitic fluid and the number of cells contained therein. In one passage series, growth of the tumor was completely suppressed by the 8th transfer. In Swiss mice the ascitic reaction was generally unaffected though the fluid was often discolored by bile salts. Liver lesions which were less advanced than in the susceptible Princeton mice but nevertheless unmistakable were unexpectedly encountered.

A suspension of washed liver tissue containing only the virus was injected intraperitoneally in Princeton mice. This series was continued for 22 passages and supplementary transfers made from time to time in Swiss mice. By the 5th to the 7th day all of the Princeton mice were acutely ill and showed a typical hepatic reaction which increased in severity as the passages progressed. The Swiss mice were all normal in appearance when killed on the 7th day and only one showed a few lesions in the liver at autopsy. Despite the lack of reaction in these mice sufficient virus was present in their livers to produce hepatic foci on back passage in Princeton mice.

The emergence of active murine hepatitis virus in mice that normally show no signs of infection is known to be promoted by the injection of *E. coccoides* (Gledhill et al.), by leukemic cells (Nelson), and by urethane (Braunsteiner and Friend). The preceding findings intimate that the ascites tumor may also result in virus activation. The neoplastic cells evidently provide an additional milieu for multiplication of the virus. The appearance of liver lesions in dually injected Swiss mice which ordinarily are not responsive to the virus is of particular interest. There is a suggestion here that the reaction may be due in part to the death of virus-infected tumor cells fortuitously carried to the liver.
Several closely related lines of research have been followed during 1956-57. All of them have been concerned with the chemistry of naturally occurring polypeptides, proteins, enzymes and hormones. A considerable part of the work has been directed toward the improvement of existing methods and the development of new methods for deriving information about the structure of this type of natural product. This part of the study has contributed toward a broader significance than that relating to this particular field since these methods have been or can be applied to widely different fields of naturally occurring substances.

In view of the great need for more precise methods of separating and proving purity with proteins, an extensive search has been made for liquid-liquid systems which will permit the fractionation of proteins by the technique of countercurrent distribution. Unfortunately, the type of system which should be technically the most selective presents conditions known to promote denaturation and transformation. In spite of this, a degree of success has been achieved by incorporating certain components into the system which promote stability, probably by binding with reactive sites and thus inhibiting the conformational change or alteration of shape associated with the first stages of denaturation.

The proteins, ribonuclease, lysozyme and serum albumin, have been successfully studied by this approach without denaturation. In a crystalline sample of ribonuclease the combined use of ion exchange chromatography and countercurrent distribution has shown the presence of six components aside from the main component present as 70% of the original. In both these proteins the
main component behaved as if it were an ideal solute in spite of the large size.

Several points of considerable technical significance were found in the serum albumin work. A system containing a low concentration of trichloroacetic gave a distribution band much more narrow than expected. This type of system developed in this laboratory several years ago for the fractionation of insulin has been of great use in other laboratories for the isolation and purification of pituitary hormones (ACTH, lactogenic hormone, growth hormone, etc.) but has often proved somewhat confusing because it often gave distribution patterns inexplicably too narrow. A logical explanation on the basis of trichloroacetic binding has come from the albumin work. This same binding phenomenon with trichloroacetic acid was also shown to provide a higher level of stability.

Systems with a high content of ammonium sulfate have shown particular promise. This type of system to which a small amount of sodium caprylate has been added as stabilizer has permitted the demonstration of at least three components in a crystalline sample of bovine serum albumin. These proteins are the largest molecules (molecular weight 66,000) thus far fractionated by countercurrent distribution. Systems similar to this offer an environment not too different from that offered by the familiar ammonium sulfate salt precipitation method.

A new approach to the quantitative study of dialysis through cellophane has been developed. For this purpose a small dialysis cell has been designed which increases the overall rate of dialysis by providing a large dialyzing surface relative to the solution volume. This has permitted dialysis rate studies to be effectively extended to considerably larger molecules. It has also permitted less porous membranes to be used with the smaller solutes. When this is done the selectivity of dialysis was found to be greatly increased.

Not only have useful separations been made with the improved technique but its value as a tool for estimating size and demonstrating homogeneity with respect to size has been shown. Once
the size of the molecule has been established, the effect of so-called
denaturing conditions on dialysis rates (such as, with urea, ex­tremes of pH or heat) now offers another parameter for studying
the interesting conformational changes which proteins undergo.
When their environment is changed, apparently a molecular ex­ansion or significant change of shape results.

An attempt has been made to gain a better understanding of the
unique structures present in the bacitracin polypeptide antibiotics.
They have been shown to lose part of their antibiotic activity
through racemization of the terminal amino acid (isoleucine).
Bacitracin B has been further purified and shown by amino acid
analysis to differ from bacitracin A by the presence of an additional
valine residue. An unexpected finding in regard to this valine in­
volves the fact that it can partly replace isoleucine as the terminal
amino acid, either valine or isoleucine furnishing the free amino
group but not both at the same time. A tautomeric shift of some
obscure nature could be indicated or a mixture resulting from a
curious inversion of amino acid sequence. Other than this, bacitra­
cin B contains the same unique thiazoline ring as well as the basic
and acidic groups of A. It also seems to contain the same type of
peptide ring system with a branched chain at the lysine residue.

An attempt has been made to isolate in pure form the substances
which serve as substrate for the enzymatic chemiluminescent re­
action of the Cypridina crustacean. Evidence has been obtained
that these substances are of relatively small size and have a chromo­
phoric structure conjugated in some way to a polypeptide moiety.
Fractionation attempts have indicated the presence of a large num­
ber of solutes capable of serving as substrate but it is hoped that
they will prove to have a common chromophoric moiety which will
simplify the problem.

Further fractionation work has been carried out with the anti­
genic proteins of ragweed pollen. The complexity of the protein
mixture obtained by ammonium sulfate precipitation has been
shown by a variety of techniques. Zone electrophoresis with a Geon
resin as the supporting agent has given a reproducible separation. Fractional dialysis has given resolution into three and perhaps four families of different molecular sizes in the range of 3000, 10,000, 20,000 and a larger but less definite size.

Laboratory of Walther F. Goebel
Tsunehisu Amano, Guy T. Barry, Margeris A. Jesaitis

Colicine K. There are a number of bacterial species which elaborate potent antibacterial agents during their growth; these agents are known as bacteriocenes. These are substances of great biological importance for they play a dynamic role in the survival of the species which elaborates them and in addition they are believed to be related to the bacterial viruses. The precise chemical nature of the bacteriocenes has never been fully established. A study has therefore been undertaken in this laboratory for the purpose of obtaining these substances in purified form so that their true nature might be established and their relationship to the bacteriophages better understood.

A strain of colon bacillus known as E. coli K235 produces the bacteriocene colicine K. When this microorganism is grown under precise environmental conditions it elaborates large quantities of colicine K into the surrounding medium. By chemical fractionation it is possible to separate this substance from the constituents of the medium and to obtain a material possessing very potent antibacterial properties. This substance, which is electrophoretically homogeneous, is a lipocarbohydrate-protein complex, and it is believed to be identical with the specific O antigen of the parent microorganism.

Colicine K can be readily broken down into its lipocarbohydrate and protein components. Upon so doing the colicine activity remains associated with the protein constituent. It can be shown that this protein is electrophoretically homogeneous, yet an immunological analysis reveals that it is a mixture of three different
serologically active substances. Quite recently it has been found that these can be separated chromatographically into two non-colicine containing fractions, and a third having very potent colicine K activity. Thus the characterization of colicine K appears imminent and awaits the futherance of this work.

In order to establish with more certainty the nature of the component responsible for colicine K activity, a non-colicinogenic variant of E. coli K235 has been isolated. This microorganism yields no colicine K, yet it agglutinates in an antiserum against the parent cell, a fact which indicates that the O antigens of the two strains must be closely related indeed. A comparison of the chemical structure of the O antigens of these two microorganisms should reveal differences in their chemical make-up which in turn can be related to the component responsible for colicine K activity.

Finally, it may be stated that a comparison of the properties of purified colicine K with those of the coli-dysentery phage T6, the virus to which the colicine is presumably related, has revealed that these two agents bear neither a chemical or immunological relationship to one another. Any similarities in biological behavior which these two substances exhibit must therefore be regarded as fortuitous.

Colominic Acid. A macromolecular substance has been isolated from the culture medium of growing E. coli K235 which has been termed colominic acid. This substance is believed to be part of the capsule of this microorganism, for a non-encapsulated variant of the parent strain does not elaborate the acid. Colominic acid contains nitrogen, acetyl, and carboxyl groups and is free of phosphorous, sulfur and methoxyl. Tests for protein, hexosamines, pentoses and hexuronic acids are negative. From the Ehrlich test it has been found that colominic acid gives such a high absorbance per unit of weight that it must be constituted solely of sialic or neuraminic acid-like substances. A crystalline material has been obtained from aqueous hydrolysates of the acid in high yield. A comparison of the elementary composition, infrared spectrum, optical rotation and
neutral equivalent of the unknown crystalline substance with that of N-acetylneuraminic acid has revealed that the two are identical. Colominic acid must therefore be regarded as a unique acidic polysaccharide composed of repeating units of N-acetylneuraminic acid. The exact arrangement of the monomer units in the macromolecule is as yet not known. The results of these studies have revealed that a sialic acid-containing substance has, for the first time, been obtained from a source other than mammalian.

The Structure of Phage Nucleic Acids. It has been demonstrated in this laboratory that the nucleic acids of the coli-dysentery phages T₂, T₄ and T₆ contain glucose and that the amount of this hexose is in each instance different. If the nucleic acids are hydrolyzed with pancreatic deoxyribonuclease and phosphodiesterase, some 20-30 per cent of glucose is liberated in the form of a mono- or diglucoside of hydroxymethylcytidylic acid. The main portion of the sugar remains bound, however, to enzyme-resistant oligo- and polynucleotides.

In order to establish more fully the nature of the glucose linkage a study of the degradation of the viral nucleic acids by chemical means has been undertaken. Upon subjecting them to mild acid hydrolysis and subsequent separation of the liberated purines by dialysis it is found that the apurinic acids contain all of the glucose originally present in the viral nucleic acids. Examination of the apurinic acids by electrophoresis in monovalent buffers has revealed that they are essentially homogeneous. Chemical analyses of these substances have shown that their content of pyrimidine bases and of glucose is the same as that of the nucleic acids themselves. The purine content, on the other hand, is decreased to within a few per cent of its original value. From all this it is evident that the purines do not serve as the site of attachment for the hexose in the viral nucleic acid molecule. If the three nucleic acids are treated with dilute alkali their glucose content remains unchanged, a fact which indicates that the hexose is not bound to phosphoric acid as a tertiary ester.
In order to ascertain whether thymine might be combined with glucose, the apurinic acids have been hydrolyzed with barley deoxyribonuclease, an enzyme present in malt diastase. After treatment with this enzyme some 30-60 per cent of the apurinic acids are converted to nucleosides and mononucleotides. Analyses of the degradation products have revealed that glucose is bound only to the hydroxymethylcytidine and to hydroxymethylcytidylic acid; thymidine and thymidylic are not in combination with this sugar. In addition, it has been found that the amount of glucose present in the oligonucleotides produced by the enzyme is related to the content of hydroxymethylcytosine. Thus it is apparent that thymine does not serve as the site of attachment of the hexose and hence the glucose component of the three viral nucleic acids must be linked only to hydroxymethylcytosine.

Laboratory of Walter A. Jacobs
S. William Pelletier, David M. Locke

During the past year this laboratory has continued its long-range investigations of the molecular structures of the aconite alkaloids and of a few problems remaining from earlier studies with the veratrine alkaloids. The latter group has occupied Drs. Pelletier and Locke and the former has been divided between Drs. Jacobs and Pelletier. During the past decade many workers in other laboratories have been attracted to these fields, and have made important contributions so that along with those of this laboratory, the structures of most of the veratrine alkaloids have been solved. In much smaller measure this is true of the aconite problem. The first clue to the general category of the aconite alkaloids, which showed their probable relationship to the diterpenes, was found in this laboratory about 14 years ago. Later studies here and elsewhere substantiated this conclusion. The aconite alkaloids fall into a number of categories: the highly oxygenated and very toxic aconitine group of ester bases, which includes the classical aconitine and
delphinine; the less oxygenated and less toxic atisine group; and
corably related aconite base, lycoctonine, was made by Przybylska and
Marion in Ottawa from X-ray studies on one of its derivatives. This
led them to an almost conclusive picture of its hexacyclic structure
with a modified diterpenoid core for its six rings and with satisfac-
tory arrangements for its ethyl-bearing tertiary nitrogen atom and
other groups. Such a structural skeleton has also been tentatively
adopted to explain data obtained with several other highly oxygen-
ated aconitine bases. The attempt was then made to reconcile the
accumulated data obtained in this laboratory from delphinine to
such a structure but without success. A different hexacyclic diter-
penoid structure was tentatively suggested last year to explain the
data. However, recent results have shown such a structure to be
incorrect. But all of the data likewise cannot be satisfactorily ac-
commodated to a lycoctonine ring system without assuming still
unexplained rearrangements during the formation of some of the
delphinine transformation products.

To shed further light on the problem and because of certain
conflicting data published elsewhere, a return was made to delphi-
nine derivatives, some previously used, in which the esterified
hydroxyl groups have been exposed by saponification. The results
continue to show that delphonine, the saponification product of
delphinine, does not contain a hydroxyl group adjoining its nitro-
gen atom. It was necessary also to repeat the study of the exhaustive
methylation of delphonine. This confirmed the previous results of
the laboratory and showed that the process could not be brought beyond the stage of the first methine base and that no appreciable amount of nitrogen-free material could be isolated, contrary to claims elsewhere.

In another direction the oxidation product of delphinine, \( \alpha \)-oxodelphinine, was found to yield a crystalline \( \alpha \)-oxodelphonine on saponification which from former studies must have a tertiary hydroxyl group adjoining the liberated secondary hydroxyl group. This substance on careful oxidation yields an intermediate crystalline \( \alpha \)-hydroxyketone, \( \alpha \)-oxodelphonone, which infrared data have shown to be a cyclopentanone derivative. Therefore the original benzoylated hydroxyl group which adjoins the free tertiary hydroxyl group in the parent alkaloid is on a penta-atomic ring carbon atom. As an \( \alpha \)-hydroxyketone, \( \alpha \)-oxodelphonone, is further oxidized with ring cleavage to a keto acid in which, from infrared data, the newly formed keto group is situated on a six carbon or larger ring. Similar data were obtained with other delphonine derivatives, a description of which is beyond the scope of this report. Studies with aconitine have also been in progress. While the results thus far obtained have contributed a great deal to our understanding of many features of these alkaloids, much remains to be done to obtain a complete and unambiguous answer to this very difficult problem.

The study of members of the atisine group of alkaloids has continued. In atisine itself the exact positions of its allylic terminal methylene and secondary hydroxyl groups seem still in question. Definite positions were provisionally assigned on the basis of the isolation of 1-methyl-6-ethylphenanthrene from the dehydrogenation of atisine and certain of its derivatives. However, an alternative arrangement seems possible and further evidence along this line is being sought. Certain degradive schemes are therefore under investigation to determine this point. Experiments designed to shed light on the stereo-chemistry of ring D in atisine are also underway.

During the course of the isolation of atisine from \( A. \) heterophyllum a new alkaloid, atidine, was encountered which was shown to
have the molecular formula, $\text{C}_{22}\text{H}_{33}\text{NO}_{5}$. Chemical and infrared absorption studies indicate that one of the oxygen atoms is present as a ketone in a six- or larger membered ring while the other two oxygens exist as primary or secondary hydroxyl groups. Also present are a terminal methylene group, a C-methyl and a tertiary nitrogen atom. An $\text{N-CH}_2\text{CH}_2\text{OH}$ group in the molecule is also indicated by the formation of glyoxal on oxidation with lead tetraacetate. These data suggest that atidine is a pentacyclic tertiary base of the dihydroatisine type containing a carbonyl group in a six-membered ring. Experiments designed to relate atidine directly to dihydroatisine are in progress.

Work on heteratisine, another minor alkaloid of A. heterophyllum, has continued and significant data as to the type of functional groups present and their relationship to each other have been obtained.

In the field of the veratrine alkaloids the program designed to confirm the identity of rubijervine as $\Delta^5$-solanidine-3$\beta$, 12$\alpha$-dial previously found by this laboratory has been completed. Infrared and optical rotatory dispersion studies on rubijervone-12 have been shown to be in agreement with the assignment. Reduction of this ketone with sodium-in-alcohol gave a new compound 12-epirubijervine, showing that the 12-hydroxyl in rubijervine is axial. Molecular rotation data for rubijervine and 12-epirubijervine derivatives were also in agreement with assignment of the 12$\alpha$- and 12$\beta$-configurations respectively to these compounds. Two previously formed dehydrogenation products of rubijervine have also been identified. The earlier interpretation of the hydrocarbon, $\text{C}_{18}\text{H}_{16}$, as 1'-methyl-1,2-cyclopentenophenanthrene has been confirmed by synthesis. The phenol, $\text{C}_{18}\text{H}_{16}\text{O}$, has been converted to the same hydrocarbon, and its infrared spectrum leads to the conclusion that it is 1'-methyl-1,2-cyclopentenophenanthrol-3. The appearance of these compounds with rearranged carbon skeletons in a selenium dehydrogenation is of some theoretical interest, since no ready explanation for their appearance can be made.
In order to convert the phenol, \( \text{C}_{18}\text{H}_{16}\text{O} \), to the parent hydrocarbon it was necessary to adapt for use with small amounts of material the Kenner and Williams method for the conversion of phenols to aryl diethylphosphates and their subsequent reduction with sodium-liquid ammonia. This method had been previously used only with larger amounts of mono- and dicyclic compounds. In addition to using this new method with the phenol from rubijervine, it has been shown to be effective on a semi-micro scale with a number of other polycyclic phenols, both mono- and dihydric. This conversion promises to be a useful new technique for the natural product chemist.

Laboratory of Stanford Moore and William H. Stein

 Structural Studies on Ribonuclease. For a number of years this laboratory has been concerned with the biochemistry of proteins, peptides, and amino acids, with particular attention to the development and application of methods for determining the chemical structure of proteins. The structure of the enzyme ribonuclease has been under intensive investigation, and as a result of these studies, coupled with the findings of Dr. C. B. Anfinsen and his associates at Bethesda, it is possible to write the nearly complete structural formula given schematically in Fig. 1.

In advancing from the partial structural formula for the enzyme, which was described a year ago, the exact sequences which have been elucidated by Dr. Hirs have now accounted for about three-quarters of the molecule. In this work, thirteen peptides formed by the action of trypsin on performic acid-oxidized ribonuclease have been the starting products. The nine smallest could be studied directly, but the four larger ones, which contained about 20 amino acid residues each, were first hydrolyzed with chymotrypsin to give 14 smaller fragments. In determining the sequence of the amino
Fig. 1. A two-dimensional diagram of the ribonuclease molecule, incorporating the amino acid sequences established to date. The sequences of 90 out of the 124 residues have been determined by Dr. Hirs. The residues in parentheses, and separated by commas, are of as yet undetermined sequence. The positions of the disulfide bonds have been established by the research of Dr. Spackman and of Drs. Ryle and Anfinsen in Bethesda.
acids in these 23 peptides, none of which contained more than eight residues, Dr. Hirs has employed a variety of chemical and enzymatic techniques. Each stage in the degradation of a peptide has been monitored by quantitative amino acid analyses.

Concurrently with the sequence work, Dr. Spackman in this laboratory and Drs. A. P. Ryle and C. B. Anfinsen at Bethesda have been attempting to determine which pairs of half-cystine residues (numbered I to VIII in Fig. 1) are connected by disulfide bonds. For this purpose, Dr. Spackman has submitted unoxidized ribonuclease (in which the disulfide bonds are intact) to the action of trypsin and chymotrypsin, and he has isolated two cystine-containing peptides from the hydrolysate. From the amino acid composition of these peptides, it could be concluded that one disulfide bond links half-cystine residues I and VI and another unites residues IV and V. Ryle and Anfinsen, proceeding in a different manner, also have found evidence pointing to a I-VI and a IV-V bond, and in addition, have succeeded in demonstrating a linkage between half-cystines II and VIII, and III and VII. All four of the disulfide bonds thus appear to be accounted for, and from their arrangement (Fig. 1), it is clear that the peptide chain in ribonuclease is tightly tied together.

With the structural work advancing toward completion, efforts have been initiated by Dr. Uziel to determine which particular portion, or portions, of the large ribonuclease molecule is responsible for its catalytic activity. To this end, attempts have been made to degrade the enzyme with trypsin and then to isolate parts of the molecule that are still active. The preliminary steps in this direction are being accompanied by study of other means of altering the molecule.

**Investigations on Other Proteins.** The structural studies that have been carried out on proteins and large peptides in this and other laboratories have not yet led to the recognition of any common plan for the arrangement of the amino acid residues in substances such as insulin, ACTH, glucagon, or ribonuclease. Nor has it been
possible to deduce very much about the metabolic machinery that synthesizes these molecules, except that it is amazingly precise. The fact that success has attended the attempts to elucidate the structures of these substances indicates that each molecule of bovine ribonuclease (or of the insulin from a given species) is exactly like each other molecule. A particularly striking example of this unique synthetic specificity has been encountered in the course of an investigation of the amino acid composition of the normal and pathological human hemoglobins. The samples analyzed by Dr. Spackman and Dr. Cole were prepared electrophoretically by Dr. Henry Kunkel. It was found that the hemoglobins prepared in this way all contained less isoleucine and less cysteine than had been reported heretofore, indicating that most of the previous samples of this protein had not been pure. The present analyses have been sufficiently precise to permit the conclusion that human adult hemoglobin contains less than 0.02 per cent isoleucine. So vanishingly small a quantity corresponds to a maximum of one residue of isoleucine per 10 to 12 molecules of hemoglobin. This same number of hemoglobin molecules contains from 600 to 700 residues of valine and from 700 to 850 residues of leucine, two amino acids that structurally are extremely similar to isoleucine. Not as often as once in all of these opportunities does the enzyme system synthesizing hemoglobin make a “mistake” and insert an isoleucine residue in place of one of valine or of leucine—a notable example of biological specificity.

The structural investigations summarized above have required the performance of hundreds of quantitative amino acid analyses, the expeditious completion of which would not have been possible without the automatic recording equipment developed over the past three years in collaboration with Dr. Spackman. This apparatus, which permits a complete analysis to be turned out every 24 hours with a minimum of labor, has been described previously in these reports.

*Reductive Cleavage of -S-S- Bonds.* Before the type of structural
analysis carried out with insulin and ribonuclease can be employed to elucidate the structure of other proteins, a method must be developed for cleaving disulfide bridges in proteins that contain tryptophan. The oxidative procedure used in the case of ribonuclease and insulin, which are unusual in that they lack tryptophan, would cause extensive decomposition of this amino acid. Accordingly, Dr. Bailey and Dr. Cole have been attempting to apply to proteins the reductive cleavage by sodium sulfite that has long been known to split -S-S- bonds in simpler molecules. Insulin and ribonuclease were studied first. By carrying out the reaction in urea, it has been possible to convert all of the half-cystine residues in both of these proteins to the -S- sulfonate derivatives. The two peptide chains thereby generated by the splitting of the insulin molecule, could be separated by ion exchange chromatography. The extension of this sulfite cleavage to other proteins is being studied.

**Chromatographic Purification of Trypsinogen.** The use of trypsin as a specific hydrolytic reagent in studies on the chemical structure of proteins has emphasized the need for preparations of this enzyme possessing a high degree of enzymatic homogeneity. Trypsin itself is difficult to purify, but Dr. Tallan has been able to chromatograph the zymogen, trypsinogen, on columns of the carboxylic acid resin IRC-50. Activation of such purified trypsinogen should provide trypsin free from other proteolytic enzymes. In the course of this work, evidence has been secured for the existence of more than one form of the zymogen.

**Cystathionine in Human Brain.** The chromatographic methods developed primarily for research on proteins have been applied in the past few years to a study of the distribution of amino acids and related compounds in physiological fluids and mammalian tissues. Of particular interest was the identification by Dr. Tallan, reported a year ago, of N-acetyl aspartic acid as a major constituent of the brain and spinal cords of higher animals, including man. The metabolic role of acetyl aspartic acid in nervous tissue is unknown, but investigations of this problem begun here by Dr. Tallan are
being continued by him in the laboratory of Prof. H. A. Krebs at Oxford, where Dr. Tallan is spending a year on a U.S.P.H.S. Fellowship.

Fig. 2. The free amino acids and related ninhydrin-positive compounds in an extract of human brain tissue. Cystathionine is seen to be one of the major amino acids in human brain. The effluent curve is a tracing of a chromatographic analysis carried out by Dr. Tallan employing automatic recording equipment. The resin employed was Amberlite IR-120, and the eluents were chosen primarily for the separation of the amino acids emerging at or near the cystathionine position.

In pursuing further the investigations of the amino acids of brain tissue, a survey was made by ion exchange chromatography of the free amino acids in extracts of human brain (Fig. 2). An unexpected major peak was observed upon the effluent curve. The compound responsible for this peak was found in smaller although measurable amount in monkey brain, but only in trace amounts in other human tissues and in the brains of other lower animals.
studied. The amino acid has been shown to be L-cystathionine, the
metabolic role of which was established a number of years ago by
the work of du Vigneaud. Until now this sulfur-containing amino
acid had not been shown to be present in measurable quantities in
mammalian tissues. The special cause for its high concentration in
human brain is not known.

Laboratory of D. Wayne Woolley
R. B. Merrifield, E. N. Shaw, J. M. Stewart, G. L. Tritsch

This laboratory is interested in the nature and modes of action of
the vitamins and other compounds which are essential to living
things. The understanding of the chemical structures of vitally
essential compounds and of their specific roles in living processes
allows one to know about the causation of natural phenomena, and
has led directly to means of controlling some of them. During the
past year the following points of interest have been studied:

How to increase specifically the serotonin of the brain. Previous
work from this laboratory had indicated that the hormone sero-
tonin was very important in the maintenance of normal mental
processes, and that disturbance in the functioning of serotonin in
the brain may be the causative factor in schizophrenia. Two possi-
bilities arose. One was that the disease was the result of a deficiency
of the hormone in the brain, and the other was that it was the result
of too much of it there. A decision between these two alternatives
could not be made by injection of serotonin into schizophrenic pa-
tients, because it was found that injected serotonin, at least in
laboratory animals, did not reach the brain. A method of increasing
brain serotonin was thus needed. This has been found. It consists
of administration of the precursor of serotonin, viz. 5-hydroxy-
tryptophane, along with a special antimetabolite of the hormone,
viz. BAS (benzyl analog of serotonin). The BAS protects the pe-
ripheral tissues, but not the brain, from excess serotonin. This BAS
Biochemistry

is the special antagonist of the hormone found in this laboratory 2 years ago. Clinical experiments with schizophrenic patients given BAS plus hydroxytryptophane are now in progress. There are indications that these experiments will reveal much about the cause of this disease, and may point the way to a useful treatment.

**New and more potent antimetabolites of serotonin.** The study of antimetabolites of serotonin initiated in this laboratory in 1951-2 has continued in an effort to find compounds better than BAS for the treatment of hypertension and certain other diseases. Emphasis has been placed on finding more active drugs of this new series. One which gives considerable promise is BAS-phenol, which, in laboratory animals, is ten times as potent as BAS. BAS-phenol has been synthesized for the first time, and its pharmacology has been investigated.

*The nature of the serotonin receptor.* This is part of a study of the basic mode of action of serotonin. Through the use of BAS and BAS-phenol it has been possible to show that the serotonin receptors in living animals are different from the tryptamine receptors, and from those for adrenaline, noradrenaline, and acetylcholine.

**Synthesis of the natural precursor of the purines.** Although it has been known several years that the immediate precursor of purines such as inosinic acid in animals and microorganisms is the riboside of 4-amino-5-carboxamidoimidazole, this substance has not been studied more extensively because it is so rare. All of it must be isolated from natural sources at great labor and expense. A chemical synthesis of this riboside has now been achieved, so that it should become readily available. The synthesis starts with inosine, which is selectively benzylated and reduced to the desired compound.

**Synthesis of the toxin of the wildlife disease of tobacco.** This laboratory spends much effort in the synthesis of new compounds because it is in this way that rare materials of great physiological significance are discovered and made available for study. It is also in this way that their structures are finally proved. One such is the
toxin of the wildlife disease of tobacco. This toxin was first isolated here. Final proof of its structure now rests on attempts to synthesize it. To this end the 4 isomers of \( \alpha, \epsilon \)-diamino-\( \beta \)-hydroxypimelic acid have been synthesized, and their configurations established.

Isolation of a new peptide with high strepogenin activity. Strepogenin is a vitamin required for optimal growth of some microorganisms, and possibly of animals. In the preceding two years a pure compound showing this activity was isolated for the first time. Its structure was established as serylhistidylleucylvalylglutamic acid. This was confirmed by total synthesis. A new peptide with somewhat greater activity has now been isolated, and its structure has been deduced from chemical degradations. It is the disulfide of leucylvalylcysteinylglutamylarginine, and it was obtained from enzymic digests of insulin.

Synthesis of peptides related to strepogenin. In an effort to learn more about the mechanism by which strepogenin promotes bacterial growth, a number of suitable relatives of serylhistidylleucylvalylglutamic acid have been synthesized chemically and investigated biologically. These studies have indicated that the presence of serine is very important. Based on this finding two very potent and specific antagonists (antimetabolites) of strepogenin have been made.

Antimetabolites of peptides are very rare, but indications are that they will be of considerable importance in the understanding of some vital processes, and possibly in the control of these processes when they go astray.

Laboratory of Sam Granick
David C. Mauzerall

Studies of porphyrin biosynthesis have been continued. The formation of an early precursor, \( \delta \)-amino levulinate by hemolyzed chicken erythrocytes supplied with glycine is enhanced when the following substances are added: pyridoxal-\( \text{PO}_4 \rangle \text{CoA} \rangle \text{glutamine} \)
>DPN (not DPNH). The effect of glutamine is inhibited by azaserine. Perhaps these substances may be found to aid in the maintenance of the life of the red cell in storage.

Certain control and protective mechanisms in porphyrin biosynthesis have become apparent: (1) The formation of δ-aminolevulinic acid in chicken erythrocytes is inhibited by a number of amino acids: cysteine > serine > arginine > proline. Conceivably a partial block in the formation of δ-aminolevulinic acid is removed when such amino acids reach a high concentration relative to glycine. Thus, in erythroblasts during rapid globin synthesis, such amino acids might be diminished to a low level and permit increased δ-aminolevulinic acid formation. (2) Pyruvate and α-keto butyrate also inhibit δ-aminolevulinic acid formation and the rate of their production or removal may also be factors that govern δ-aminolevulinic acid production. (3) The relatively high glutathione content of erythrocytes serves to maintain the activity of at least two of the known SH-enzymes of porphyrin biosynthesis: the enzyme which condenses δ-aminolevulinic acid to porphobilinogen and the enzyme which decarboxylates uroporphyrinogen to coproporphyrinogen. In addition glutathione serves to decrease the autoxidation of the colorless porphyrinogens which are the actual intermediates at these stages of porphyrin biosynthesis. (4) The high protein content of red cells serves both to stabilize the δ-aminolevulinic acid enzyme which is relatively unstable in dilute solution, and to protect porphobilinogen from undergoing spontaneous condensation reactions.

An enzyme fraction which converts type III coproporphyrinogen to protoporphyrin is present in relatively low activity in the particulate portion of chicken erythrocytes and in high activity in the particulate portion of Euglena. O₂ is necessary for the oxidation but the reaction is not cyanide sensitive. As expected, coproporphyrin is inactive. The enzyme preparation is also inactive towards type I coproporphyrinogen. This fact might well explain the reason for the absence of a long sought for protoporphyrin
isomer other than that related to type III, even in congenital porphyrinuria where coproporphyrin I is produced in red cells in relatively large amounts.

We have also investigated the intriguing photoreduction of certain dyes (e.g. thionins and flavins) by amino acids of the ethylenediaminetetra-acetic acid type reported by various workers. Our results still do not decisively distinguish between direct reduction of the photoexcited dye by the organic acid and reduction by water. The latter reaction would be of great interest in relation to mechanisms of photosynthesis. In these experiments we have profited greatly by talks with Dr. G. Osper of Brooklyn Polytechnic Institute.

Laboratory of Gertrude E. Perlmann  
Olga O. Blumenfeld, Mary J. Mycek

The study of protein transformation by selected enzymes whose attack on specific linkages in the molecules leaves the protein relatively intact has been continued, emphasis being placed on the type of modifications an enzyme undergoes while still retaining its biological function.

The action of “pepsin on pepsin” as a model for such reactions has been selected for investigation. The findings of the past year, together with those previously made, will be summarized briefly: if pepsin, at temperatures above 20°C., is exposed to concentrated urea solutions, an appreciable loss of activity occurs over a period of 24 hours. This loss of activity is accompanied by a shift of the absorption maximum of the protein from λ2780 to λ2750 Å and by the appearance of non-protein material. Although exposure to aqueous urea causes denaturation of proteins, in the case here autodigestion was postulated as the underlying mechanism.

This hypothesis is further supported by the following observations. Dr. Blumenfeld has shown that pepsin in 8.0 M urea at 37°C. remains fully active for a period of 30 minutes but that
appreciable amounts of trichloroacetic acid soluble and dialyzable material are formed within 2 to 5 minutes after contact.

In contrast with other proteolytic enzymes, such as trypsin and chymotrypsin, the optical rotation of pepsin is not altered by prolonged exposure to urea. Since such rotation is considered to be a sensitive index of structural changes one must conclude that pepsin is relatively stable in this regard although the spectral shift noted above suggests that some hydrogen bonds involving the tyrosine residues are affected by urea. Thus the picture derived from these results is that only a few hydrogen-bonded crosslinks have to be broken to render certain peptide bonds accessible to enzymic hydrolysis and to initiate autodigestion.

As shown previously with the aid of electrophoretic analysis, the non-dialyzable material of pepsin, exposed to urea, consisted of a protein P' with the mobility of pepsin and two additional components, p₁ and p₂. This mixture has a specific activity similar to that of the parent substance. Although the possibility remains that P' is unmodified pepsin, the experiments described below suggest otherwise. Dr. Mycek has separated the active component, P', by zone electrophoresis on polyvinyl resin, Geon 426, with 0.1 N sodium acetate of pH 4.6 as solvent. In comparison with the unmodified enzyme the best preparations of P' thus far obtained have an activity of 140 to 150% per unit nitrogen or 180 to 200% per unit of protein tyrosine as measured by UV absorption.

Preliminary amino acid analysis, carried out by Dr. Spackman, revealed a difference between P' and pepsin not only in the content of tyrosine but also in that of lysine, arginine, glycine and proline. An entirely new and unexpected finding was the occurrence, in the chromatogram, of a peak corresponding to hydroxyproline. Using chromatography on Dowex 50, 8% cross linked, and a colorimetric method specific for this amino acid, Dr. Mycek has shown that two different preparations of P' contained hydroxyproline. This is the first evidence for the occurrence of this amino acid in proteins other than those of the collagen group.
From the present observations it is not possible to state whether the urea-modified pepsin represents a mixture of molecules which differ from each other by small changes in their amino acid composition brought about by autodigestion or whether the original pepsin molecule has been broken down to smaller units which reaggregate to form a complex of a molecular weight of 30,000 to 35,000.

It is clear that the biological activity of pepsin requires a specific configuration within an as yet undefined portion of the molecule and, therefore, the emphasis is being placed on the question of the smallest possible unit which still functions as an enzyme.

BIOPHYSICS

Laboratory of Frank Brink, Jr. and Detlev W. Bronk
Clarence N. Connelly, S. C. Cheng, William P. Hurlbut

Nerve cells are studied usually in order to understand the activity of the nervous system of which they are the constituent units, or to investigate the nature of physical and chemical processes which are relevant to the physiology of cells in general. Our research has been directed primarily to the latter objective, although our ultimate aim is to understand more fully the cellular basis of the nervous control of the body.

The excitability of the axons of nerve cells and the resultant conduction of nerve impulses are dependent on chemical sources of energy. We have given especial attention to the questions: How do these sources of energy maintain the capacity of nerve cells to respond to excitation, and what is the relation of the electrochemical process of impulse conduction in a nerve fiber to its oxidative metabolism? All of our experiments have been performed on the readily accessible and easily excised nerve of frogs.

Relations between rate of oxygen consumption and the ionic content of nerve. Nerve cells have a relatively high concentration of
potassium and low concentration of sodium; this is of especial significance because the distribution of these ions inside and outside a nerve fiber is related to the electro-chemical processes involved in the conduction of a nerve impulse. It is a reasonable hypothesis that these differences of concentration across the surface of a nerve are maintained by energy derived from chemical processes which utilize oxygen.

We previously reported a loose correlation between the degree of depression of the rate of respiration and the rate of loss of potassium and rate of gain of sodium. As a continuation of our studies on the relationship between the oxidative metabolism and the ionic distribution in peripheral nerves we have investigated the reversibility of the changes in ionic contents induced by chloretone, azide and anoxia. It was found that peripheral nerves placed in oxygenated Ringer's solution slowly reverse the changes in ionic contents that had been induced by five hours of anoxia or treatment with azide. The effects of chloretone at concentrations which act primarily through respiratory interference (less than 15 mM) were also reversible, while the changes induced by high concentrations of this narcotic were completely irreversible. Thus, the metabolic machinery for reconstituting the ionic content of the nerve is not destroyed by suppression of oxidative metabolism for many hours. This is further evidence for the crude physical picture of a nerve as an electro-chemical machine coupled to a distinct set of energy-yielding chemical reactions. A brief study of the effect of temperature has been made. Reducing the temperature from 20° to 2°C. reduced the resting rate of respiration of frog nerves by 80%, but produced only small changes in the ionic contents of the tissue. The effects of azide and anoxia on ionic contents were much reduced at the lower temperature. Again, chloretone at concentrations less than 15mM appeared to affect the ionic distribution primarily as a result of respiratory interference, while higher concentrations produced large changes in ionic contents apparently unrelated to oxidative metabolism.
Effects of activity on the electrical properties of nerve and its ionic content. Potassium moves out of nerve and sodium moves in during the conduction of a train of nerve impulses; there is a reverse movement during the recovery of the resting state of nerve. In order to further our understanding of these processes, we have observed the electrically measurable properties of nerve during activity and recovery. Studies of the slow change in membrane potential (the positive after-potential) that accompanies and follows activity in frog nerve fibers have continued and correlations between this hyperpolarization and increase in oxygen utilization have been extended.

The amplitude of hyperpolarization depends upon frequency of response below about 25 impulses per second but appears to approach a maximum level at higher frequencies. Above 25 impulses per second, the longer the sequence of impulses the longer hyperpolarization persists afterward, as if continued activity were producing in the nerve an accumulation of a substance. This interpretation is based on the assumption that the removal of the material during recovery has associated with it a hyperpolarizing electromotive force. The area under the hyperpolarization-time curve varies approximately linearly with the duration of tetanus, suggesting that under the conditions of the experiments the magnitude of hyperpolarization is a linear measure of the rate of a recovery process. The hypothesis that this process is the outward transport of sodium ions is supported by the observation that substitution of lithium for sodium in the bathing solution almost completely eliminates hyperpolarization. The increase in oxygen uptake that is normally associated with activity is similarly effaced by this procedure. This evidence will be strengthened if it can be demonstrated that during recovery from activity lithium ions are extruded from frog axons much less rapidly than are sodium ions.

After a tetanus of frog nerve at a frequency greater than 25 impulses per second, the time course of hyperpolarization does not follow a simple exponential curve back to zero nor do recovery curves after tetani of different durations have the same form. An
Biophysics

The analysis of recovery in terms of a saturable ion-transporting reaction mechanism fails to predict the shapes of recovery curves observed after long tetani. A considerable body of electrical evidence indicates that the ionic exchanges that occur during activity in myelinated nerve take place across the nerve membrane at the nodes. If the transport processes associated with recovery are also confined to the nodal areas, then ions that enter during activity but are not immediately extruded must diffuse along the axis cylinder into the myelin-protected internode and cannot be extruded until they diffuse back to the node during recovery. To investigate the effect of diffusion in the internode on the kinetics of ionic recovery a vacuum tube analog circuit has been utilized. It has been found that families of observed hyperpolarization curves may be largely duplicated by a circuit which is an analog of a saturable ion-extruding mechanism at a node associated with a diffusion field in the internodal spaces.

Additional chemical characteristics of active nerve. An attempt to separate the adenine nucleotides and creatine phosphate from a single sciatic nerve was unsuccessful. We are dealing with millimicromoles of substances, and the ion exchange method is found unreliable in this range. However, it did give some information confirming reports in the literature that the adenosine triphosphate and creatine phosphate levels change when nerves are stimulated anaerobically. After two hours of anaerobic stimulation at 50 impulses per second, there was a drop in the concentration of adenosine triphosphate and creatine phosphate and a rise of adenosine diphosphate concentration. Continuation of these studies is based on the determination of adenosine triphosphate with the enzyme luciferase. This method, in our hands, can measure $10^{-10}$ mole of adenosine triphosphate. Adenosine diphosphate can be determined by converting it to adenosine triphosphate through the myokinase reaction, and creatine phosphate by conversion through the phosphocreatine kinase reaction. Utilization of these sensitive specific methods may provide direct evidence for the sequence of processes that couple impulse conduction to oxidative phosphorylation.
The retina of the eye is more than a simple mosaic of light-sensing receptors; it is a nervous center as well. Cellular elements in neighboring retinal pathways interact so that the ultimate patterns of optic nerve activity are not mere reproductions of the patterns of light and shade on the receptor mosaic. Instead, certain significant features of the retinal image are accentuated by this integrative action. Sudden changes in light intensity give rise to vigorous bursts of optic nerve activity; the movements of lighted and shaded areas over the retina are signalled, and the contrast at borders and edges is enhanced.

In the vertebrate retina these complex integrative reactions are the result of an elaborate interplay of both excitatory and inhibitory interactions. In a more primitive invertebrate eye (*Limulus*) the interaction of neighboring retinal elements is purely inhibitory, and its effect on the pattern of optic nerve activity is therefore simpler and more readily analyzed.

The activity of each receptor unit (ommatidium) in the compound eye of *Limulus* depends not only on the amount of excitation furnished by the illumination upon its facet, but also on the amount of inhibition exerted on it by its neighboring receptors. This depends on the number of active neighbors and on the degree of their activity, which is, for each one, the resultant of its excitation and whatever inhibition is exerted on it in turn by its neighbors. The responses of the receptor units are thus mutually interdependent in a manner that can be described concisely by a set of simultaneous equations.

We have made a detailed quantitative study of the interactions of receptor units in the eye of *Limulus* under steady conditions of illumination. When only two neighboring units are active we have found, by recording simultaneously the activity of each, that the
amount of inhibition each exerts on the other is a linear function of the frequency at which it discharges impulses in its optic nerve fiber. When more than two units are active, the augmented set of simultaneous equations that are required can be constructed by including in the equations for the activity of each receptor linear terms representing the inhibitory influences from all the other receptors, combined by simple addition. These equations permit us to account quantitatively for the inhibitory effects of two small spots of light shining on the eye in a variety of configurations near a receptor from which optic nerve fiber activity is being recorded (Hartline and Ratliff).

When the pattern of illumination on the receptor mosaic of the Limulus eye is changed, the pattern of optic nerve fiber activity changes as the receptors react to the new levels of excitation and as they readjust their activity to the altered pattern of mutual inhibition. The transient changes that accompany the reaction and readjustment are complex. Indeed, we have found it possible to reproduce in an eye of Limulus some of the kinds of response patterns characteristic of the vertebrate retina. By carefully balancing the relative amounts of light on a receptor and on its near neighbors, conditions can be found for which short bursts of impulses are elicited only at the onset of illumination or at its cessation, or both. Thus by controlling the excitation on a small group of receptors and utilizing the inhibitory interaction that is a property of the eye, the interplay of these two opposing influences can be manipulated to obtain responses very similar to those observed in a complex eye where the interplay is determined by neural processes within the ganglionic structures of the retina (Ratliff and Mueller).

The histologic structure in the eye of Limulus that appears to mediate the inhibitory interactions is a plexus of nerve fibers interconnecting the individual ommatidia. There are no ganglion cells to be found in this plexus, but electron microscopy has revealed regions of densely matted neuropile scattered throughout the network along axons from the ommatidia. Within the fine nerve
branches that compose these clumps of neuropile are typical “synaptic vesicles.”

Studies are also being made of the ultra-structure of various invertebrate photoreceptors. The eye of the scallop, _Pecten_, is especially interesting. It possesses a double retina, the distal layer of which is composed of sense cells that respond only to a decrease in illumination (evidently subserving the vigorous shadow reaction that these animals exhibit). These distal sense cells, we have found, possess concentric or coiled laminated appendages that are derivatives of cilia, as is clearly shown by the electron microscope. We believe these organelles are the sites of photoreception in the sense cell. If this is so, they are analogous to the outer limbs of the rods and cones of the vertebrate retina, which are also derivatives of cilia, and they differ markedly from the rhabdomers of the arthropod photoreceptors which we have shown are comprised of numerous microvilli of the receptor cell surface, densely packed in a honeycomb-like structure (Miller).

Electrophysiological studies are being conducted on the responses of invertebrate photoreceptors to light. The eyes of the sandworm, _Nereis_, have been shown to yield a slow retinal action potential when illuminated. This is one of the most primitive eyes from which electrical responses have been obtained. It has been found that the time course of dark adaptation in this eye is very similar to that observed in more complex forms (Enger).

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Biophysics

ods to the solution of problems in biology and medicine. The principal effort of the laboratory has gone into the development of an ultraviolet color-translating television microscope. This instrument gives a color representation of the distribution of various chemical constituents in living cells, making use of their differing characteristic absorptions in the ultraviolet. The instrument uses a combination of sequential color television and ultraviolet microspectrophotometric techniques. The specimen is illuminated, on the stage of a microscope having catadioptric reflecting objectives and condensers, with three selected wavelengths in the ultraviolet or visible. The image is reproduced on a color television receiver. The minimum spectral bandwidth in the present instrument is $5\mu$ which permits the differentiation of small absorption shifts in the specimen. The ultraviolet dosage to the cells is kept to a minimum by ultrafractionating the light into approximately $1$ millisecond bursts $1/60$ of a second apart. Previous methods required photography with the specimen immobilized or constant illumination using an ultraviolet sensitive vidicon. There is evidence to indicate that the intermittent illumination reduces or eliminates radiation damage to the specimen. The difficulties which were previously encountered with the low sensitivity of the vidicon camera used and with the retention of the image from frame to frame have been overcome by the use of a special ultraviolet sensitive image orthicon tube and a black and white standard image orthicon camera chain which has been converted in our laboratory to sequential color operation at a 20 cycle repetition rate for each field. The brilliance of the light sources has been effectively increased by pulsing them during the vertical retrace time of the system. With these alterations the instrument has now been developed to the stage of a practical tool for the study of cells and tissues in their natural unaltered state. For the first time, it has been possible to take motion pictures in color from the screen of a color television receiver which closely represent the natural absorptions of living material in the ultraviolet. In order to explore the potential appli-
cations of this instrument motion pictures have been made showing capillary circulation, the protoplasmic streaming of amoeboid cells, the manipulation of Kupfer cells containing phagocytized colloidal iron and a number of connective tissue elements present in living mesentery. The results we have obtained have previously been possible only on a monochromatic basis using monochrome television techniques. The availability of this instrument as a research tool has made possible the initiation of new investigations into the morphology of exfoliated cells from the lining of the stomach and the buccal pouch (and the attempted observation of absorption changes in single muscle fibres as a result of multiple excitation). This work is being carried on in collaboration with staffs of the New York Hospital and Western Reserve University School of Medicine.

At the present stage of development of the instrument, the resolution limitation is in the ultraviolet optics and not in the electronic portions of the equipment. This points out the necessity for the development of improved apochromatic refracting objectives. Several optical firms here have been apprised of this problem and have expressed an interest in it.

Another problem is the improvement of motion picture technique. At the present time, we are using a non-synchronous camera which produces blanking bars. A synchronous movie camera which should lead to improved recording of the monitor picture is to be developed.

A number of commercial organizations have expressed an interest in producing the complete instrument for investigations outside the Institute.

Another project of the laboratory has been the development of a series of short-range ingestible telemetering capsules which broadcast physiologic data from the interior of the gastrointestinal tract or similar organs. The first "radio pill" transmits information as to ambient pressures. The pill has been successfully used on a number of volunteers and patients and records representing cyclic
variations in hitherto inaccessible regions of the small intestine have been obtained.

The first versions of the capsule use a miniature nickel-cadmium battery as the power supply for the miniaturized complete FM transmitter. The battery life limits the operations to approximately 16 hours. An improved passive capsule energized from a power source external to the body is under development. The operation of this capsule depends upon variations in the electrical characteristics of the capsule core. This will permit continuous measurement for long periods. Consideration is being given to methods of measuring, by similar means, other physiologic data of interest such as pH, temperature and the recognition of particular colors such as would indicate the presence of internal bleeding.

The use of electronic computers in public health data correlation and as a diagnostic aid in case history symptom diagnosis is being explored in another investigation. Previous work has shown the feasibility of suggesting possible diagnosis by the mechanical correlation of case history data using punched-card sorting. The use of a large scale digital type computer enables the storage of more data and permits greater flexibility in the logical processes used. Initial work has been confined to the field of hematology and a computer program has been written and is being tested through collaboration of members of the staffs of a number of local hospitals and the RCA-Bizmac computer facility in Camden, New Jersey. After successful completion of the work in hematology, it is hoped to extend the program to other fields of medicine.

The field of ophthalmology, through the medium of photoelectric and optical transducers has a number of problems in which electronic methods show promise. As an example, we have succeeded in obtaining, in collaboration with A. M. Potts, Department of Ophthalmology, Western Reserve University, continuous television display of the fundus of the eye with continuous illumination using an improved sensitive image orthicon tube. This work is to be continued in Cleveland with equipment we have made
available to Western Reserve University. The enlarged image obtained on the monitor facilitates the demonstration of salient features of the images obtained through an ophthalmoscope for instructional and measurement purposes. The possibility of compensating for involuntary tremors of the eye electronically is being considered.

Another ophthalmologic problem is the measurement of intraocular pressure changes as influenced by pharmacologic and other agents without physical contact with the eye. An initial attempt at measuring very small changes in radius of curvature of the cornea by interferometric methods showed the feasibility of picking up changes which reflected the respiration and cardiac rates of the experimental animal used which was immobilized in a Horsley-Clarke apparatus. Other optical methods which permit some degree of eye motion are being investigated. Ultrasonic apparatus for determining ocular pressure which would be reflected in the time of transmittal of sound pulses between two points on the eye and which would require only moderate contact are also being investigated. This work is being done in collaboration with I. Lowenfeld and O. Lowenstein, Department of Ophthalmology, Columbia University College of Physicians and Surgeons.

In any borderline field such as medical electronics, literature searching is a particular problem since no specific publications are prime sources of reference material. The Medical Electronics Center has, therefore, undertaken as a project the compilation of a bibliography representing source material on medical applications of electronics. We have found these references an invaluable aid in answering the numerous inquiries received from workers in this field. The scope of the bibliography is being expanded with the collaboration of a number of workers in this field outside the Institute. This bibliography is to be published by the Institute of Radio Engineers.

To facilitate communication in this interdisciplinary field, the Medical Electronics Center has collaborated in the organization
and program of the Tenth Annual Conference on Electrical Techniques in Medicine and Biology, held in Boston, Massachusetts, and, in cooperation with the Professional Group on Medical Electronics, in a Symposium on Uses of Computers in Biology and Medicine at the National Institutes of Health. Conferences being planned include an International Conference on Medical Electronics, a Conference on Artificial Internal Organs, and a Conference on Problems in Ophthalmology.

CYTOLOGY

Laboratory of Keith R. Porter and George E. Palade
Montrose J. Moses, Philip Siekevitz, Jean-Gabriel Lafontaine, Carlo Bruni, Russell J. Barrnett, James B. Caulfield, John Luft, Goetz W. Richter, Steven L. Wissig, Lee D. Peachey, and Peter G. Satir

As in the past the staff of the laboratory has continued the structural and functional analysis of the animal cell by the convergent use of electron microscopy, cell fractionation procedures and cytochemical tests made in situ. General improvements in preparatory techniques, especially in fixation and cytochemical tests, have permitted the extension of electron microscopical studies to a wider variety of materials and to a number of experimental studies on physiological problems.

Fixation. It has been found, for example, that increasing the tonicity of the standard osmium fixative by the addition of sucrose improves the quality of fixation of a number of important tissues, including the growing points of plants (Caulfield and Porter). It is important, also, to test other fixatives because the proof of authenticity of the OsO₄ preserved form depends in part on finding similar structure after the use of other reagents. Thus, it is significant that the continuing search has shown permanganate to be remarkably valuable for the preservation of the membranes that
are part of cell fine structure (Luft), and further that the structure revealed after its use is identical in most respects to that obtained after OsO₄.

**Cytochemical tests for electron microscopy.** Cytochemical tests must also be devised for direct electron microscope observation so as to carry the inquiry into the chemical composition of the cell structures down to the level of resolution of this microscope. It is required of such tests that the reaction products have a high density and that the structure of the specimen be well preserved. During the last year this potentially important field of research has been explored and a satisfactory procedure was found for the demonstration of the succinic dehydrogenase systems (Barrnett and Palade). The test, which utilizes potassium tellurite as electron acceptor and succinate as substrate, was carried out on fresh material and was followed by OsO₄ fixation. The best results were obtained with heart muscle where reduced tellurite, the end product of the reaction, could be visualized in the electron microscope as dense crystals or fine particulate deposits on, or in close relationship to, mitochondrial membranes.

**Morphological analysis.** A major object of interest among the newly revealed components of tissue cells has been the complex reticular system of membrane-limited channels referred to as the *endoplasmic reticulum*. Electron microscopy continues to reveal new patterns in the organization of this system, patterns that are expressions of cellular differentiation and sources of clues to the functions of the system.

One of the more interesting of such patterns is encountered in striated muscle where the elements of the reticulum are disposed around the myofibrils in a characteristic and precise structural relationship to the bands of the sarcomeres (Porter and Palade). During the past year the relation of this *sarcoplasmic reticulum* to the sarcolemma has been explored for continuities, but thus far only close appositional “contacts” of the membranes have been found. Experimental work is now under way to determine the functions
of the system and metabolic transport and impulse conduction are among the possibilities being examined (Porter and Peachey).

In investigations into the functional significance of elements of cell fine structure some use is being made of induced or natural pathological states. It is known that certain azo dyes and especially 3'-methyl-4-dimethylaminoazobenzene, when included in the diet of rats, is toxic and carcinogenic for the animal's liver. There is also good evidence that such azo compounds are concentrated in the microsomal component (which includes the elements of the endoplasmic reticulum) of the liver cell and that the dye interferes with intracellular systems functional in the formation of cell-specific proteins. During the past year studies have been initiated which aim to define, at the fine structure level, the morphological response of the liver cell to the administration of these dyes (Porter and Bruni). Thus far the observations are preliminary, but describe profound changes in the organization of the endoplasmic reticulum, and a reduction in the normal association of the endoplasmic reticulum with ribonucleoprotein particles, known to be important in protein synthesis. It is planned to follow the liver changes through to the development of tumors.

It is valuable in any electron microscope investigation of the distribution and origins of macromolecular components of cells to have such components "labelled" with a heavy atom. Within the period covered by this report this device has been used for a study of hemosiderin granules and their relation to ferritin (Richter and Porter). Here atomic iron serves as the dense marker. Hemosiderin deposits induced in kidney and liver cells of rats by repeated injections of hemoglobin and identified by cytochemical tests for iron were found to consist of innumerable small dense particles about 55Å in diameter. Similar particles not localized in discrete aggregates or "siderosomes" were found scattered throughout the cytoplasmic matrix. Compared with ferritin molecules, the hemosiderin particles were found to be identical.

A few years ago the fine structure of blood capillaries was inves-
tigated with the electron microscope and large numbers of small vesicles (400Å) were found in the cytoplasm of endothelial cells immediately below the cell membrane facing both the capillary lumen and the pericapillary spaces. The relationship of these vesicles with the cell membrane ranged from complete isolation to contact and continuity, in the last case the content of the vesicles being continuous with the extracellular media. To explain these findings, the hypothesis was advanced that the vesicles are active in the transport of fluid and solutes across the capillary wall (Palade). Now this hypothesis is experimentally tested by using large molecules dense enough to be seen in the electron microscope (ferritin) and by increasing the density of blood plasma (Wissig and Palade).

A systematic study of events taking place in the acinar cell of the pancreas (guinea pig) during starvation and upon feeding was carried out to determine the time at which intracisternal granules appear, and the time and mechanisms involved in the formation and discharge of zymogen granules (Palade). The information obtained was used as a starting base for the project described in the next paragraph.

**Combined morphological and biochemical analysis. (A) Cell fractionation procedures.** The work started a year ago on protein synthesis in the pancreas of the guinea pig was continued, with the aim of finding out what part is played by the various cell components in the synthesis and subsequent handling of the digestive enzymes produced by this gland (Siekevitz and Palade). Fractionation procedures adapted to the pancreatic tissue during the last year were perfected by centrifuging the tissue brei in discontinuous density-gradients and by systematically controlling the cell fractions with the electron microscope. As a result, a satisfactory fraction of zymogen granules was obtained, in addition to the usual mitochondrial and microsomal fractions, and to the recently defined post-microsomal fractions of “free” ribonucleoprotein (RNP) particles.
The distribution of ribonuclease (RN-ase) and trypsin-activatable proteolytic (TAP-ase) activity among the various cell fractions was studied and the results obtained were confirmed by the actual isolation of a protease precursor (chymotrypsinogen) from the same fractions by column chromatography. The major part of chymotrypsinogen and of RN-ase and TAP-ase activities was recovered in the zymogen fraction (\(\sim 40\) per cent), the specific activity of which was \(\sim 10\) times higher than that of the other cell fractions. The trypsin inhibitor described some years ago by Kunitz was localized in the final supernatant.

An intriguing variation in the RN-ase and TAP-ase activity of the microsomes led to a comparative analysis of this fraction in starved and fed animals. The results showed that the total and specific activity of the microsomes rise sharply one hour after feeding, approaching the corresponding values for the zymogen fraction. Simultaneously the morphology of the endoplasmic reticulum is changed in the intact glandular cell by the enlargement of the cavities of this system and by the appearance therein of dense, relatively large (0.2 to 0.3\(\mu\)) intracisternal granules. For this reason, microsomes isolated from fed animals were treated with a low concentration of deoxycholate in order to "solubilize" their membranes and were subsequently subfractionated to yield a heavy, intermediate, and light subfraction. The heavy subfraction, which proved to be a concentrate of intracisternal granules, was found to be as active as the zymogen fraction, but considerable TAP-ase and especially RN-ase activity appeared to be associated with the other microsomal components, namely the attached RNP particles, the content and possibly the membrane of the microsomes.

All these findings could be satisfactorily explained by assuming: that the enzymes a) are produced by the rough surfaced part of the endoplasmic reticulum; b) are temporarily segregated in the cavities of the system in the form of intracisternal granules; c) are packed in smooth surfaced membranes in the Golgi region; and
d) finally stored as zymogen granules in the apical region of the cell, ready for discharge at the next meal.

To test this hypothesis, the time course of the incorporation of leucine-\textsuperscript{14}C into the mixed proteins of the various cell fractions was studied \textit{in vivo}. The results indicated that the microsomes, and especially their attached RNP particles, are the loci of the most active early incorporation, and the relationship of the curves of the various fractions suggested that the newly synthesized proteins follow the pathway: attached RNP particles → microsomal content → zymogen granules. As a final step in verifying the hypothesis mentioned, the time course of the labeling of chymotrypsinogen by leucine-\textsuperscript{14}C is now followed in parallel in the microsomal and zymogen fractions.

While working on the general project of enzyme synthesis in the pancreas, two observations of potentially general interest were made and are now followed up by appropriate experimental work.

The first observation (Siekevitz and Palade) concerns a marked difference between the free RNA particles of the cytoplasmic matrix and the RNP particles attached to the membrane of the endoplasmic reticulum, viz., microsomes. The latter are considerably more active in the early incorporation of labeled amino acids into proteins. The finding suggests that each type of particle is involved in the synthesis of a different set of proteins, the attached particles being probably connected with the production of enzymes for “export” and the free particles with the synthesis of proteins for intracellular use.

The second observation (Palade) concerns the smooth surfaced membrane of the mature zymogen granules. It is derived from the stacks of flat vesicles in the Golgi region and, upon zymogen discharge, it becomes part of the plasma membrane of the apical region of the cell. The finding indicates that the membrane limiting the cell and the intracellular membranes of the Golgi zone are interchangeable, therefore either identical or closely related chemically.

(B) \textit{Cytochemical tests in situ}. Electron microscopy and cyto-
chemical tests carried in situ were successfully combined for the investigation of several problems of nuclear morphology. The first of these problems concerns the structure of the chromosome “core” (Moses).

The earlier discovery in this laboratory of a surprising array of parallel filaments (the “core”) extending along the axes of meiotic prophase chromosomes has been followed up in an attempt to define the morphology and, if possible, to discover the function of these new structures. So far, cores have been seen in the chromosomes of primary spermatocytes of a wide variety of vertebrate and invertebrate species. It is therefore unlikely that they represent a structural peculiarity of limited interest; on the contrary it appears that they are structural elements of widespread and possibly general occurrence. A core is fundamentally a tripartite group of filaments disposed, with evident bilateral symmetry, in a single plane, the central filament occupying the position of the long axis of the chromosome. On account of these features and its common occurrence in primary meiotic chromosomes, it can be reasonably assumed that the core is involved either in chromosome splitting or in chromosome pairing. The choice in this alternative can be made by finding out whether the core is part of paired or unpaired chromosomes, and this inquiry hinges in turn on the difficult problem of identifying with certainty in the electron microscope the meiotic stages during which chromosomal cores are present. The problem was solved by using the light microscope to study thick, stained sections of easily identifiable material immediately adjacent to the thin sections examined with the electron microscope. The results show that the core lies between closely paired chromosomes and suggest that the filamentous structure is implicated in chromosome pairing and disjunction and in related, genetically important events such as crossing over. The origin and fate of the cores is now under investigation. A broad survey has failed to reveal thus far a corresponding structure in mitotic chromosomes.

The smallest structural unit, the chromosome fibril, so far iden-
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tified in mitotic and meiotic chromosomes is a long twisted fibril with a diameter of 100Å or less (Moses and Lafontaine). It appears to be present in all chromosomes and in some cases it represents their major component. The arrangement of the fibrils in mitotic chromosomes has not yet shown any clear pattern or degree of order, but in meiotic chromosomes the fibrils seem to emerge from the lateral filaments of the core and appear to be radially, rather than longitudinally, disposed in relation to the whole structure. The \( \sim 100\) Å fibrils represent, most probably, the deoxyribonucleoprotein of the chromosomes, because they are found wherever deoxyribonucleic acid (DNA) can be localized in situ, and are lacking where DNA is absent. Moreover, after fixation with formaldehyde, a reagent known to fix satisfactorily nucleoproteins, the fibrils are preserved and can be demonstrated in the electron microscope without further treatment. The finding indicates that, in keeping with their assumed DNA-protein content, the fibrils have high intrinsic density.

The results of this investigation on chromosome structure are of considerable cytological as well as genetic importance. They indicate the association of genetic material (nucleoprotein) with a morphological entity (the chromosomal fibrils) and the existence of connections between these entities and the filaments of the core. With these findings we are brought closer to the devising of a new chromosome model that could be tested by experimental work in cytogenetics.

To learn more about the organization of mitotic chromosomes a correlated electron microscopical and cytochemical study of the mitotic cycle was carried out on the root meristem of the onion \textit{(Allium cepa)} and of the broad bean \textit{(Vicia faba)} (Lafontaine). Good correlation was established between the light and the electron microscope images of the nucleus and chromosomes throughout the various stages of the cycle. The observations made concern the progressive condensation of the fibrillar material of the chromosomes throughout the prethelophase stages, the fate of the nu-
Development of organisms—the stepwise transformation of an egg into a functioning mature individual—is so enormously complex that understanding of the underlying mechanisms of growth, differentiation and elaboration of shape and internal architecture depends on our ability of resolving this intricate fabric into simple elementary components which can be studied separately. We have recently concentrated on the following.

Cell Contact Relations. When cells derived from different epithelial tissues or organs are made to meet in various combinations, either during wound healing or in tissue culture, they show remarkably discriminative behavior towards each other in that those of like kinds merge, whereas those of different character bypass each other and fail to associate. Something in their surface constitution, therefore, enables cells to recognize their mutual kinship or strangeness. Because of the fundamental importance of this property in the building or dissolution of compound structures, and also because secondary surface estrangement from its mother tissue is presumably the cause of the pernicious faculty of the cancer cell to metastasize, we have undertaken to study the behav-
ior of cells on mutual contact more thoroughly by direct visualization in phase-contrast time-lapse microcinematography. The results of these observations discount any theory of selective attraction between like cells or repulsion among unlike ones; rather do they indicate that cells make contacts entirely by chance as they move about, but that once contact has been established, they react differentially so that matching kinds will remain combined, whereas non-matching combinations will secondarily break apart. Such discriminative behavior leads to the gradual sorting out of cell types within a mixed population. Dr. Moscona has added to his earlier demonstration of the fact that, in this reaction, differences of cell type override even species differences. We also found that association of cells with their own kind reduces their motility, growth and multiplication. This means that a wound engenders reparative growth automatically by depriving the cells near the wound edge of the growth-repressing association with their former neighbors. In support of this view, we observed in tissue culture that cell divisions are preferentially localized at the free border of a cell colony or at the line of encounter between two colonies of different types.

Cell Orientation. Since some of the most important formative functions of cells are achieved by shifts and movements, a further study has been given to the three major components of this problem: (a) the motile mechanism of the cell; (b) the orientation of the motile cell relative to its environment; and (c) the direction of advance along the line of orientation. Evidence was found that the motile energy is produced within the cell in rhythmic spells and is translated into protrusions of the free cell surface or of the cell margin along interfaces between the substratum and the medium. But just how the ordinary tissue cell creeps is still obscure. The mechanism of orientation, on the other hand, has been essentially elucidated. After having shown in earlier experiments that orientation can be imposed on cells at will by providing them with various microscopic or submicroscopic filaments as guides, we have now succeeded in
observing the details of the process of orientation by taking motion
pictures of cells aligning themselves on special glass gratings de-
vised by Dr. Taylor. Since differential adhesiveness of the cell to
its surroundings is instrumental in producing orientation, we have
also resumed a study of the problem of cellular adhesiveness to
wettable and non-wettable surfaces (cholesterol, paraffin, vaseline,
silicones, etc.), but we have as yet discovered no unifying principle.

Cell Locomotion. The direction in which a cell proceeds along
a given guideline has commonly been ascribed to gradients of elec-
tric potential ("galvanotaxis") or of concentrations of chemical
substances (positive and negative "chemotaxis"), and the outwan­
dering of cells from a tissue fragment in tissue culture has been
explained in those terms. New observations in our studies of popu­
lations of free cells, dispersed on glass in tissue culture, have now
not only disproven those earlier speculative notions, but revealed
the true explanation of why cells move in one direction rather than
the reverse. Neither electric nor chemical gradients are involved,
but the movement is primarily directed by mutual cell contact.
Contrary to epithelial cell groups, which, as mentioned above, tend
to stay united, the motile cells of the connective tissues and related
kinds react quite differently on mutual contact. When two of them
meet, the portions of the cell border which have made contact
are put into a state of temporary contraction and paralysis. Since
the other parts of the cell keep moving, each cell of the pair is neces­sarily towed away from the point of collision until the paralyzed
sector recovers its own motility and stalls a further advance. In a
uniformly distributed cell culture, cells thus shuttle back and
forth among one another, without resultant overall displacement.
However, in a cell population that grades off in density, the prob­
bility of collisions is greater for a cell moving in the direction
ward the denser portion than in the reverse direction and, as a
result of this statistical falling off of collision frequencies from
center to periphery, the cells gradually shift peripherally until their
distribution has become equalized. This explanation of cell move­
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ment in terms of population dynamics bears close resemblance to the diffusion of molecules in gases and liquids from regions of higher concentration to regions of lower concentration. Having thus ascertained the mechanism of "negative chemotaxis," at least for this case, we are now preparing to examine the nature of "positive chemotaxis" [e.g., of leucocytes].

Tissue Architecture. One of the most obscure and ill-explored problems is the manner in which growing systems acquire a definite shape, that is, a specific orderly arrangement of component parts in a defined space pattern. While we are investigating mechanisms of morphogenesis in several organ systems, especially the nervous system and the kidney, two other objects have recently provided us with information of particular interest in its potential bearing on the understanding of basic formative forces. The first example pertains to the submicroscopic realm and deals with the structure of the basement lamella under the epidermis of amphibian skin. Electronmicroscopic studies, combined with enzymatic and other chemical treatments of the live skin prior to fixation, have revealed a physical and chemical architectural system of great regularity. According to this analysis, the basement membrane consists of a ground substance of mucopolysaccharide (dissolved by hyaluronidase) built in layers, plywood fashion, each layer containing cylindrical fibers of collagen, all parallel within a given layer, but with the axis orientation changing by ninety degrees from layer to layer. To discover the manner in which such an orderly fabric is being formed, we study the mending of a hole in it in the course of wound healing. We had previously found that the membrane is repaired cooperatively by both the underlying connective tissue cells and the overlying cells, the former furnishing the fibrous material in random disarray, the latter imposing progressive layering upon the feltwork of elementary units. To these two tributaries, a third one has now been added, as we could show by transplantation experiments that the orientation of the fiber axes within the individual layers is determined by neither of
the two cell layers between which the membrane is sandwiched, but by the fiber orientation in the old membrane surrounding the hole that is being filled. This demonstration of axial orientation by pre-existing crystalline axes has strengthened our earlier hypothesis that macrocrystalline forces are involved in ordering the structural units in the building process.

On the tissue level likewise, an example has been found which points to the presence within the individual cells of factors which direct their definite geometric arrangement when they are brought together in groups. As previously reported, cells from tissues that have been dissociated, either mechanically or by the enzymatic treatment developed by Moscona and further elaborated by him in our laboratory, can after reaggregation and culture in vitro give rise to highly organized and typically differentiated body parts. In extending these tests to the precursor cells of cartilages of different destinations, we could now show that the blueprint for building a cartilage of particular geometric structure resides, as it were, in each individual cartilage cell. Normally, cartilage of the limb grows in whorls, whereas the cartilage around the eye has the shape of a plate. When the loose precursor cells of these two types of cartilages were dissociated by trypsin, then each reaggregated and plated out in tissue culture, they turned into structures which had not only the general histological characteristics of cartilage, but each had recognizably the type of architecture characteristic of its origin: the one formed whorls, and the other, plates. In this manner, architectural characteristics of whole tissues could be referred to properties that are inherent in the individual cells and enable them to build up the complex product by collective action.

Wound Healing. Because the reconstitution of skin is an ideally suited object for the study of elementary processes of growth, regeneration and reconstitution, we have given intensified study to the problems of wound healing, especially since this is one area where gains in fundamental and practical regards are intimately interrelated. We have found the skin of the chick embryo to offer
unique advantages for the experimental analysis of the factors involved in wound healing, as well as for the testing of agents of potential stimulatory or inhibitory effectiveness: a dramatic change in healing capacity occurs in this skin about the eleventh day of incubation. Wounds made after that date heal normally as in hatched and older birds. However, wounds made prior to that critical date remain raw and open. Wounds in this latter class begin spontaneously to heal as soon as the embryo reaches the critical age. Since cell multiplication goes on at all ages, the crucial difference is the inability of the earlier cells to migrate across the lesion, which, in all wound healing, is the primary event. An extensive program is being planned to utilize this natural system for a study of the relation between cell migration and cell multiplication in the repair of wounds and ulcers in general.

**Differentiation.** Work under this heading is concerned chiefly with factors that can switch cells from one course of development into another. Since prior experiments (in which transformation of skin from a horny into a slimy differentiation course had been induced by a brief bath of embryonic skin cell suspensions in Vitamin A) had not crucially excluded the possibility that vitamin had been absorbed into the cells and then acted later from within, we made fresh attempts to trace residual amounts of the vitamin by fluorescence microscopy. Uptake could be observed in detectable amounts only in injured or dead cells, indicating that the metaplastic effect had not been due to storage of appreciable amounts of the vitamin in the affected cells.

**Cell Growth.** The theory that the growth of each organ in the body is progressively retarded by specific inhibitors produced by that particular organ for its own cell types has now been formulated in rigorous mathematical terms, ready to be subjected to quantitative experimental tests. Confirmatory evidence had been reported by Professor Teir (Finland). Following our invitation, Teir repeated and expanded his tests in our laboratory (with Dr. Taylor), using eye glands of the rat as test objects to assay growth
stimulation or inhibition by injections into the animal of extracts from homologous glands, either fresh or after various stages of necrosis. Despite long continued tests, the results have been too erratic to be as yet acceptable as proof of our theory of growth control. Similarly, an attempt to assay a decrease of kidney inhibitor concentration in the circulating fluids of the body after removal of one kidney by the growth of kidney cell cultures imbedded in permeable capsules in the nephrectomized animal, has failed to give conclusive results. These experiments will be resumed at a later time.

Miscellaneous. Dr. Taylor, who had done pioneering work on the survival of deep-frozen cells, has started to explore the possible enhancing effect of increased hydrostatic pressure during the freezing process.

Professor Randall (University of London) has continued his work on the electronmicroscopy of the structural units of protozoans and of muscle.

Professor Gustafson (Wenner-Gren Institute, Stockholm) has studied the breakdown of tail muscle in metamorphosing frogs under the influence of thyroid hormone, with particular attention to the localization and sequence of structural modifications and their relation to the population of mitochondria.

Professor Bucher (University of Lausanne) developed a sensitive statistical technique for the detection of effects on cell size in tissue culture.

Professor Hintzsche (University of Bern), who had described the phenomenon of rotation of cell nuclei in many cell types, collaborated with Dr. Weiss in developing a mechanical model of the mechanism of rotation.

Professor Martinovitch (Atomic Energy Commission of Yugoslavia), after transecting the necks of early chick embryos to suppress direct communication between the pituitary and thyroid glands, discovered a remarkable ability of the severed parts to become organically interconnected again, which led to his later ex-
Dr. Kavanau has continued studies on the use of yolk breakdown products, the free amino acid pool, vitamins, and cofactors in the synthesis of proteins during the early stages of development.

Dr. Moscona, besides continuing his work on the selective aggregation of dissociated embryonic cells, has elaborated my previous concept of cellular exudates as integrating factors in intercellular relations, and has now identified some of these exudates in tissue culture as mucopolysaccharides, hence a common feature of the ground substances of the body.

The reported samples of many different investigative lines, carried on concurrently in close interrelation, well illustrate the growing realization that the field of development stands to gain more from a many-sided empirical advance, guided by ideas and observations on all three levels, the organismic, the cellular and the molecular, and carried out by the conjoint use of a considerable variety of techniques, than from a single-tracked approach, guided by the illusory hope that there may be a single key answer to all problems of development. Sobering though this realization may be, it is also challenging in that it proves that we are still in the logarithmic phase of growth, during which the problems multiply faster than the solutions.

**ECOLOGY**

Laboratory of Howard A. Schneider

Derek Hobson, Johanna M. Lee, Henry N. Wood,
William F. Arndt, Jr.

One of the great overriding facts of biology is that living things exhibit the phenomenon of variation. No matter how minute and fractional are the schemes by which we classify biological phenomenon, an examination of a series of samples always reveals diver-
gence and diversity. This characteristic state of affairs in biology pursues us when we confront problems of disease, for we find variation in host resistance and susceptibility and in pathogen virulence as well. To come to grips with these problems, so that we may the better understand the diseases of man and, with such understanding, gain a mastery—has been the preoccupation of Dr. Schneider's laboratory for many years.

Analysis of both infectious and non-infectious disease has been implemented by studies of so-called "model" diseases. The model diseases most intensively investigated in this laboratory have been diseases of mouse populations which are counterparts of human disease, i.e., infectious salmonellosis and non-infectious acute disseminated encephalomyelitis in mice, as murine images of human typhoid fever and multiple sclerosis respectively. In both of these diseases the problem of variation has been shown to have two important and interacting components, heredity and the nutritional environment. During the past year the activities of this laboratory have continued their focus on this nutritional environment as it operates within defined genetic frameworks.

**Salmonellosis.** A vitamin-like nutritional factor (SRF) has been detected in natural foodstuffs which has a profound effect in increasing natural resistance to this disease. This vitamin is present in some natural materials in very small amounts, about one part per million in the best sources. This has posed obvious problems in isolation, purification and identification. During the past year a technique has been developed for purification of the vitamin by counter-current distribution and a study of the properties of SRF continued. Problems of supply of SRF have been solved by studies leading to the specification of conditions of bio-synthesis of SRF by microbial means. Interestingly, *in vitro* studies have shown that SRF is not an antibiotic, but exerts its favorable effects for the host in some other way.

**Acute disseminated encephalomyelitis.** In this mouse model of an allergic non-infectious disease of the central nervous system, a
counterpart of human multiple sclerosis, this laboratory has discovered mouse genotypes which are 100% susceptible or 100% resistant. The susceptible mice have been shown to be nutritionally dependent on several vitamins, folic acid, biotin, and vitamin B₁₂, in order to achieve their genetic potential of 100% susceptibility. By defining such genetic and nutritional benchmarks in this disease evidence has accumulated during the past year that an hitherto unknown environmental factor is operative in this disease. An epidemiological investigation was begun to define this new factor.

At the base of this allergic disease is an allergen, a constituent of the central nervous system tissues, to which the animal responds with the disease emergent as a consequence. Progress has been made in chemical fractionation studies leading to the chemical description of this entity.

Hemorrhagic lung disease. From time to time, and usually during the winter months, some of the mouse stocks of this laboratory have been subject to a fatal illness characterized by hemorrhagic lungs. Investigations have shown that a more general phenomenon underlies this disease than the classical view of a specific, directly responsible, pathogenic agent. Results thus far indicate that no single microbial species is uniquely associated with the disease, but a variety of bacteria, all of them gram-negative, are to be found in this context. Purified gram-negative bacterial endotoxin, on injection, has been found to elicit the hemorrhagic disease at times coincident with its seasonal spontaneous appearance. The relationship of gram-negative bacterial endotoxin to this and other hemorrhagic phenomena in mice has been pursued and a mouse genotype ideally suited for such studies has been identified.

Susceptibility of mice to pleuropneumonia-like organisms. In collaboration with Dr. John B. Nelson, Dr. Wood has demonstrated the capacity of some unsaturated fats, when fed in a synthetic diet, to markedly increase the multiplication of these organisms when they are injected into the brains of mice. This
nutritional promotion of susceptibility mimics the effect of certain brain necrotizing viruses and possible common biochemical features are being investigated further.

**General Physiology**

Laboratory of Moses Kunitz

Work was continued during the 1956-1957 academic year on the purification and crystallization of enzymes isolated from baker’s yeast.

*Purification of Alkaline Yeast Phosphatase.* Alkaline yeast phosphatase has been intensely studied in this laboratory for several years. A great deal of effort has been exerted in purifying the enzyme with the aim of being able ultimately to isolate the enzyme in crystalline form. Preparations more than 10,000 times as pure as the original yeast extract have been obtained. The yield of the purified material, however, decreased rapidly on continuous purification, with the result that it became impossible with the present laboratory facilities to collect enough material required for extensive search for appropriate conditions for the crystallization of the enzyme. It was therefore decided to postpone, for the present at least, further work on yeast phosphatase, and to try instead some other convenient source for this enzyme. Work has been initiated on the purification of an alkaline phosphatase extracted from chicken intestinal mucosa, crude preparation of the enzyme being available commercially.

*Crystalline Inorganic Pyrophosphatase.* Crystalline inorganic pyrophosphatase was first isolated by Kunitz in 1952 from baker’s yeast. The enzyme has the property of bringing about the hydrolysis of inorganic pyrophosphate ions into orthophosphate ions. This enzyme proved to be of great value to enzymologists as an important reagent necessary in the study of a series of recently discovered enzymatic reactions which can be carried out experimentally *in*
vitro, such as synthesis of polypeptides, nucleic and fatty acids, sulfate transfer (Robbins and Lipmann), etc. All these reactions involve the use of adenosine triphosphate or other nucleotides, and are accompanied by the formation of inorganic pyrophosphate as one of the end products of the reaction. The liberated pyrophosphate has, however, the tendency to inhibit the reactions unless it can be removed from the reaction mixtures as it is formed. Crystalline pyrophosphatase produced in this laboratory has been used to great advantage to destroy specifically the pyrophosphate formed, thus allowing the reactions to proceed to completion. Crystalline pyrophosphate is still unobtainable commercially, and it was found necessary to continue the preparation of the enzyme in Dr. Kunitz’ laboratory so as to be able to satisfy the repeated requests from various laboratories, here and abroad, for samples of the reagent necessary for the study of the various reactions involving the formation of inorganic pyrophosphate.

**Crystalline Hexokinase.** Hexokinase is an enzyme which brings about the transfer of phosphorus from adenosine triphosphate to dextrose or fructose, thus initiating the metabolism of these sugars. The enzyme was first isolated in crystalline form from baker’s yeast by Kunitz and McDonald in 1946. Since then, the enzyme has been used extensively in various laboratories in the study of carbohydrate metabolism. Crystalline hexokinase, which is unavailable commercially, is being prepared in Dr. Kunitz’ laboratory on a scale large enough to satisfy the repeated requests for samples of the enzyme.

**The Proportion of Mutants in Bacterial Cultures.** (Dr. Northrop and Dr. Kunitz.) Dr. Kunitz cooperated with Dr. John H. Northrop during 1956-1957 in developing mathematical equations for the growth of mutants in bacterial cultures and for the ratio of mutants to the wild cells producing them. As will be seen from his more detailed report elsewhere in this volume, Dr. Northrop found the derived theoretical expressions to agree well with his experimental results.
We have been studying the biochemical syntheses that occur in the cell nucleus. In the past few years we have used for our experiments nuclei isolated from thymus lymphocytes. With preparations of isolated nuclei certain experiments can be done which would at present be impossible with nuclei in intact cells. Suspensions of isolated thymus nuclei incubated in suitable media synthesize proteins and nucleic acids, as indicated by the uptake of $^{14}C$-labelled amino acids and $^{14}C$-labelled orotic acid and adenosine. The work done during the past year has been a continuation of this line of investigation.

It is well known from studies on protein synthesis in cytoplasmic systems that ATP (adenosine triphosphate) and other nucleotide polyphosphates are essential components in the synthetic process. These triphosphates are energy sources for synthesis. In our experiments we have found that ATP and related compounds are present in the cell nucleus, that they are required for protein synthesis and that there is a phosphorylating system within the nucleus.

Work done during the past year has given us some new information about nucleotide phosphorylation within the nucleus. It has been found that DNA (desoxyribonucleic acid) is a “cofactor” in ATP synthesis. This was shown by two kinds of experiments: in one experiment the nucleus is deprived of its DNA; in another experiment DNA is restored to the nucleus. DNA can be removed from isolated nuclei by means of pancreatic desoxyribonuclease. Nuclei deprived of their DNA are no longer able to synthesize ATP. When such nuclei receive a DNA supplement they are again able to synthesize ATP. Revival of ATP synthesis occurs even when the nuclei receive a supplement of yeast RNA (ribonucleic acid), surely a very different polynucleotide from the DNA originally present in the nuclei.
These experiments show clearly that one function of polynucleotides, including both ribo- and desoxyribonucleic acids, is to mediate the synthesis of adenosine triphosphate. This function of nucleic acids is essentially non-specific, for our experiments show that nucleic acids of quite different character are equally effective as “cofactors” for ATP synthesis in isolated nuclei.

It is now widely accepted that nucleic acids have highly specific properties which in some way determine the specificity of protein synthetic reactions. In our studies of synthetic processes in the nucleus we are trying to distinguish between the specific and non-specific activities of nucleic acids.

**Laboratory of John H. Northrop**

**The Proportion of Mutants in Bacterial Cultures (with M. Kunitcz).** The equations reported last year, which predicted the proportion of mutants in bacterial cultures, have been slightly modified and extended to cover the case in which mutants appear without cell division.

The results may be summarized as follows:

The proportion of mutants in a growing culture of organisms will depend upon (1) the rate at which the wild cells produce them (with or without growth), (2) the back-mutation rate, and (3) the growth rates of the wild and mutant cells.

If the mutation rate without growth and the back-mutation rate are neglected, the growth of a mutant is expressed by

\[
M = \frac{2\lambda AW_0}{B-(1-2\lambda)A}[e^{Bt}-e^{(1-2\lambda)At}] + M_0e^{Bt} \tag{3}
\]

and the ratio of the mutant to wild by

\[
\frac{M}{W} = \frac{2\lambda A}{B-(1-2\lambda)A}[e^{B-(1-2\lambda)At}-1] + \frac{M_0e^{B-(1-2\lambda)At}}{W_0} \tag{4}
\]
in which $\lambda = \text{mutation frequency rate constant, "mutation rate"}$

$A = \text{growth rate constant of wild cells} (W)$

$B = \text{growth rate constant of mutant cells} (M)$

If the term $[B-(1-2\lambda)A]$ is positive, the proportion of mutants increases continuously.

If it is negative, the proportion of mutants reaches a constant value

$$\left(\frac{M}{W}\right)_{eq} = \frac{2\lambda A}{A-B} \quad (6)$$

If mutation is assumed to occur without growth at the rate $C$ then the corresponding equations are (11), (12), and (14).

$$M = \frac{CW_0}{A-B-C} [e^{(A-C)t} - e^{Bt}] + M_0 e^{Bt} \quad (11)$$

$$\frac{M}{W} = \frac{C[e^{(B+C-A)t} - 1]}{B+C-A} + \frac{M_0 e^{(B+C-A)t}}{W_0} \quad (12)$$

If $(B+C-A)$ is negative and $t = \infty$

$$\left(\frac{M}{W}\right)_{eq} = \frac{C}{A-B-C} \quad (14)$$

If $C << A$

$$\left(\frac{M}{W}\right)_{eq} = \frac{C}{A-B} \quad (14a)$$

If $(B+C-A)$ is positive, the mutants will overgrow the culture.

Equations (3) and (4) are very similar to those previously deduced by others, but differ in that they contain the constant 2. This number derives from the fact that, when a bacterial cell divides, there is an increase of 1 in the total population, but an in-
crease of 2 in the number of new cells. In bacterial cultures, therefore, the increase in the number of new cells is twice the increase in the total population, instead of equal to it, as in the case of higher organisms.

This fact appears to have been overlooked. There is very little difference in the final equations as long as the mutation rate is very small. If it is large enough to be significant with respect to 1, however, the factor 2 must be used; otherwise the equations fail completely.

*The Proportion of Terramycin-Resistant Mutants in B. Megatherium Cultures.* The number of terramycin-resistant mutants present in *B. megatherium* cultures, at various times and in the presence of various concentrations of terramycin, has been determined. The number of mutants present under all conditions agrees quite well with the equations derived by Northrop and Kunitz. The values for the constants used in these calculations agree with the values determined by entirely independent experiments. This fact adds a great deal to the significance of the results.

*The Effect of Ultraviolet and White Light on Growth Rate, Lysis, and Phage Production of Bacillus Megatherium.* Cultures of *megatherium* 899a, growing under different conditions, were exposed to ultraviolet or white light.

Cultures exposed to ultraviolet light and then to white light continue to grow at the normal rate. Cultures exposed to ultraviolet light and then placed in the dark grow at the normal rate for varying lengths of time, depending on conditions, and then lyse with the liberation of from 5 to 1000 phage particles per cell, depending on the culture medium.

Increasing the time of exposure to ultraviolet light results in an increase in the fraction of cells which lyse in the dark. The lysis time decreases at first, remains constant over a wide range of exposure, and then increases. The lysis can be prevented by visible light after short exposure, but not after long exposures.
The time required for lysis is independent of the cell concentration.

**Effect of temperature.** After exposure to ultraviolet the cell concentration increases about 4 times at 20°, 30°, or 35° C., but only 1.5 to 2.0 times at 40-45°. This is due to the fact that the growth rate of the culture reaches a maximum at 38° while the lysis rate increases steadily up to 45°.

Terramycin decreases the growth rate and lysis rate in proportion.

At pH 5.1, the cultures continue to grow slowly in the dark after exposure to ultraviolet light.

*Megatherium* sensitive cells infected with T phage lyse more rapidly than ultraviolet-treated 899a, and visible light does not affect the lysis time.

The results agree with the assumption that exposure to ultraviolet results in the production of a toxic (mutagenic) substance inside the bacterial cell. This substance is inactivated by white light.

**Origin of Bacterial Viruses.** The following facts are now reasonably well established. (1) The virus particle has no individuality of its own. The virus is therefore a product of the cell metabolism (Bordet, Northrop, Lwoff, Welsch, Raettig, Boyd, Bawden). (2) The production of virus by lysogenic bacteria is a genetic property of the bacterial cell and the virus particle transmits genetic information, just as does the transforming principle (Freeman, Fredericq, Lederberg). (3) In lysogenic cultures only a few cells produce phage at any one time (Burnet, Lwoff), but all cells are potentially able to produce virus (Bordet, Northrop). Those cells which are not producing virus, contain no virus particles (Gratia) nor any virus antigen (Miller and Goebel). (4) The proportion of cells producing virus is increased by mutagenic agents (Lwoff et al.).

The assumption that the virus is the result of a mutation of the
bacterial cell is a simple and adequate explanation of these facts. From this point of view, “infection” of a cell represents the transfer of a genetic character, and phage production is an “hereditary” lethal mutation. This assumption may be tested by comparing the effect of mutagenic agents on the proportion of phage producing cells with that on the proportion of cells which are known to be mutants; since mutagenic agents in general increase the proportion of all mutants to about the same extent.

Cultures of *B. megatherium* contain a few terramycin-resistant cells. The work reported last year proved that these cells were true mutants. If the phage producing cells are also mutants, then ultraviolet light and hydrogen peroxide should cause similar increases in the proportion of terramycin-resistant cells, and of phage producing cells.

Preliminary experiments show that this is the case. In addition, the equations which correctly predict the proportion of terramycin-resistant mutants, predict the proportion of phage producing cells.

*Optically Active Compounds from Racemic Mixtures by Means of Random Distribution.* Recent work indicates that many of the building stones of living organisms may have existed on the earth before the appearance of living systems. This simplifies somewhat the problem of the origin of life; but, in order to fulfill the conditions necessary for the occurrence of a vital reaction, an optically active solution must be provided. Ordinary chemical reactions cannot meet the requirement, since, if optically active substances are formed at all, the two optically active isomers are produced in exactly equal proportions, and their solution will be optically inactive.

The classical methods of Pasteur for the isolation of an optically active compound from such a mixture will not suffice, since they depend directly or indirectly on living organisms. This fact has been cited as evidence that living organisms could not have been formed from inanimate matter.

An optically active solution may be obtained simply by random distribution of the individual crystals from a racemic conglomerate
(a mixture of individual \( d \) and \( l \) crystals in equal proportions). It is only necessary that the individual crystals be separated far enough from each other so that their solutions do not mix when the crystals dissolve. If the crystals are dissolved under these conditions, each optically active crystal will provide a small amount of an optically active solution.

Laboratory of W. J. V. Osterhout

Work was continued on some of the topics mentioned in the last report. In addition new experiments have been made to determine the effects of electrical forces on living protoplasm. Our knowledge of this subject is relatively meager and it seems important to carry on experiments whenever favorable opportunity occurs.

These experiments were performed on the fresh water alga, *Spirogyra*. Each cell contains a thin layer of cytoplasm surrounding a central vacuole filled with sap in which lies the nucleus. Embedded in the cytoplasm is a long spiral chloroplast containing chlorophyll, which extends from one end of the cell to the other.

The poles of the battery were placed on opposite sides of the living cell so that the current passed through the cell. These cells were placed in \( 0.001 \text{M} \) sodium chloride solution which brought about no visible change in the chloroplasts. When a small amount of direct or alternating current (less than 0.2 Milliampere) was passed through the solution each spiral chloroplast in less than ten minutes became detached, straightened, and contracted until it formed a small rounded mass. This contraction continued even after the current was turned off. A cell whose chloroplast was not visibly affected by the current sometimes showed signs of contraction after the current was turned off. This was not reversible. If the direct current was reversed, there was no additional effect.

In order to make certain that these effects were not due to the heat produced in the circuit, the following was done. A drop of gelatin whose melting point was \( 35^\circ \text{C} \) was placed in the path of the current along with *Spirogyra* cells. Since the gelatin did not
melt the temperature was less than 35°C. If a drop of gelatin was placed in a Stender dish containing some water at 35°C, it melted. Cells of *Spirogyra* exposed to a temperature of 40°C for one half hour in a Stender dish of water showed no signs of contraction. These results indicate clearly that the contraction of the chloroplasts was not caused by a rise in temperature but by electrical forces.

These results might be explained on the ground that the chloroplast may be altered by the passage of the electric current so that water is lost and the chloroplast contracts. These changes may continue after the current is turned off. Such changes may be initiated in the nonaqueous surface layer of the chloroplast.

On another topic, experiments have been made to determine if the movement of water affected the rate of entrance of solutes into the living cells. The aquatic plant, *Nitella*, was used. Each cell was over five centimeters long. It was possible to observe under the microscope the movement of particles inside the cell.

A living cell of *Nitella* was divided into two parts, A and B, by means of a vaseline seal at the center. A solution of the acid dye, acid fuchsin, was placed at A and water at B. There was no penetration of dye into the cell and there was no visible entrance of water into the cell. The water at B was then replaced by 0.25M sucrose solution. The water at A rushed into the cell. The water travelled rapidly inside the cell from A to B carrying particles to B where they collected while the water escaped from the cell at B. In spite of this rapid inrush of water into the cell, the acid fuchsin did not penetrate the cell unless it was injured. These experiments indicate that the rate of penetration of acid fuchsin is not dependent on the rate of penetration of water.

This was confirmed by the following experiments with the basic dye, brilliant cresyl blue. If a solution of this dye at pH 5.5 in which the dye was chiefly in the form of ions was placed at A and if water was placed at B no dye entered the cell and there was no visible entrance of water into the cell. When the water at B was replaced by 0.25M sucrose solution, the water at A rushed into
the cell, and travelled inside the cell from A to B where it came out of the cell. In spite of this inrush of water, no dye penetrated the cell.

If a brilliant cresyl blue solution at pH 9 was used at A, and 0.25M sucrose solution at B, the dye penetrated rapidly into the cell. At this pH value the dye was chiefly in the form of undissociated molecules but on reaching the acid sap the dye became dissociated and travelled to B. If the pH value of the sucrose solution at B was at 5 the dye rapidly escaped from the cell at B, but if the sucrose solution was at pH 9, the dye collected inside the cell at B. Water rushed in and out of the cell independently of the pH values.

These experiments indicate that the rate of penetration and that of exit of the dye is dependent chiefly on the selective permeability of the protoplasmic surfaces and not on the rate of entrance and exit of water. This is important since it deals with the normal functioning of the cell.

These experiments also indicate that the dyes do not enter the cell through pores, since if they did so the penetration of dyes would run parallel with the penetration of water. We may therefore conclude that solutes enter the living cell through the non-aqueous layer of protoplasm. This may involve solubility and chemical combination.

GENETICS

Laboratory of Rollin D. Hotchkiss
Maurice S. Fox, Norton D. Zinder, Muriel Roger, Chester DeLuca, Sanford F. Lacks, Elena Ottolenghi, Chandler Fulton

Bacterial transformation. The sequence of events brought about when deoxyribonucleate (DNA) from one strain of bacteria induces a genetic change in a different but related strain has been investigated at several points: (1) effect of alterations or degrada-
tions of the molecules of extracellular nucleate upon their subsequent biological activity in genetic transfer, (2) properties of the initial reaction between transformable cells and DNA, (3) fate of the genetic units introduced in transformation, and (4) nature of the modifications in enzyme structure which are specified by genetic units contained within certain deoxyribonucleate molecules.

Chemical and physical studies are under way to correlate the biological inactivation of transforming DNA with molecular changes that occur when it is denatured by various agents. With this aim in mind, light-scattering and viscosity measurements have been carried out with intact and altered calf thymocyte and pneumococcal nucleic acid. Information obtained from these measurements relates to both molecular size and configuration. The molecular weight of 5 to 8 million and high intrinsic viscosity obtained for both thymus and pneumococcal DNA are in good agreement with results from other laboratories.

Short exposure of pneumococcal DNA to dilute acid (pH 3.5) or dilute base (pH 11) does not affect activity. However, prolonged exposure under these conditions results in a slow inactivation, and when the DNA bears two or more genetic markers a differential rate of inactivation of the markers may be observed. When the linked sulfanilamide resistance markers are assayed after such mild degeneration, it is found that the destruction of linked regions occurs considerably faster than that of individual markers.

Mild heat treatment (80-90° C) of transforming nucleic acid also brings about differential inactivation of diverse markers, and results in a collapse in the extended structure of the molecules with little, or no, change in molecular weight. The concomitant 90% inactivation indicates that features connected with molecular shape are as important a requirement for activity as is molecular size.

The enzymatic degradation of pneumococcal DNA by very small
quantities of specific nuclease has been measured by the decrease in intrinsic viscosity as well as loss in activity. A tentative conclusion is that in this process activities of two different markers fall in parallel fashion at a time when viscosimetric measurements show little evidence of molecular breakdown.

In developing methods of preserving viable pneumococci by freezing with glycerol, it was observed that their transformability, ordinarily a transient property, was well preserved. Such transformable bacteria react with the nucleic acid in such a manner that the number of cells in which transformation is initiated is linearly proportional to the time of exposure to the nucleic acid.

This linear rate of initiation of transformation is proportional both to the concentration of the nucleic acid and the concentration of bacteria, and at high concentrations of either the rate approaches a maximum. From the observation of the saturation of the rates it is concluded that there must exist preceding its incorporation an equilibrium state of nucleic acid adsorbed to the bacteria. A formal analysis of the kinetics has permitted a calculation of the equilibrium constant between the bacteria and the nucleic acid as well as the quantity of nucleic acid bound by the bacteria in the equilibrium state. In addition, it has been possible to estimate the rate constants for the formation and destruction of the equilibrium state and for the incorporation of the nucleic acid.

A preliminary attempt at direct measurement of the amount of nucleic acid bound by the bacteria in the equilibrium state, by permitting adsorption to occur at a temperature sufficiently low that incorporation cannot occur, has given quantities in agreement with those calculated from the kinetic data.

The property of high sulfonamide resistance in pneumococcus is transferable from strain to strain by transforming DNA, but usually becomes changed in the process. This system, developed in collaboration with Miss Audrey Evans of the Media Department, has been shown to have a fine structure consisting of three discrete genetic determinants. Transformation occasionally results
in the transfer of all three, but more commonly only two, and still more frequently only one, of the linked units is introduced into a given transformant. The single units are transferred without modification, and can be recombined into all of the possible pairs, and the triplet, by transformation. Since each unit and each combination gives rise to a characteristic level of sulfanilamide resistance in the transformant which receives it, this linked system provides an opportunity to follow the fate of specific individual fragments of genetic material.

It appears that each cell transformation is an individual event at which the complete cellular genome is challenged, more or less successfully, by the transforming DNA particle which comes from without. The studies just outlined, and earlier ones, all suggest that at the initial stages of the interaction only the essentially intact nucleic acid molecules become available to the cell. The success with which the genetic units carried by this molecule invade or modify the cell genome and become established is determined by additional factors besides their intactness. Although the origin of one end of an “inserted” fragment may be randomly determined by spacings, the position of the other apparently depends upon size limitations. Quite simply, of the DNA molecule which enters the cell, it is more likely to be a small piece rather than a large one which becomes genetically established. In fact, there seems to be a maximum size of fragment which can achieve this transfer. It is believed that such size limitations apply to genetic crosses within intramolecular regions in such other systems as viruses, bacteria and various fungi, and account for many of the quantitative anomalies which seem to have arisen in these systems.

The seven transformant strains, differing in genetic fine structure, have each a characteristic degree of resistance to sulfanilamide. This resistance can be shown to be attributable to a series of changes in a single enzyme involved in the conversion of \( p \)-amino-benzoic acid to folic acid. Measurement of the strain differences can be made more precise in terms of the sensitivity of this enzyme
to sulfanilamide inhibition. In this way, certain properties of the prosthetic group of this enzyme may be investigated, as they are genetically modified.

In particular, by systematically using chemical variants of sulfanilamide, it is possible to explore more and more precisely the nature of the changes induced in the p-aminobenzoic utilizing enzyme. A tentative conclusion is that one of the genetic units determines the nature of a basic group at the active center of the enzyme, while the other two control spatial and structural features of the protein.

Fine structure has also been genetically analyzed in the DNA determining formation of a pneumococcal enzyme hydrolyzing maltose to glucose. Eight independent mutants have been obtained which fail to make active enzyme. Their DNA's have suffered alteration in eight different regions, closely linked with one another, and showing size-dependence of transformability similar to that found with the sulfonamide resistant series.

Host relationships of bacterial viruses. A model of the effects of viral infection upon living material is presented by the viruses that attack bacteria (bacteriophages). Not only do bacteria provide a host cell within which viral growth may be studied, but living cells often survive the infection and emerge with altered properties. The virus itself may often be modified in some respects by growth on particular hosts. The work of Dr. Zinder has been concerned with these more subtle aspects of cell-viral interaction.

The organisms used have been members of group B of the Salmonellae, particularly S. typhimurium and the bacteriophages of group A, particularly P22. Three phenomena have been studied, the immunity exhibited by lysogenic cells to superinfection, lysogenic conversion which is the appearance of a new cellular property following lysogenization, and transduction which is the transfer of fragments of bacterial genetic material by bacteriophages.

The immunity to superinfection of lysogenic bacteria was found to be inevitable only when the superinfecting phage was identical
to the one carried. A number of phage genes have been found to play an important role in the determination of this property. Some phage genes confer to the cell immunity to superinfection by all other related phages, others invoke in the cell immunity only to themselves. These genes have been mapped by phage crosses. With the breakdown of superinfection immunity one of the best criteria for lysogenicity is gone. When a cell is not immune to superinfection by a related phage the same events occur that follow infection of non-lysogenic cells, including phage substitution.

Following infection by P22 or its relatives a new surface antigen appears in the lysogenic bacteria. It is the somatic antigen O1, long known as one of the diagnostic antigens of this group. Following the discovery of this fact, it was found that even in those cells which are going to be destroyed in time by the viral infection the antigen appears. Thus the phenomenon is not a consequence of stable lysogenization. In fact, the antigen is sufficiently developed eight minutes after infection to be detectable long before a stable lysogenic condition exists. The relationship of this effect to the cellular immunity is being studied.

In the analysis of transduction particular attention was paid to the activity of phage obtained following the stable lysogenization of bacteria in comparison with the effect of that obtained following the direct growth of phage. It was found that transduction of most bacterial genes was independent of the method of viral preparation. However, for the genes concerned with galactose fermentation the phage obtained from lysogenic bacteria had a unique effect; the efficiency of transduction was markedly increased. This result may indicate a special relationship between the phage genes and the genes affecting galactose fermentation in bacteria. An equivalent relationship is known to exist for another unrelated bacteriophage, the phage lambda attacking strains of E. coli. Comparison of the special transduction of galactose fermentation with the general transduction of other bacterial genes may help to reveal the nature of transducing particles, especially as to the completeness of the phage genome which they carry.
The mechanism of sensitization is being pursued with vigor in a number of laboratories, particularly with regard to the principal problem of the separateness of the mechanisms involved in evanescent and in delayed type manifestations of allergy. The induction of experimental allergy by means of chemicals (drugs) as opposed to the use of native proteins has permitted a wider range of observations. We have been able to show, for example, that delayed-type hypersensitivity persists after antibody appears in the circulation, although it is commonly supposed that delayed-type hypersensitivity disappears with the onset of antibody production and is “replaced” by Arthus-type dermal reactivity. When protein is used to sensitize an animal and is then injected into the skin to determine the type of hypersensitivity, determination of persistence of delayed-type reactivity is not properly to be made, since the effective ‘dose’ of antigen is consumed in the initial, evanescent reaction that occurs as soon as antibody has been produced. Other studies, employing the transfer of lymphocytes from sensitized to normal guinea pigs, are applicable to the question of the mode of sensitization, as described previously. New experiments made in collaboration with Dr. Leonard Hamilton of the Sloan-Kettering Institute, have been initiated in order to trace the fate of transferred lymphocytes and nuclear material from these, by means of \textit{in vivo} tritium-labelling of the cells of the lymph nodes.

\textit{Hereditary susceptibility of guinea pigs to allergens.} (Dr. Chase)

Segregation of selected characteristics through breeding produces stocks of guinea pigs that can be either more or less susceptible to experimental sensitization than the pen-inbred stock of origin. Some of the colonies of genetically variant guinea pigs that we are carrying were described before: in addition to the specific purposes for which they were bred, they have been an excellent source of
material for studies on experimental granulomas, as described below. Certain additional lines are being segregated from the Rockefeller Institute albino stock, with the hope that animals of heightened susceptibility to sensitization will serve to predict in greater measure the allergenic properties of chemicals that are intended for use on human beings. We have succeeded already in setting up a "line" that is regularly sensitized by allyl isothiocyanate (mustard oil) in contrast to the behavior of almost all varieties of guinea pigs, and have thus regained a susceptibility which Jacobs, Kelley and Sommers (1942, Proc. Soc. Exp. Biol. and Med., 48: 639) showed could be obtained by selective breeding. We are endeavoring to select animals to possess ready and high degree of susceptibilities to picric acid, and to the water-soluble allergen thioglycolylamide. Another line of guinea pigs, Family II of Sewall Wright, is also being developed for genetic studies.

Further observations on the development of granulomatous nodules in tuberculin-sensitized guinea pigs. (Dr. Chase, Miss Slizys) In the Annual Report for 1955-56, there was described the finding of granulomatous lesions in guinea pigs into which paraffin oil containing killed tubercle bacilli had been injected, along with various compounds made from simple chemical allergens. The tubercle bacilli potentiated the development of dermal, contact-type hypersensitivity to the respective allergens. When contact-type tests with the allergens or Mantoux tests with tuberculin were performed, granulomas would develop in some of the animals around the periphery of the sites, usually within 7 to 14 days at a time when damage ensuing from allergic inflammation had undergone repair in the healing phase; shortly thereafter, certain animals would be seen to have disseminated granulomata scattered over the flank as isolated nodules, which later became confluent and yielded aggregates and cords of tissue. The lesions are found firmly attached to and forming part of the subcutis, although they occur predominantly within the areolar tissue underlying the subcutis, especially in relation to lymphoid spaces and
a greatly increased number of fat cells; they are composed principally of epithelioid cells and giant cells, with some eosinophiles and lymphocytes. The process is now found to commence spontaneously with appearance of swelling and transient formation of nodules in the skin lateral to the ankle of the hind limbs around the 16th day, and development of inflamed nodules in the genital area 10 to 15 days later. The animals that show these features are those subject to translocation of nodules at will to various areas of the skin, in the wake of specific allergic reactions or non-specific skin irritations. Non-specific stimuli (e.g., applications of cantharidin) appear to be effective for the first two or three weeks, but specific allergic stimuli serve over a considerably longer period of time. In simplest form, the injection of dead mycobacterial cells (either Myco. tuberculosis var. hominis or saprophytic Myco. butyricum) in hydrocarbon is sufficient to “prepare” animals. Apart from the use of non-specific stimuli, effective translocation of granulomas to the flank has been secured in various measure with (a) unheated tuberculin adsorbed to alumina cream, (b) Old Tuberculin, or (c) the tuberculin preparation ‘PPD’. No transmissible factor of disease has come to light as an explanation of the development of granulomas. The role of heredity in susceptibility appears to be supported by unlike incidence among different guinea pig “families”, varying from 12 to 90 per cent. One special stock of guinea pigs (Wright’s Strain II) has not yet been found to produce nodules, and in these animals it happens that tuberculin sensitization is not easily induced. There is evidence that an allergen may occur in the experimental granulomatous tissues.

Study of the reactive “test antigen” in human sarcoidosis. (Dr. Chase, in collaboration with Dr. J. G. Hirsch and Dr. Louis E. Siltzbach) There are ostensible similarities in histological pathology and time of development between the granulomatous nodules mentioned above in guinea pigs and the granulomatous nodules that develop slowly when minced human sarcoidal tissues are injected into the skin of persons ill with sarcoidosis (the so-called
Nickerson-Kveim reaction. It appeared useful to examine the reactive "test antigen" employed in efforts to diagnose human sarcoidosis, and to collaborate in studies on human sarcoid being carried out in the Hospital of the Rockefeller Institute under Dr. J. G. Hirsch and at Mt. Sinai Hospital under Dr. Louis E. Siltzbach. In preliminary studies, test antigens have been prepared from human sarcoidal lymph nodes and spleens in several ways; some progress has apparently been made in separating components of the splenic extracts.

The mechanism of induced "immunologic unresponsiveness" to allergenic chemicals. (Dr. Ritts) It has been known since 1944 that preliminary feeding of certain chemical allergens to normal guinea pigs will prevent these animals from being rendered hypersensitive to the same chemical and from making antibodies to the same chemical grouping when it is attached to guinea pig protein. The effective "blockade" of the animal's responses persists for at least a year after cessation of the feedings. No serum factor has been uncovered in such "blocked" animals that can prevent a recipient guinea pig from developing an active sensitization. The most likely explanation for the phenomenon would appear to be retention of the chemical groupings coupled to tissue in or adjacent to sites of antibody synthesis or of lymphocytic maturation. In an attempt to locate the path of entry and final fate of the allergens that are fed, allergenic chemicals are being employed having radioactive carbon ($^{14}\text{C}$) within the benzene ring or hydrogen ($^3\text{H}$) attached to it. Studies to date indicate that when $2:4:6$ trinitrochlorobenzene (picryl chloride) is fed only minute amounts of the allergenic "picryl" grouping persist to confer unresponsiveness. Much of the radioactivity passes through the gut or is absorbed and re-excreted as picric acid and perhaps other picrated complexes. It is therefore necessary to use allergens of high specific radioactive labelling in order to localize sites of deposition, and experiments with compounds of still higher labelling are in progress. There is some evidence, not yet sure, that radioactivity is
localized within the spleen, areas of the liver, and lymph nodes. It is known, however, that “immunologic unresponsiveness” can be established as readily in splenectomized as in normal animals.

**MEDICINE**

Laboratory of Edward H. Ahrens, Jr.

J. Hirsch, W. Insull, Jr., M. L. Peterson, and W. Stoffel

This group is engaged in clinical and laboratory studies of fat metabolism. The major clinical study is concerned with certain metabolic effects caused by carefully controlled nutritional variations. Two main findings have been made: (1) the ingestion of certain unsaturated fats causes lower serum levels of cholesterol and phospholipids than diets containing isocaloric amounts of saturated fats, and (2) replacement of fat by carbohydrate may cause striking increases in serum triglyceride levels, even without marked alterations in cholesterol levels.

The first finding, made originally in 1954, has been greatly extended in tests with a wide variety of fats of natural origin. All data are compatible with the tentative conclusion that the fatty acid structure of the dietary fat is responsible for these effects. Thus, the degree of depression of serum cholesterol and phospholipid levels reflects the degree of unsaturation of the fed fat. The possibility that these effects are due to minor components or trace materials in the dietary fats is currently under critical test, using glycerides synthesized from highly purified fatty acids. If it is shown that fatty acid structure is the major determining factor, further tests with “synthetic glycerides” are planned in order to determine whether the effects are due (1) to the content of linoleic acid, an “essential fatty acid”, or (2) to the number of double bonds per unit weight of fat.

The mechanisms involved in these changes are currently under investigation. The fate of ingested fatty acids in man may be
described first by defining the pattern of fatty acids in the various fatty acid-containing groups of the serum. Preliminary results show that the fatty acids of the phospholipids, cholesterol esters and triglycerides are all readily affected by the chemical character of the fed fat, and that the triglycerides are most responsive to dietary influences. The subtle relationships between qualitative and quantitative effects remain to be determined.

In collaboration with Drs. Hellman and Rosenfeld at the Sloan-Kettering Institute, experiments have been carried out to determine whether synthesis of cholesterol is altered when serum levels are decreased by dietary means. No evidence of altered synthesis has been obtained. However, it has been shown that excretion of cholesterol (and its end-products, the bile acids) is increased when serum levels fall and is decreased when serum levels rise. The net gains and losses in circulating cholesterol are thus largely ascribable to changes in excretion through the gut. These findings serve to focus attention on intestinal excretory mechanisms rather than on altered synthesis rates, and in addition they demonstrate that the cholesterol which leaves the serum, when unsaturated fat is fed, is not sequestered in other tissues.

A second clinical study, under way for a year, was designed to study the transfer of fat from the serum to the milk in nursing mothers. The relative quantitative aspects of fat synthesis de novo in the mammary gland, as compared to transfer from the blood, have not been defined in any species. Since milk fat is formed and excreted within a 3-4 hour period, it is “trapped” out of the mainstream of dynamic metabolic processes. A number of original findings have been made: the fatty acid pattern in the milk lipids is dramatically affected by the quantity and by the quality of the mother’s dietary fat, but also is influenced by over- and undernutrition. Thus, a mother ingesting a diet containing an excess of calories but no fat produces milk fat rich in saturated acids of $C_{12}$ and $C_{14}$ chain length. If insufficient calories are fed and the mother loses body weight, her milk fat comes to resemble her adipose tissue
in its pattern of fatty acids. In caloric balance the ingestion of corn oil produces a milk whose lipids closely resemble corn oil fatty acids in distribution. Studies in progress aim at defining diurnal changes, rapidity of response to alterations in maternal diet, species of serum lipoprotein which most strikingly affects milk composition, effect of maternal diet on infant serum lipid levels, etc.

Laboratory studies are focussed primarily on development of technics for separation and quantitation of various lipids. Dr. Hirsch has successfully standardized silicic acid chromatography for separation of complex lipid groups. He is currently exploring the use of other supporting media and eluants, as well as devices for monitoring effluent curves in a completely automatic manner. Dr. Insull has made wide use of gas-liquid chromatography of fatty acid esters with apparatus set up in this laboratory by its co-inventor Dr. A. T. James (Medical Research Council, London) last Spring. The continuing development of these methods of analysis is central to the success of both clinical programs.

Laboratory of Reginald M. Archibald
D. Dziewiatkowski, H. Jaffe, S. Klebanoff, H. Rasmussen, C. Rich

Study of some of the hormonally controlled metabolic processes involved in growth and maturation of bone and cartilage has entailed:

1. purification of parathyroid hormone—one of the hormones which has a tremendous influence on metabolism of bone mineral;
2. investigation of some of the effects on bone and cartilage metabolism which result from administration of estrogens and thyroid hormone (i.e. hormones which are already available in pure state);
3. in vitro synthesis of and measurement of the excretion of chondroitin sulfate—one of the chief and characteristic constituents of cartilage; and,
(4) identification of some of the minute structural and biochemical changes in bone, cartilage and erythrocytes damaged by application of heavy doses of X-radiation.

The hormone of the parathyroid gland appears to control the blood concentration of calcium and phosphate. When secretion of this hormone is excessive the blood level of ionized calcium rises and of phosphate falls. The urinary excretion of both calcium and phosphate ions increases to the point where damage to the urinary tract may result from precipitation of calcium phosphate. Whether the hormone acts primarily on bone to release excessive amounts of calcium into the blood, or whether it acts primarily on the kidney to cause excessive excretion of phosphate, or whether it acts simultaneously and directly at both locations, and whether the actions at the two sites are caused by the same or by different hormones, are questions which so far have not been answered definitely, partially because no pure preparation of parathyroid hormone has been available.

Dr. Rasmussen has undertaken to purify the hormone of the parathyroid glands. When beef glands are extracted with an aqueous solution of hydrochloric acid an active preparation which contains parathormone A can be obtained. If acetic acid rather than hydrochloric acid is used, one can derive from the extract an apparently different and more stable active preparation, parathormone B. Interconversion of parathormone A to B or B to A has not been achieved. Activities of both preparations are assayed by measurement of the increase in the blood concentration of calcium which they can induce in parathyroidectomized rats. By use of ultra filtration followed by zone electrophoresis it has been possible to separate from the acetic acid extract an ultrafilterable, active polypeptide which migrates as a single component in the ultracentrifuge.

Dr. Rich has conducted extensive investigations on the effects of endogenous parathyroid hormone on calcium and bone metabolism before and after partial parathyroidectomy had been performed on a patient with hyperparathyroidism. From these studies
conducted with the aid of tracer doses of radioactive calcium it was concluded that excessive secretion of parathyroid hormone results in an abnormally high rate both of resorption of bone mineral and of deposition of bone mineral. The resorption rate was faster than that of deposition. After partial parathyroidectomy when the rate of secretion of parathyroid hormone was more nearly normal, the rate of deposition of bone mineral, though less than before the operation, was still much greater than normal and was very much less than that of bone resorption. The patient was then in “positive calcium balance” because of the severe depletion of bone mineral which had occurred before operation. In normal adults (i.e. in individuals who are in “calcium balance”) the rates of resorption and of deposition of bone mineral are equal.

Administration of estrogenic hormones to children or growing animals is known to hasten closure of the epiphyses, i.e. it hastens the disappearance of cartilage from the epiphyseal plate and the replacement of this cartilage with bone. This action of estrogens appears to account, at least in part, for the fact that growing girls, at any given age, have a more mature skeleton than boys of the same age. One of the characteristic components of cartilage is the mucopolysaccharide known as chondroitin sulfate. During the period of growth of long bone some of the constituents of the chondroitin sulfate of cartilage are transformed to, or incorporated into, bone. This has become apparent through study of radioautographs of bones of growing animals into which radioactive sulfur in sulfate ($S^{35}O_4^-$) was injected. Administration of the estrogen, 17$\beta$-estradiol benzoate, to growing rats has been found to inhibit resorption of metaphyseal bone thereby favoring accumulation of such administered tags as $S^{35}$ in the metaphyseal bone.

Because cartilage is a precursor of bone and because the characteristic component of cartilage is chondroitin sulfate there has been great interest in the measurement of this acid mucopolysaccharide in the urine of patients with diseases which are characterized by pathological processes in those tissues (cartilage, bone and skin) which contain appreciable concentrations of chondroitin
sulfate. The rate of acid mucopolysaccharide excretion has been found by Dr. Rich to be slightly elevated in patients with lupus erythematosus and greatly elevated in patients with leukemia, lymphoma and myeloma.

Some of the conditions required for in vitro synthesis of chondroitin sulfate have been studied by Drs. Klebanoff and Dziewiatkowski. Incorporation of $\text{S}^{35}$ sulfate into chondroitin sulfate has been found to take place intracellularly in rib cartilage slices suspended in salt solutions. The pH optimum for this process is 7.0. Addition of ascorbate, especially in the presence of cupric ion, inhibits this process chiefly because of the production of hydrogen peroxide which attends ascorbate oxidation. That this peroxide is an important inhibitor is indicated by the fact that addition of catalase (which destroys hydrogen peroxide) prevents this inhibition, whereas addition of azide (which inhibits catalase) potentiates it.

Hydrogen peroxide produced by other means also is likely to result in a disturbance of chondroitin sulfate synthesis. Ionizing radiations, including X-rays, cause production of hydrogen peroxide in aqueous systems which absorb them. Dr. Klebanoff found that X-radiation of human erythrocytes resulted in a decreased concentration of reduced glutathione as evidenced by a marked decrease in glyoxalase activity. Addition of hydrogen peroxide to erythrocytes caused similar changes.

Because of its high mineral content, bone absorbs more energy from an X-ray beam than does soft tissue or an erythrocyte suspension. Patients who receive therapeutic doses of X-ray are particularly susceptible to pathological fracture of bones which have been in the irradiated field. Because chemical and histological evaluation of radiation damage to human bone is seldom feasible, the effects of X-rays on mouse bone have been studied by Dr. Dziewiatkowski in collaboration with Dr. Woodard of Memorial Center. Those epiphyseal cartilage plates which were shielded from X-rays decreased in width with age and had an orderly columnar arrangement of chondrocytes and a metachromatic matrix. Twenty-one
days after irradiation with 2000 r, relatively few chondrocytes re­
mained. Even these few were in a very disordered distribution. By
the 60th day the matrix showed fissures and was almost ortho-
chromatic. The impairment of uptake of Sr\(^{89}\) by the irradiated bone
paralleled loss of phosphatase activity. Impaired uptake of
\((S^{35}O_4)^-\) by the irradiated cartilage plates was believed due to
damage to, and loss of chondrocytes wherein chondroitin sulfate
and its precursors are synthesized.

Laboratory of Vincent P. Dole
I. L. Schwartz, E. L. Bierman, P. Elsbach

Studies of obesity have continued during the past year, and have
added further evidence to the hypothesis that marked obesity arises
from a fault in intermediary metabolism rather than from simple
overeating. This distinction perhaps needs to be explained. It is
obvious that all body tissue ultimately comes from food eaten, but
it does not follow that appetite necessarily is abnormal. The func­
tion of appetite is to maintain a supply of fuel for working cells.
The immediate source of this fuel is material stored in tissues,
especially fat tissue. Food molecules replenish the tissues, and thus
maintain the supply of energy, but they have only an indirect con­
nection with the stream of molecules serving as fuel, or what might
be called the effective diet of the cells. Thus an accumulation of
fat may arise as a compensation for deficiencies in the function of
adipose tissue, rather than from dietary overload. The crucial
question is whether the abnormal quantity of adipose tissue in
obese subjects serves a purpose, or whether it is simply a burden
of excess matter.

To answer this question we are studying a small group of fat
people before, during and after reduction in weight. We aim to
find out whether reduction brings the fat person to a state of nor­
mal body composition, or whether after weight loss the proportion
of fat, protoplasm and water in his body remains abnormal. In
terms of function it is important to determine whether the reduced
person has a normal metabolic rate, normal performance of liver, stomach, thyroid, and other organs, normal control of blood sugar and lipids—and, intangible but important, normal vigor with a sense of well-being.

Although the study will not be completed until next year, it is already clear that some “reduced-fat” persons are not functionally normal. Metabolic rates—both the basal rate, measured in the conventional way, and the total number of calories required for maintenance—generally fall with weight loss, and in some cases become markedly subnormal. This explains why these people regain weight by eating only a normal amount of food. To hold their reduced weight they must limit themselves to subnormal diets.

A second approach to the problem has been a study of the factors controlling mobilization of fat from tissue stores and its transport in the bloodstream. The work has brought out the importance of a hitherto neglected fraction of blood lipids, the non-esterified fatty acids (NEFA). Control of this fraction is abnormal in diabetes, and apparently it is an acute failure of this control that precipitates a dangerous state of diabetic acidosis. It was also of interest to observe that both the stabilized diabetic and the obese, non-diabetic tend to have a higher than normal concentration of NEFA in blood taken before breakfast. The significance of these findings in relation to metabolic function or long term health remains to be determined.

Laboratory of René J. Dubos
Diethelm H. Boehme, James G. Hirsch, Derek Hobson,
Cynthia H. Pierce, Russell W. Schaedler, Harold J. Simon,
Curtis A. Williams

Most of the progress in the control of microbial diseases has come so far from the development of techniques to interfere with the event of infection, or to treat the sick patient. In contrast, little is known of the factors which control natural resistance to disease. Yet there is no doubt that infection often fails to evolve into overt
disease under normal circumstances and that, furthermore, disease once established often regresses spontaneously. For example, while some 60 million persons are infected with virulent tubercle bacilli in the United States today, only one million exhibit signs or symptoms referable to this infection, and no more than 250,000 to 350,000 suffer from active tuberculosis. Similarly, while approximately half the population carry virulent staphylococci in their nasopharynx, only a very small percentage of those infected suffer from boils, osteomyelitis or other conditions caused by these bacteria. Several projects bearing on the problem of natural resistance to disease have been instituted in our laboratory in an attempt to contribute knowledge to this neglected field.

One phase of the program is concerned with normal tissue substances which kill or suppress multiplication of invading bacteria. Studies are continuing on mechanisms by which polymorphonuclear leucocytes destroy many of the organisms that they engulf. A new method for separation of large quantities of these cells from peritoneal exudates in rabbits permits biochemical and microbiological investigations on leucocyte extracts. These extracts contain at least three antibacterial materials: lysozyme, organic acids and phagocytin. Present knowledge suggests that lysozyme and acids act primarily on Gram-positive microbes, while phagocytin exerts a lethal effect on Gram-negative enteric bacteria. Considerable progress has been made towards isolation of phagocytin. Present preparations contain no detectable material other than protein, and are highly active; as little as 0.1 microgram per milliliter has been found sufficient to kill certain coliform bacteria.

It has also recently been discovered that low concentrations of hemoglobin (0.1 microgram per milliliter or less) exert a lethal effect on some Gram-negative bacteria provided the reaction medium is of low pH and ionic strength. This bactericidal action is due to the globin portion of the molecule; it is blocked by certain polysaccharides as well as by haptoglobin, the normal hemoglobin-binding protein of serum.

One of the important conditions which influences the fate of
microbes in leucocytes is the intracellular pH. Previous investigators, using indicator dyes or microelectrodes, have observed a reaction near pH 4.5 in phagocytes. Studies now in progress in our laboratory are aimed at measuring acidity within intact rabbit polymorphonuclear cells. The new technique employed for this purpose makes use of the known relationship between the distribution of a weak electrolyte and the difference in pH on two sides of a cell membrane.

Clinical experience strongly suggests that many types of disturbances and stresses increase susceptibility to infection in a non-specific manner. The factors which bring about a breakdown of resistance are being investigated in this laboratory by the use of experimental infection models in albino mice. The susceptibility of these animals to infection is being measured in terms of two criteria: (a) survival time of the infected animals following intravenous or intraperitoneal injection of a standardized dose of bacteria; (b) fate of the bacteria in the organs at various intervals of time after infection. It has been found that susceptibility can be modified at will by submitting the animals at the time of infection to agents and procedures as varied as: hormones, chemical or bacterial toxins, asymptomatic allergic reactions, nutritional upsets, and mildly irritating smogs. In all cases the infection-enhancing effect is non-specific, modifying in a similar manner the response of the animals to many types of Gram-negative bacilli, to staphylococci, or to tubercle bacilli. Furthermore, this effect occurs rapidly and is readily reversible, the animals recovering their normal state of resistance within a few days after cessation of the disturbance. Recovery of resistance may occur even though the disturbance be continued, provided the animal has had the time to become adapted to it before administration of the infective microbial agent.

Among factors that influence natural resistance, emphasis is being placed presently on the intake of nutritional proteins. As is well known, protein deficiencies constitute the most important nutritional problem in the world today, particularly in econom-
Medically underprivileged countries. It is certain moreover that the deleterious effects of protein deficiencies are most notable among children. For this reason, experiments have been instituted to test the effect of protein nutrition on the resistance of young animals to infection. These studies have revealed that animals receiving a diet containing 20 per cent casein are much more resistant than those receiving only 8 per cent of this protein. Not all proteins are equally effective in this respect. For example, animals fed commercial pellets containing 22 per cent protein, but chiefly of plant origin, are more susceptible to infection than those receiving 20 per cent casein. Supplementation of the diets with the proper kind of synthetic amino acid mixtures increases resistance to infection. It is of interest that the effect that the various experimental diets exert on resistance to infection bears no relation to their effect on the weight curve of uninfected animals.

An accidental discovery has recently opened up another approach to the problem of non-specific resistance to infection. In the course of studies on the development of a non-living vaccine against tuberculosis, it was found that injection of certain cellular constituents of the tubercle bacillus can affect in a dramatic manner the response of experimental animals to unrelated bacterial infections, increasing either their susceptibility or their resistance depending upon circumstances. The mechanism of this effect is now under investigation. It has been found that the cellular fractions of tubercle bacilli which modify resistance to infection also affect profoundly the activity of the reticulo-endothelial system as measured by uptake of carbon particles by the living animals. Attempts are being made to separate by chemical procedures the components of tubercle bacilli which are capable of eliciting immunity to tuberculosis and those which have an effect on other infections and on certain physiological activities.

Dr. Dubos' and Dr. Horsfall's laboratories are jointly conducting clinical studies on respiratory diseases. Three programs are currently active: (1) Studies on sputum cytology in respiratory
infections, (2) Investigation of the relationship between metabolic rate and recovery from tuberculosis, and (3) Studies on disseminated sarcoidosis.

The study of sputum cytology in respiratory infections is being conducted in collaboration with Dr. George Papanicolaou, Cornell University Medical College, who recently described in some sputum films the presence of a peculiar type of degenerated ciliated epithelial cell, called ciliocytophthoria (CCP). This finding was usually associated with pulmonary infections of obscure etiology. Some of the evidence also suggested that CCP might be correlated with subsequent development of lung cancer. By studying sputum films from patients with various types of respiratory infections and other pulmonary diseases, including cancer, the significance of CCP is being assessed. The findings thus far indicate that CCP occurs in association with numerous respiratory infections of viral origin, e.g. influenza, but not with acute or chronic bacterial infections of the lung. Further studies are required to establish the relationship between CCP and neoplasia, and to determine whether sputum cytology will be of use for early diagnosis of pulmonary infections.

Studies on patients with tuberculosis have been directed towards better understanding of the relationship between thyroid function and host resistance. Observations have been made on the recovery from moderately far advanced active pulmonary tuberculosis of 8 patients to whom triiodothyronine was administered intermittently to induce alternating cycles of hyper- and euthyroidism. All were treated continuously with combined antituberculosis chemotherapy. In no patient was there demonstrable acceleration of the rate of recovery during periods of induced hyperthyroidism.

A long-term study of disseminated sarcoidosis has been initiated. Sarcoidosis is a chronic disease, or group of diseases, characterized by the appearance of disseminated granulomatous lesions, most commonly involving lymph nodes and lungs. Although formerly considered rare, sarcoidosis is now recognized with increasing fre-
quency, especially in certain ethnic groups. In young adult American Negroes, for example, recent evidence suggests that sarcoidosis occurs almost as commonly as tuberculosis. The etiology is unknown; at present it cannot even be placed in a disease category, i.e., infectious, toxic, allergic, etc. By investigating various aspects of the epidemiology, natural history and pathogenesis of this mysterious malady, it is hoped that meaningful information will be gathered which may eventually lead to understanding of its causes and provide logical approaches to its treatment.

Laboratory of Frank L. Horsfall, Jr.
Igor Tamm, James S. Murphy, Marjorie M. Nemes,
Suydam Osterhout

Of the common infectious diseases of man, those which are most poorly controlled are the large group caused by viruses. Useful preventive measures are available for only a few. Effective treatment is not available for any. Antibiotics and chemotherapeutic substances, so useful in the treatment of many bacterial diseases, are ineffective against virus diseases. Many occur so commonly or so frequently that they are generally accepted as being ubiquitous and inevitable.

Contrary to common opinion, virus infection and virus disease are not identical. Disease does not develop unless virus infection occurs but infection does not regularly lead to a diseased state. With certain viruses infection which does not result in disease is more frequent than infection which goes on to disease.

Neither infection nor disease occurs unless the virus multiplies. Virus disease depends also upon a gross host response to the infection. This occurs commonly in some instances; infrequently in others. To understand the mechanisms which lead a virus to induce disease, it is necessary to learn how the virus multiplies and how the infected host reacts to this process.

Multiplication of viruses is studied directly in dynamic and bio-

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chemical experiments; indirectly by altering the process with chemical compounds of known structure. The multiplication of some viruses can be examined in the absence of gross host cell reactions. This makes possible the separation of infection from disease and an examination of the features of the antecedent events without the sequelae. It is not possible to induce disease without a preceding infection, although gross tissue reactions can be produced in the absence of multiplication in non-susceptible hosts. Although such reactions may not be precise models of events in nature, their study illuminates the toxicity of virus materials.

The dynamics of and the biochemical alterations accompanying virus multiplication have been extensively investigated. Understanding of the mechanisms of biosynthesis of virus nucleic acid and protein and their assembly into new virus particles is no more advanced than for other cell components. The mechanisms of the pathological responses of the infected host have received relatively little close scrutiny, in part because of the inadequacy of available techniques.

The dynamics of multiplication of human, animal, plant and bacterial viruses are closely similar, if not identical. Only the time parameter seems to vary. In each case, the infecting particle is disrupted and loses its identity in the process of initiating multiplication in the infected cell. The nucleic acid of the virus, not the whole particle, orients biosynthetic processes in the cell which then produce precursor virus components. Certain components, particularly nucleic acid and protein, are elaborated separately, both in space and time, and only later are assembled into new virus particles. The capacity to infect another host cell is acquired mysteriously and is the very last property added. How the completed particle leaves the cell is not yet clear.

With some human viruses, the final step in the multiplication process, i.e., the acquisition of the infective property, is controlled by a reaction comparable to a feed-back mechanism. Virus particles themselves, when they reach a sufficient concentration, inhibit the
production of more infective particles but do not otherwise greatly affect the dynamics of multiplication. The emergence of non-infective particles does not maintain the infection and may contribute to recovery.

Certain compounds of known chemical structure, i.e., chlororibosyl derivatives of benzimidazole, inhibit the biosynthesis of ribonucleic acid. Such compounds effectively inhibit the multiplication of human viruses, e.g., influenza and poliomyelitis, which contain ribonucleic acid. They are effective only during the early interval when the precursor nucleic acid is produced in the infected cell. With chemical inhibitors of protein synthesis, analogous results are secured. These inhibitors are active chiefly during the interval when precursor virus protein is produced in the infected cell. None of the inhibitors yet discovered is capable of diminishing virus multiplication without also affecting some metabolic activity of the host cell. Whether it will be possible to develop compounds that can specifically and selectively prevent the biosynthesis of a virus component and not interfere with the metabolism of host cell materials remains to be seen.

Some synthetic compounds of known structure, i.e., polyhydroxyalkyl derivatives of benzimidazole, augment the multiplication of influenza virus without affecting ribonucleic acid metabolism. Such an effect is not secured with any known cell metabolite. This suggests that multiplication depends in part upon a metabolic system which is as yet unidentified.

Pathological changes in infected host cells do not appear during the period when precursor virus components are being produced. This is the interval during which chemical inhibitors are effective. Pathological alterations usually are not recognizable until new virus particles are assembled and mature particles emerge from the infected cell. Tissue lesions commonly do not appear until the concentration of virus particles has approached maximal levels. By this time, the process of multiplication is almost if not entirely completed.
Whether it is the process of multiplication and the associated biosynthetic alterations or the products of the process that lead to damage of the infected cell is not yet clear. The nature of both the virus and the host contribute to the development of damage. Variant strains of the same virus may be pathogenic or non-pathogenic even though they multiply. The multiplication of certain viruses leads regularly to severe lesions in one host species but causes no lesions in another. Furthermore, a virus infection may induce disease in one individual but not in another. Except that it may result in immunity, infection by a virus need be of no concern to the infected host if it does not lead to lesions or disease.

Laboratory of Henry G. Kunkel
A. G. Bearn, E. C. Franklin, H. R. Holman, R. Trautman

A study of some peculiar antibody-like proteins found in the blood of patients with various forms of arthritis represented the major project of the past year. Rheumatoid arthritis and allied disorders remain diseases of completely unknown etiology, and it is hoped that the specific alterations under investigation may furnish a clue as to the disease mechanism. Initial observations, particularly by Dr. Franklin, demonstrated the presence of an unusual high molecular weight material in the serum and plasma of many patients with rheumatoid arthritis. This fraction was not observed in the blood of normal individuals or patients with other disorders and accounted for a number of serological reactions known to be characteristic of rheumatoid arthritis. Isolation and detailed studies of the high molecular weight material indicated that it represented a complex of two different proteins. It could be dissociated readily by urea, acid buffers and numerous other reagents. One portion of the complex had the chemical, physical, and immunological properties of a high molecular weight antibody and accounted for the serological properties of the complex. The other fraction is as yet uncharacterized. The accumulated evidence sug-
suggests that the high molecular weight material circulating in the blood of these patients represents an antigen-antibody complex. Just what role it plays in the manifestations of rheumatoid arthritis remains to be determined.

Detailed studies of another peculiar group of proteins found in the blood of patients with the related disorder, lupus erythematosus, have indicated that these probably represent auto-antibodies to different components of the patient's own cell nuclei. This hypothesis was first suggested from observations of Dr. Holman with the fluorescent antibody technique where isolated cell nuclei and nucleo-protein were found to be specifically coated with protein when exposed to serum of patients with this condition. Further studies employing complement fixation reactions in collaboration with Dr. W. C. Robbins showed that a whole group of independent factors existed which reacted with different components of cell nuclei including histone, deoxyribonucleic acid (DNA) and whole nuclei. The reaction with DNA was of particular interest because it was completely non-specific in regard to the species used as the source of the DNA; preparations from herring sperm reacted just as well as those from human sources. Similar factors have not been demonstrated clearly in experimental animals and these patients appear to represent an experiment of nature through which the problem of anti-nuclear antibodies may be investigated.

Dr. Bearn continued his studies of problems in human genetics, particularly those related to alterations in blood problems. Investigations centered about the haptoglobins, a group of proteins which vary in normal individuals and are inherited according to Mendelian Laws. Partial isolation and characterization of these proteins was accomplished. Difference in molecular weight were observed with the ultracentrifuge. The technique of Smithies for starch gel electrophoresis aided considerably in determining the genetic differences in these as well as other serum proteins.

A combined genetical, clinical, and biochemical study of 25 families with Wilson's Disease initiated several years ago was con-
continued throughout the year. The importance of the environment in modifying the biochemical expression of the mutant gene was examined. It has now been observed that rarely a low serum ceruloplasm (the characteristic biochemical abnormality) may be increased to supernormal levels by an alteration in the patient’s own internal environment, e.g., the administration of estrogens. The converse, namely a similar biochemical abnormality in members of these families with no overt manifestations of the disease, has also been found. These observations underscore the necessity of evaluating the modifying influences, both genetic and environmental, which influence the expression of the phenotype.

Laboratory of Maclyn McCarty
Rebecca C. Lancefield, Richard M. Krause, William C. Robbins, Lewis W. Wannamaker

The continuing studies on the biology of group A streptococci can be divided into two general categories: those related to the surface of the bacterial cell and those concerned with the numerous extracellular products produced by these organisms. The analysis of the cell surface is being approached by investigations of the antigenic structure, chemical composition and biological significance of the cell wall and of the various substances which appear to be attached to it. This includes a continuation of previously reported studies on M protein and its antibody, on the nature of bacteriophage receptors and on the properties of an enzyme present in bacteriophage lysates of group C streptococci which is capable of lysing group A cell walls.

Further evidence has been obtained to support the view that the primary determinant of serological specificity of the cell wall carbohydrate of group A streptococci is N-acetyl-glucosamine, probably occurring as side chains. Additional studies have confirmed the finding that the substance removed from the carbohydrate by an induced bacterial enzyme which destroys serological reactivity is
indistinguishable from authentic N-acetyl-glucosamine. It appears that the N-acetyl-glucosamine is attached to the remainder of the carbohydrate molecule through $\beta$ linkages, since the enzyme will attack synthetic $\beta$-but not $\alpha$-glucosaminides. Furthermore, phenyl-N-acetyl-$\beta$-glucosaminide effectively induces formation of the enzyme by the soil bacillus while the corresponding $\alpha$ compound is inert. The relative simplicity of the antigenic determinant made it possible to apply the classical diazo antigen technique to the problem. It was shown that antigens prepared by coupling p-amino-phenyl-N-acetyl-$\beta$-glucosaminide to various proteins, such as crystalline egg albumin or bovine serum albumin, cross-react consistently with group A streptococcal antisera but not with antisera of other groups. N-acetyl-$\beta$-glucosaminides specifically inhibit the precipitin reaction with either group A carbohydrate or the synthetic diazo antigens. These results supply additional evidence for the importance of N-acetyl-glucosamine as an antigenic determinant in group A carbohydrate.

Analysis of the total complement of extracellular substances produced by group A streptococci during growth in fluid media has indicated that the known enzymes and toxin can account for only a fraction of the bacterial protein released. A significant finding arising from this study is that these organisms produce three electrophoretically and serologically distinct desoxyribonucleases. In certain cases, at least, a single strain is capable of producing all three enzymes simultaneously. Each of the desoxyribonucleases is magnesium activated, but they differ from one another in the pH of optimum activity. It remains to be determined whether they attack different linkages in the substrate macromolecule.

Rabbit antisera obtained by immunization with electrophoretically separated preparations of the three nucleases specifically inhibit only the homologous enzyme. Examination of human sera shows that one of the enzymes, designated desoxyribonuclease B, is the most consistent stimulator of antibody production. Most individuals have detectable serum antibody to this enzyme, and a
high percentage of patients show increases in titer following streptococcal infection. On the other hand, antibody to desoxyribonucleases A and C is much less common and the response after natural infection is irregular. This is consistent with earlier studies in this laboratory which indicated that the antibody response to streptococcal desoxyribonuclease in human beings is highly variable, since it can now be shown that the strain employed in these studies produces predominantly desoxyribonuclease A.

The Hospital

The Hospital maintains 55 beds for selected patients with diseases that are under study by the staffs of the Laboratories of Medicine. It also maintains an out-patient clinic for the continuation and extension of investigations on patients. The study of certain common and important diseases of human beings has been carried on constantly in the Hospital throughout 46 years. The application of the methods and techniques of the basic sciences to the problems of medicine and the opportunity to pursue free inquiry in depth have yielded important information and led to the development of new concepts of disease.

The major disease categories under study are: cardiovascular diseases, including coronary heart disease, and abnormalities in fat metabolism; endocrine disorders, particularly those of the thyroid and parathyroid glands; hepatic diseases, including various forms of cirrhosis and abnormalities in metal storage; respiratory diseases, particularly pulmonary tuberculosis and those initiated by viruses; and rheumatic diseases, including lupus erythematosus and acute rheumatic fever.

The chief objectives of the clinical and laboratory investigations bear on the following problems: the rates and pathways of fat metabolism and the storage and utilization of fats in relation to cardiovascular diseases; the mechanisms by which hormones influence metabolic processes affecting growth and maturation as
seen in endocrine disorders; the mechanisms responsible for defects in protein metabolism associated with hepatic and certain other diseases; the mechanisms of susceptibility and resistance to infection and the relation of virus reproduction to respiratory and other diseases; the genesis of rheumatic fever and host reaction to infection as bearing on rheumatic diseases.

The laboratories of Doctors Archibald, Dole, Dubos, Horsfall, H. Kunkel and McCarty carried out the study and investigation of patients admitted to the Hospital as well as those followed in the out-patient clinic. Physicians on the staffs of these laboratories and the post-doctoral Graduate Fellows who participate in clinical investigations undertook responsibility for the care and treatment of patients. A number of consulting physicians, associated with other university teaching hospitals, generously made available their special training and competence whenever these were needed.

During the year, 223 patients, of whom 195 were admitted during this period, were cared for and studied in the Hospital. The number of patients in each major category was: cardiovascular diseases, 64; endocrine disorders, 21; hepatic diseases, 59; respiratory diseases, 44; and rheumatic diseases, 35. The number of patient-days in hospital was 12,673. The average period of hospitalization was 65 days.

The long period of hospitalization reflects the protracted nature of many of the investigations, not the severity of the illnesses of the patients. As the study of chronic infectious, degenerative and metabolic processes has progressed, the need for long continued comprehensive investigations has increased. Since 1952-1953, the average period of hospitalization has been extended from 34 to 65 days, i.e., by 91 per cent.

The number of visits to the out-patient clinic was 3,082; 631 were new patients; 2,451 were return visits. The large proportion of return visits reflects the protracted character of the follow-up studies that are required by many of the studies. The number of clinic-visits by patients in each of the major categories was: cardio-
vascular diseases, 398; endocrine disorders, 1,365; hepatic diseases, 511; respiratory diseases, 392; and rheumatic diseases, 414.

Poliomyelitis vaccine was made available to all members of the staff and their immediate families; 747 immunizations were carried out in the clinic.

Seminars in Medicine, conducted by members of the Faculty or by eminent investigators from sister institutions, were held in the Hospital each week during the academic year. The Journal Club, an informal association for the discussion and exchange of scientific information, continued to hold biweekly meetings as it has for nearly 40 years.

The complete records of more than 12,000 patients who have been studied in the Hospital are being reproduced on microfilm and will be made available on microtape mounted on cards. This conversion will greatly reduce the space required for their storage and will provide complete protection against their loss or destruction.

The facilities, furnishings and equipment of the out-patient clinic and the equipment of the wards of the Hospital were increased and improved through funds provided by a grant from the Ford Foundation.

The more significant new findings which resulted from studies on disease in human beings are summarized in the reports of the Laboratories of Medicine.

**NEUROPHYSIOLOGY**

Laboratory of Herbert S. Gasser

In a study of the differentiation of nerve fibers it is as important to consider the properties that all fibers have in common as to determine the variations in behavior of the several classes. To the century old differentiation of nerve fibers in accord with their medullation, a recent addition has been the division of the un-
medullated fibers into two subgroups on the basis of their origins: those arising from sympathetic ganglia (s.C), and those arising from the ganglia on the dorsal roots (d.r.C). Unique among nerve fibers is the large positivity recorded immediately after the spike of the d.r.C fibers. When it was first observed the information was lacking which would permit satisfactory correlation of this feature with an aspect of the action potential, known for the generality of nerve fibers. Recent work in the laboratory has been directed toward the supplying of this information. It required some new studies of the A group of medullated fibers as well as experiments on C fibers.

Postulation of two loosely linked processes is necessary for an understanding of the configuration of the action potential; and two are sufficient for interpretation of all the changes in configuration observed during tetani of mammalian nerve fibers in a physiological condition. They are designated as the spike process and the after-potential process. In both, manifestation of action starts with a negative deflection and is continued through a subsequent stage of positivity to restoration. The positivity after the spike is called the first positivity (P₁), and that following the negative after-potential the second positivity (P₂). Experimentation consisted in recording tetani at film speeds adequate for revelation of how the development of the processes enters into the shape and position of each successive component of the tetanus. Both frequency and duration of the tetanus are important variables in the obtaining of information.

If the frequency is high enough, in all fibers P₁ increases at the start of a tetanus, with the rate of increase greater as the frequency is augmented. The increase is easy to demonstrate in A fibers, more difficult in C fibers. In the d.r.C fibers the difficulty is derivative from the fact that the postspike positivity is already so great in a single response; in the s.C fibers it is for another reason.

As a tetanus progresses, that the negative after-potential increases is readily visible in the successive changes in the compo-
ments of the tetanus. Even during a tetanus the P₂ reaction appears and at the end of a tetanus P₂ is uninterrupted and long lasting. In the algebraic summation of events the effects of the changes in the two processes as influenced by frequency and duration are complex, but resolvable. As would be expected, the sequence of changes is quantitatively different for each of the sorts of fibers.

For the difference between the A and s.C fibers, cathodal electrotonus curves recorded on these two sorts of fibers were illuminating. On the s.C fibers the ratio of slow electrotonus to fast electrotonus was found to be much higher than on the A fibers. Slow electrotonus is produced by the after-potential process, fast electrotonus by the spike process. Correlated with the slow electrotonus, a large negative after-potential and its subsequent P₂ have a tendency to obscure the relatively smaller P₁ so that in some situations the latter cannot be separately identified in s.C fibers. In the course of a tetanus the negative after-potential-P₂ sequence dominates the events. Nevertheless P₁ can be brought out at an appropriate frequency. On the ground of the negative after-potential alone, it would be instructive to have an electrotonus curve of the d.r.C fibers. But no nerve is known on which it can be obtained. It certainly would be different from the one on the s.C fibers.

The outcome of the survey was to show that d.r.C fibers conform with other fibers. In spite of its unparalleled size the postspike positivity is still a P₁. It can be caused to increase when the fibers are forced into activity at a frequency higher than any that would probably be demanded in physiological functioning. There is no visible negative after-potential in a single response. However, during tetani at frequencies permitting a large part of the course of the P₁'s to be observed, a negative after-potential develops. It starts by filling in the troughs of the P₁'s, with the result that the P₁'s seem progressively to get smaller. Later a visible shape to the negative after-potential appears; and if the tetanus is long enough there is after the end of the tetanus a clearly defined and slowly decrementing P₂. Its visible beginning is at a sharp bend in the positive
after-potential after the terminal $P_1$. Absence of supernormality during the negative after-potentials is accounted for on the basis that even at the negative after-potential maxima the net state of the fibers is one of hyperpolarization.

Laboratory of David P. C. Lloyd  
Vernon B. Brooks, Susumu Hagiwara, Victor J. Wilson

During the year the work of this laboratory has expanded into a new and very promising field while continuing to be concerned with the problems of neural organization and of excitatory and inhibitory phenomena in various relatively simple neural pathways. This new endeavor, utilizing the simplest of techniques in its initial stages, concerns the activity of sweat glands and the neural control thereof. It has already provided an unequivocal answer to the controversial question of reabsorption from the lumina of sweat glands.

*Observations on sweat emergence in the central foot-pad of the cat.* (Lloyd). Reabsorption of water and of sodium has been postulated to account for concentration differences between slowly and profusely secreted sweat, or for those between plasma and sweat. Direct evidence of reabsorption is lacking and the ducts of sweat glands usually are regarded as mere channels from the secretory tubules to the surface.

If one were to assume that the ducts of truly resting sweat glands are empty, then, on activation by stimulation of the motor nerve supply, sweat *formation* would begin, but a phase of duct filling would antecede sweat *emergence* at the skin surface. At some time following cessation of stimulation formation would cease as would emergence, but the condition existing prior to stimulation would not be regained before the ducts again became empty through re-absorption.

Sweat emergence at the surface of the cat’s foot-pad in response to stimulation of the secretomotor nerves has been observed visually and its latency in various circumstances of stimulation measured.
According to visual inspection stimulation at 10 per sec. after prolonged rest may require up to 60 sec. for beginning sweat emergence. Repetition of the stimulation after a brief rest causes sweat to emerge in 2.5-3 sec. As the rest period between stimulations is increased emergence latency increases initially rapidly and linearly with duration of rest, and then more slowly. A final relatively constant value for latency has not yet been encountered despite observation after rest periods approaching 90 min.

If one applies ‘conditioning’ stimulations of 1 min. duration at 10 per sec. frequency, and, after constant rest periods of 2 min., applies ‘test’ stimulations of variable frequency then latency of sweat emergence in response to the test stimulation is found to vary inversely as the frequency in the range of frequencies between 30 per min. and 10 per sec. At low frequency (6 per min.) there may be no visible emergence.

These observations indicate that the ducts of sweat glands do, in fact, reabsorb; that sweat formation is a relatively rapid process, sweat reabsorption being a relatively slow process; that the minimum emergence latency with ducts full represents the latency of sweat formation; and that a balance can be struck between formation and reabsorption.

Some effects of rhythmic stimulation upon excitatory and inhibitory actions in the spinal cord. (Wilson) As a rhythmic train of impulses travels through a simple reflex pathway there is during the train a depression of response and following it there may be a period of enhanced response. Both these phenomena are traceable to action in the afferent rather than the motor side of the reflex pathway. These effects, depression and potentiation, can be employed as tools to investigate certain fundamental and controversial aspects of excitatory and inhibitory action. Two of the problems upon which the present experiments throw light can be posed as follows. Are there two phases in excitatory action at the junction between nerve cells? Does the inhibitory path in the reflex pathway of the knee jerk, unlike the excitatory path contain a connector
nerve cell intercalated between afferent cell and motor cell?

Observations have been made on the occurrence or non-occurrence of depression and potentiation of not only the brief transmitter action, but also of the more prolonged facilitator action of afferent impulses, and also with regard to these effects a comparison has been made between the direct inhibitory pathway and an inhibitory pathway known to contain a connector cell (a disynaptic pathway).

Briefly put, transmission and facilitation in the excitatory paths, and inhibition in both sorts of pathway are all potentiated following a rhythmic train of impulses. The early phase of excitation, associated with transmission, is far more sensitive to depression during a rhythmic train than is the more prolonged facilitator action. Hence there is one more valid reason for making a distinction between two phases of excitation. Direct inhibition does not suffer depression during the course of rhythmic action, but the known disynaptic pathway does. As it is likely that the inhibitory junctions (at the motor nerve cells) in both pathways are similar, it is supposed that depression in the disynaptic pathway occurs at the excitatory junctions between the afferent nerve cells and the connector cells resulting in fewer connector cells being active and hence a reduction of their inhibitory action upon the motor nerve cells. Failure of the direct inhibitory pathway to suffer depression is consistent with indications that it does not contain a connector cell.

Limitation of stretch reflex action by recurrent inhibition. (Brooks and Wilson) The stretch reflex, of which the knee jerk is a well known example, has long been known to be highly restricted to the muscle that is stretched. Its restriction is, in fact, greater than can be accounted for simply by the known anatomical limitation of reflex connection between afferent nerve cells and motor nerve cells in its (monosynaptic) reflex pathway.

The motor nerve cells possess, in addition to their axons going to the muscles, axon branches that return into the gray matter of the spinal cord (recurrent collaterals). These are active whenever
the motor cells are active and their action gives rise to a repetitive discharge of impulses in connector cells. It is known that some motor nerve cells in action depress response of neighboring motor cells. The depression could be due to known current flows about the active motor nerve cells, or to an inhibitory action of the connector cells. The experiments here described permit a choice between these two hitherto acceptable alternatives. A substance, dihydro-\(\beta\)-erythroidin, is known to reduce markedly the response of the connector cells in question. In these experiments the substance has been shown to reduce the depression of neighboring motor nerve cells markedly while having little if any effect upon the current flows about the active motor nerve cells. Hence it is concluded that the connector cells rather than the current flows are responsible for the depression, and because of this that the action is probably one of physiological importance rather than a chance phenomenon. It is suggested that this ‘recurrent inhibition’ plays a role in securing the characteristic limitation of stretch reflex action.

To test the hypothesis use has been made of the fact that trains of impulses in the stretch afferent cells of one head of a muscle (medial gastrocnemius) lead to discharge of motor cell impulses to that head of the muscle, but ordinarily cause little or no discharge of motor cell impulses to the other head (lateral gastrocnemius) despite the anatomical fact of excitatory connection to those latter motor cells. If the hypothesis is valid then injection of di-hydro-\(\beta\)-erythroidin, by blocking the recurrent inhibitory action of motor nerve cell impulses, should result in an increase in, or appearance of, motor cell discharge to the other head of the muscle, which in fact it does.

**Input-output relation in a flexor reflex.** (Lloyd) Knowledge of the relation between impulse input to any given nerve center (the independent variable) and impulse output (the dependent variable) from that center is extremely useful for an understanding of the afferent channel to that center and of the workings of the
center itself. That part of the spinal mechanism subserving flexor reflex transmission is quite complex, but basically, leaving detail aside, the input-output relation is simple. Some 6 to 12% maximal input, is required before there is output from the flexor reflex center and, ‘threshold’ having been reached, the relation is linear up to maximal input, which is to say that flexor reflex afferent nerve cells are rather uniformly distributed throughout the population of afferent cells. This population contains cells afferent for a variety of reflex actions, but in no case that has been studied rigorously has this same even distribution been found. It is further of interest that this same population subserves a variety of sensations. The problem now is whether one particular sensation associable with flexor reflex action is rather evenly represented throughout the population, or whether a variety of sensations are associable with flexor reflex production, but not with other actions reflex or sensory.

**Functional organization of the lower sacral segments of the cat spinal cord.** (Wilson) The terminal segments of the spinal cord are of particular interest from the standpoint of neural organization for they innervate midline structures, such as the tail, rather than lateral structures (the limbs). Consonant with this distinction there are anatomical differences between these lower sacral segments and those segments that innervate the limbs. The present study reveals the manner in which some of the reflex paths for bilateral control of the midline structures are connected.

Low threshold afferent fibers, presumably from stretch receptors, possess direct excitatory connection with motor nerve cells of the same side and direct inhibitory connection with motor nerve cells of the opposite side. Slightly higher threshold fibers possess a powerful relay through one connector cell to the motor nerve cells of the same side and less powerful excitatory connection, also through a single connector cell relay, to the motor cells of the opposite side. These (disynaptic) paths from the two sides converge at the motor nerve cells but not, insofar as tests can reveal, at the connector cell level. The connector cell nuclei thus appear
to be bilaterally independent structures. Still higher threshold afferent fibers connect through a complex connector cell (polysynaptic) pathway to the motor nerve cells. There is clear evidence that bilateral convergence of these polysynaptic pathways occurs at the connector cell level.

Synaptic potentials in the motor giant axon of the crayfish. (Hagiwara) The ‘synaptic potential’ is an enduring electrical change, associated with enhanced excitability, that occurs in a nerve cell as the result of excitatory impulses impinging upon it from other nerve cells. It is usually considered a necessary step in the generation of an impulse, but this is debatable. Further it is usually considered to have an ‘active’ rising phase and to decay passively over a time course determined by the electrical time constant of the nerve cell membrane, a view that is no longer tenable for all junctions.

In the present detailed study of the junctions between giant axons in the crayfish it is shown, by means of intracellular recording, that the slow membrane depolarization taking place in the motor giant axon fulfills all the requirements for designation as a ‘synaptic potential,’ but, unlike a potential that decays passively, it redevelops after impulse conduction; it is much more enduring in time course than the measured time-constant of the membrane and, furthermore, tests show that the membrane conductance change is not confined to the early phase, as at the neuromuscular junction for example, and as demanded by the hypothesis of passive decay, but lasts until the late phases of the synaptic potential. There is, in short, at this junction a prolonged action upon the nerve cell rather than merely a brief transmitter action.

Recovery of the cerebral cortex following activation. (Brooks) Following stimulation of the somato-sensory cortex, or of the afferent pathways to it, there is a cyclical recovery of responsiveness to a subsequent testing stimulus of either sort, just as has been described for the optic cortex. The periodicity is that of the normal electrical waves of the cortex, the conditioning stimulation appear-
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ing to reset the normal rhythm as from the time of impact. During the cyclical recovery responsiveness varies between normal at the maxima and extreme depression at the minima. Injection of strychnine, at subconvulsive dosage, restores responsiveness during the depressed phase suggesting, in line with other observations upon the action of strychnine upon the spinal cord, that the depression is due to active inhibitory processes rather than being a consequence of prior activity. Afferent paths appear to mediate the inhibition, for a recurrent collateral inhibitory system between motor cells in the cortex, comparable with that in the spinal cord, has failed of demonstration.

Laboratory of Rafael Lorente de Nó
G. A. Condouris, J. García-Bilbao, L. M. H. Larramendi, F. D. Vidal

During the past year this laboratory has been engaged in the study of the role of sodium in nerve function and of the mechanism of anesthesia of nerve by drugs of the type of cocaine. The two problems are closely related since abundant evidence has been obtained that cocaine blocks conduction by competitive inhibition of some step in the chain of reactions in which sodium plays a part, directly or indirectly.

In a sodium-free medium the nerve fibers lose their ability to conduct impulses because they lose to the medium a part of their internal sodium, while in a cocaine solution the nerve fibers retain their normal sodium content. Nevertheless, cocaine and related compounds bring the nerve to a state that in many important respects is similar to that which develops after the nerve has been kept in a sodium-free medium for some time.

In an attempt to establish a relationship between chemical structure and ability to substitute for sodium, a systematic study has been made of a series of organic compounds having a basic nitrogen atom (guanidines, hydrazines, hydroxylamines, amines and qua-
ternary ammonium bases). The problem is still obscure. For example, two compounds as different chemically as hydrazine and guanidine promptly restore the ability to conduct impulses to sodium-deficient fibers of the somatic system (A fibers) and yet methylguanidine and methylhydrazine are unable to substitute for sodium. No less remarkable is the fact that trimethylamine, triethylamine and tetraethyl-ammonium restore the ability to conduct impulses to sodium-deficient fibers of the sympathetic system (B and C fibers) while tetramethyl-ammonium and choline fail to substitute for sodium.

The work on the extraction of nitrogen bases from acid hydrolysates of ox brain has been continued. Highly purified extracts are now available that restore to sodium-deficient sympathetic fibers the ability to conduct impulses, and what is perhaps more remarkable the ability to produce a large positive overshooting after the end of an applied cathodal current. This overshooting is the equivalent of the positive after-potential after the end of a train of nerve impulses.

PARASITOLOGY

Laboratory of Norman R. Stoll

*Bacteria-free cultivation of Entamoeba invadens, a pathogenic amoeba of snakes in a cell-free medium.* Determination of conditions, especially nutritional, favoring the growth of parasites in pure culture without contaminants (i.e. axenically) is the continuing interest of this laboratory, with major attention presently directed to *Entamoeba invadens*. The latter organism, which in nature produces a disease in snakes similar to amoebiasis in man caused by *E. histolytica*, is ordinarily maintained under laboratory conditions in cultures of mixed bacterial species. The fact that members of this genus have been found reluctant to grow in vitro
except in the presence of associated microorganisms, gave birth to the idea that they were incapable of surviving in the absence of other living cells. A step away from this conception was made when Miller (1951, 1953) established a bacteria-free strain of *E. invadens*, apparently capable of transfer in series indefinitely, using fresh liver tissue in serum-saline. While it was not clear that the parasite survived in his cultures longer than the liver cells remained viable, Miller did thus demonstrate the first *Entamoeba* species to be grown in serial cultures bacteria-free. He was not able, however, to maintain similar serial cultures in cell-free media, when sterilized extracts of liver were substituted for the tissue.

This next step, namely, of maintaining serial cultures indefinitely of *E. invadens* in cell-free axenic media is here reported.

Beginning with a study of the Miller type culture (original obtained through the kindness of Dr. E. Meerovitch, Institute of Parasitology, Macdonald College, P.Q.,) there was subsequently initiated axenic growth in a cell-free medium. Serial cultures have now (July 1) been maintained during the 12½ months since with no diminution of vigor. Besides the capacity for successive transplants, some individual cultures reserved for the purpose, have shown amoebae able to survive for several months and be successfully transferable.

The basic medium employed is a heat-sterilized broth made from liver, supplemented with RLE, a whole extract of raw liver. RLE is processed cold, as an acid infusion, and sterilized with a Seitz filter. Besides the heat-stable and heat-susceptible liver infusion components, optimal growth has required in addition a small amount of mucin. Other supplements have been tested with less gratifying results. No anti-biotics are used, and the cultures are vaseline-sealed to favor anaerobiosis.

It will now be of interest to determine whether the ability to grow in cell-free media is applicable to other species of the genus *Entamoeba*. In another direction, the avidity for mucin displayed
by *E. invadens* in axenic culture, as found earlier by Ratcliffe (1931) in mixed bacterial cultures with this parasite, may contain a basic suggestion in regard to the natural state of affairs in the host-parasite relation.

Laboratory of William Trager
Maria A. Rudzinska, Ira Singer

This laboratory is concerned with the physiological relationships between parasitic microorganisms and their hosts, with special reference to intracellular protozoan parasites. Knowledge of the conditions necessary for the extracellular development *in vitro* of an intracellular parasite may be expected to lead toward a better understanding not only of parasitism but also of the nature of the medium within which the organelles of the normal cell maintain their activities.

**Extracellular development in vitro of the bird malaria parasite Plasmodium lophurae.** (Trager) By means of methods previously developed, *P. lophurae* can be removed from its host red blood cells and maintained extracellularly in culture medium for several days. Its growth requirements under these conditions include a number of materials of known chemical nature and also unknown substances present in a concentrated extract of duck erythrocytes. The extent of nuclear multiplication of the isolated parasites during the first day of incubation *in vitro* is dependent on the concentration of the red cell extract, thereby providing a method for assay of the active substance or substances. The activity of the erythrocyte extract was found to be non-dialyzable, suggesting that proteins may be concerned. By means of starch electrophoresis, a fraction free from hemoglobin was prepared which had one third to one half the activity of the extract and only about 1% of the protein. Certain partially purified preparations of yeast hexokinase could also be used to replace in part the erythrocyte extract.

**Folic acid and malaria.** (Trager) Since some of the active anti-
malarial drugs are analogues of folic acid, it is believed that the folic acid group of compounds is of exceptional importance in the metabolism of malaria parasites. Folinic acid has been found in previous work to have a favorable effect, evident on the 4th day, on the extracellular in vitro maintenance of *P. lophurae*. Measurements of the folic and folinic acid contents of the red blood cells of normal ducks and of ducks infected with *P. lophurae* have now shown the latter to contain much more of these materials than the former. Only about one quarter of the increase was in the bodies of the parasites, the remainder evidently being in the infected cells. Invasion of an erythrocyte by a parasite appears to alter the folic acid metabolism of the host cell in a direction of benefit to the parasite.

*Intracellular phagotrophy by malaria parasites.* (Rudzinska and Trager) Electron microscopy of thin sections of duck red blood cells infected with *P. lophurae* showed clearly the location of the parasites within the cytoplasm of the host cell. Inside the parasites was a characteristic oval “residual body” containing the granules of hemin. Detailed study of this structure revealed the unexpected fact that malaria parasites are intracellular phagotrophs. They ingest into food vacuoles portions of the host cell cytoplasm. Within these food vacuoles, which eventually constitute the residual body, digestion of hemoglobin and the concomitant formation of hemin granules occur.

*Culture of hemoflagellates.* (Singer and Trager) The hemoflagellates form a group of parasitic protozoa which includes parasites of invertebrates with a relatively simple life cycle and relatively simple nutritional requirements and parasites, such as the trypansomes of African sleeping sickness, with a complex life cycle involving alternate vertebrate and invertebrate hosts, and with seemingly very complex nutritional requirements. Thus the blood stream forms of the pathogenic African trypanosomes have never been cultured in vitro. In experiments directed toward this aim, conditions have been found under which the number of trypano-
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osomes would in some instances double during the first 24 hours of incubation in vitro and the organisms would be maintained up to 48 hours. In a medium containing horse serum, a synthetic mixture of salts, vitamins and amino acids and a small amount of blood from an infected rat, the serum, the blood, and an initial pH close to 7.2 were all essential to maintenance. Beneficial effects were noted of closing the culture flasks with rubber stoppers, of adding 1% bovine albumin to the synthetic medium, of including purines and pyrimidines in the synthetic medium, and of preventing the pH from drifting to the acid side. Allowing free access of air to the culture flasks, gentle agitation on a rocking machine, or passage of a current of air with 5% CO₂ all had deleterious effects.

An ordinarily non-pathogenic trypanosome, Trypanosoma theileri, which occurs in cattle throughout the world, has been cultivated in vitro at 37°C. in a medium of bovine erythrocyte extract. These results, obtained at the University of Florida in collaboration with Dr. M. Ristic, constitute the first successful cultivation of a blood stream trypanosome of a mammal at body temperature.

A hemoflagellate of intermediate complexity, Leishmania tarentolae from African lizards, has been grown in a medium of known chemical composition. This organism requires hemin, 17 amino acids, purines and pyrimidines, riboflavin, thiamin, pantothenic acid, nicotinamide, biotin and a relatively high concentration of folic acid. In addition it must have either pyridoxal or the combination pyridoxine plus choline. The results indicate a role of pyridoxal in the synthesis of choline by this flagellate.

The fine structure of a free-living predacious protozoan, Tokophrya infusionum. (Rudzinska) Young overfed organisms of this species showed several characteristics remarkably similar to those observed in old normally fed organisms. The most striking were: (1) the frequent occurrence of hemixis (the presence of two or more macronuclei); (2) an accumulation of small dense particles, possibly insoluble waste products, occupying large areas of the cytoplasm; (3) a highly characteristic honeycomb-like structure
of some of the chromatin bodies of the macronucleus. The occurrence of this unusual degree of organization of the chromatin, visible in electron microscopy of thin sections, in overfed as well as aged *Tokophrya*, makes possible its experimental production at will.

Electron microscopy of thin sections of *Tokophrya* has yielded new information concerning the structure of the contractile vacuole. The vacuole was found to be connected to the outside by a canal consisting of a pore, a channel and a very fine tubule located in a papilla extending into the cavity of the vacuole. During diastole this tubule is closed, but at systole it is broadly open, allowing escape of the vacuolar contents. Numerous fine fibrils, radially disposed around the canal, may be responsible for the opening and closing of the tubule. Near the contractile vacuole and also in other parts of the cytoplasm are closely packed vesicles arranged in long, narrow piles. These as yet unidentified structures may be concerned in the transfer of fluid from the cytoplasm to the vacuole.

**PATHOLOGY**

Laboratory of Philip D. McMaster
Robert E. Franzl

The Formation of Antibodies. Dr. McMaster and Dr. Franzl are continuing studies on the mechanisms of antibody formation. No satisfactory explanation of the process can be proposed until more is learned about the fate of the antigens—bacteria, viruses or foreign substances—which stimulate antibody production once they gain entrance into the human or animal organism. In which organs or cells do the first stimuli to antibody production arise? What is the nature of these stimuli, and especially, as is so well known, how does prolonged antibody formation continue long after antigen has apparently disappeared from the body?

To find answers to these questions blue tracer antigens have
been prepared in this laboratory, injected into animals, and the sites of localization of the labeled protein have been studied histologically. Previous reports have shown that these markers are taken up by phagocytic cells throughout the body especially in lymphoid and splenic tissue, both of which are known to be active in antibody formation. These tissues consist not only of phagocytic cells—macrophages—and reticular cells—which take up antigen, but also, and to a larger extent, of non-phagocytic cells, the lymphocytes and plasma cells. All these types of cells have been considered capable of forming antibodies, although the uptake of the antigen by the phagocytic cells seems to be the first step in antibody formation.

In the past year, to gain insight into the processes involved in this first step and perhaps also in succeeding ones, the fate of a tracer antigen, injected into mice rendered incapable of forming antibodies, has been studied and compared with that previously determined in animals actually forming antibody. For the purpose the hormone cortisone—which, in large doses, has a destructive effect upon the lymphoid and splenic tissues—was given to mice in doses sufficiently large to suppress antibody formation even when the animals were stimulated to produce it either by injections of the tracer antigen or of other antigens as well. Under these circumstances it seemed likely that the behavior of the tracer antigen might indicate the types of cells injured by the cortisone and therefore involved in antibody formation. Should the uptake and the fate of the antigen remain the same, both in animals forming antibody and in those unable to make any, the finding would indicate that the functions of the phagocytic cells had not been harmed by the cortisone and that the suppression of antibody formation was not due to injury to them, but to the non-phagocytic cells.

Experiments begun a year ago and alluded to in the preceding report and now completed, have shown that the lymphoid and splenic tissues of cortisone treated mice became reduced to as little as one-tenth of their initial size. However, the shrivelled spleens
and lymph nodes of such animals, when injected with the tracer antigen, took up as much of it as the same organs of normal mice. The blue antigen was stored in the same types of cells, and it was not destroyed or eliminated any faster from the shrunken organs. The withdrawal of cortisone from such animals allowed the affected organs containing the blue tracer antigen to regenerate until they became as large as those of normal mice that had received similar amounts of the tracer at the same time. The organs of all the mice contained similar amounts of the tracer.

It was also demonstrated in the past year that cortisone did not inhibit antibody formation either by impairing the antigenicity of a native antigen, or by inducing a more rapid destruction of the antigen than occurs in animals given no cortisone. For example, mice injected twice with a foreign protein antigen, bovine gamma-globulin, together with an adjuvant, and thereby strongly stimulated to form antibody, were prevented from doing so by continuous dosage with large amounts of cortisone for various periods of time up to 26 days. When antibody formation was inhibited for 12 days after the last injection of antigen and the cortisone was then withdrawn, antibody formation appeared after another 12 days. Clearly the antigen had remained in the organs of the mice in a form capable of inducing antibody formation for as long as 24 days after its injection, a period of persistence fully as long or longer than that found in animals given no cortisone. These findings, taken in conjunction with the previous ones, indicate that cortisone does not inhibit antibody formation by interfering with the distribution and storage of antigen, nor by its rapid destruction. Since the phagocytic cells, even under the influence of cortisone, continue to localize and store the tracer antigen, their undisturbed function suggests that, although they partake of the first step in antibody formation by capturing and holding the antigen, they do not form antibody. Rather the cells of the lymphoid series, which are greatly injured and reduced in number by cortisone, seem to be the units that form antibody.
Intracellular Mechanisms of Antibody Synthesis. (Franzl) The research so far described, in this and previous reports, has attempted to indicate the organs and types of cells involved in the various phases of antibody formation. Newer work initiated by Dr. Franzl with Dr. McMaster is now endeavoring to show something about the intracellular mechanisms involved.

It is now generally agreed that antibody protein, which is a gamma-globulin, is not formed from existing globulins or other proteins, but is synthesized de novo from amino acids by specialized cells of the lymphoid tissue, among them the precursors of the plasma cell. The process must entail the utilization of many enzyme systems in correlation with the specific antigenic stimulus. Dr. Franzl has been using the techniques of cell fractionation to study the intracellular distribution of serologically active antibody early in its development within the cell. He is determining from isolated cell fractions such as nuclei, mitochondria, microsomes or the fluids in which such fractions have been present, the site of the earliest formation of demonstrable antibody, and the enzyme systems involved in its formation. Several means have been devised to obtain the types of cells suspected of forming antibody and to separate them in relatively high proportions from other types of cells which probably do not form antibody. But utilizing quantitative chemical and physical methods the active protein components of the cell fractions are being characterized and their properties compared with the extracellular, fully developed antibody after its discharge from the cells into the blood stream. The early findings, so far obtained, cannot be clearly presented until further data has been assembled.

Laboratory of Eugene L. Opie

The aim of studies in progress has been to obtain information concerning the movement of water and other substances between cells and extracellular fluid by measuring water exchange when tissues
are immersed in different solutions under varied conditions. Water movement has been determined by changes in specific gravity of immersed tissues, by changes in weight, by measurement of wet and dry weight and by depression of the melting point of tissues as contrasted with that of blood serum. The attempt has been made to compare the changes which follow immersion *in vitro* with those which occur during life as the result of injuries which increase the water content of tissues, for example, cloudy swelling, so-called hydropic degeneration, etc.

When thin slices of liver, kidney cortex, pancreas or salivary gland are immersed in solutions of sodium chloride of varied concentrations they do not, like red blood corpuscles, attain water equilibrium in a solution of sodium chloride having the concentration of that in blood plasma, as heretofore usually assumed, but with solutions having a concentration of approximately twice this strength. As abundant evidence shows that the electrolyte contents of the extracellular fluid is almost the same as that of blood plasma this higher level of isotonicity indicates that the secreting cell of the liver and of other tissues maintains an osmotic pressure considerably greater than that of the fluid surrounding them. This pressure may supply the energy needed for secretion or other functional activity. When urea or creatinin is administered in toxic quantity to white rats it enters hepatic and renal cells so that the level of isotonicity demonstrable in liver or kidney tissue is raised. This relation suggests that products of metabolism may have a part in maintaining intracellular osmotic pressure. When substances which selectively injure liver cells have been administered (chloroform or carbon tetrachloride), the level of isotonicity has fallen and when an agent which selectively injures the tubules of the renal cortex is given (potassium chromate), a similar result follows. When the animal recovers from the injury it has received isotonicity of the affected tissue rises to its normal level.

When tissue slices are immersed in a solution of sodium or of potassium chloride isotonic with it, isotonicity is maintained dur-
ing from 15 to 20 minutes but with longer immersion they take up water, doubtless as the result of injury. In the attempt to obtain conditions more favorable for the maintenance of tissue cells in vitro, tissue slices have been immersed in a buffered solution with electrolyte contents approximating that of blood plasma (Ringer’s solution) but with molar concentration adjusted by addition of sodium chloride to twice that of physiological salt solution. Oxygen has been bubbled through this medium kept at 38°C. In this solution which is isotonic with the tissue, water equilibrium is maintained during at least three hours. Under similar conditions with varied molar concentration of the immersing medium it has been found possible to test the effect of various agents upon the water exchange of the tissue slices. Permeability to water referable to changes in the plasma membrane of the parenchymatous cell has been conspicuously increased by heat at temperatures 48° to 56°C., by anoxia, by alcohol and some other dissolved substances, by bacteria and bacterial products including a highly purified somatic antigen. In view of current speculation concerning the effect of histamine upon the movement of water in the body and its relation to cell injury, inflammation and allergic phenomena, its action in vitro has been studied. The attempt is made to improve methods for the study of cell permeability in vitro. Autolytic changes in the immersed tissue must be prevented. The relation of electrolytes and of some other substances to water movement and their distribution in cellular and extracellular water of the tissue is under investigation.

Laboratory of Peyton Rous
Betty S. Roof

In the last report the importance was stressed of the cellular changes whereby “benign” tumors often become malignant and prove fatal. Our experiments had made plain that the occurrence of cancerous changes in the harmless tumors of mouse lung is
unaffected by prolonged exposure of these growths to the chemical agents inducing them. Nor is the worsening of mammary cancers of the mouse, through cell alterations, determined by such exposure. Obviously the secondary changes undergone by the growths mentioned must be due to some cause other than that responsible for their primary state and may differ therefrom fundamentally. In 1955-6 one of us (P.R.), together with Dr. Allen, undertook to transplant the benign tumors induced on the skin of mice by tar, as a step toward learning why these growths (papillomas) so frequently become cancerous. They are all of one sort, yet highly various, and hence their individual transfer to many hosts would be necessary to obtain a uniform test material.

Cutaneous papillomas were the first growths to be purposely induced by investigators of tumors, but in the 43 years since then all attempts to transplant them have failed. This is understandable. They are “conditional tumors,” as past work of this laboratory has shown, dependent upon abnormally favorable tissue conditions such as the agents inducing them provide, if they are merely to persist. Nevertheless our effort to transplant them has succeeded. The growths were induced by tarring mice of homogeneous breed and small pieces of them were implanted in sucklings and weanlings of the same stock, instead of the mongrel adults of previous workers. They have not only established themselves after implantation at deep sites, but by progressive enlargement have almost always killed their hosts while still retaining their “benign” character. Six have now been maintained for nearly 2 years in five to ten successive groups of mice. The numerous cancers originating from them have been left behind at every transfer by a rigorous selection of grafts.

The papillomas have been closely studied because of the large facts they disclose. Though endowed with the ability to proliferate endlessly at the expense of the host their cells retain more or less completely the two prime capabilities of normal epidermis—to elaborate a protective layer (scurf, where thick on the skin) and
to cover raw areas by lateral spread. As induced on the body surface they are protruding, wart-like growths topped with scurf, which never grow big and usually vanish after a while, whereas when grafted to deep situations they line the little pockets in which they are placed, and forming scurf on the inner side of the lining, enlarge because of its central accumulation, shoving outwards by pushing inwards, as one might say. They are introverts in a wholly material sense of this term, not extroverts as when on the skin surface. Becoming huge they kill their hosts through the demands of their active tissue.

The diametrically different behavior of the papillomas after transfer from superficial to deep situations, from spots where they fail to spots where they succeed, tells much concerning the unnatural history of tumors. No innate change takes place in the papilloma cells after transfer to deep sites; all that happens there is that the local conditions turn the growths in on themselves and hence their progressive enlargement. Their growth is indirectly promoted, not directly as in the case of those prostatic cancers of man consisting of cells that are urged on by the male sex hormone. The course of most tumors can be better understood in the light of the distinction now made; for most are susceptible to favoring extraneous influences. Beyond doubt they are often subjected to dual promotion, that of both sorts combined.

Whether promotion or some other cause is responsible for the intrinsic cell changes that take place when papillomas become cancerous has now to be determined.

PHYSICAL CHEMISTRY

Laboratory of Lewis G. Longsworth

The motion of charged particles in solution under the influence of an electric field, electrophoresis, and the settling of suspended matter in a gravitational or centrifugal field, sedimentation, are
examples of transport processes. Another such process, one that has not been extensively studied in liquids, is that of thermal diffusion. For example, if an aqueous solution of a salt is brought into contact with a hot metal plate above and a cool one below, heat flows through the liquid. Coupled with the flow of heat is a movement, termed thermal diffusion, of the salt, generally to the cold plate. This movement tends to concentrate the salt at that plate and proceeds until a balance is attained between the separating effect of the thermal diffusion and the remixing effect of ordinary diffusion, which is still another transport process resulting from the concentration difference. In this balanced or steady state the fractional change in concentration between the hot and cold plate, divided by the temperature difference, is the Soret coefficient, so named after a pioneer investigator in the field.

Although the concentration changes accompanying the heat flow are small they may be followed with the aid of the optical methods, based on the interference of light, that have been used in this laboratory for the study of electrophoresis and ordinary diffusion. Moreover, during the past year an optical method for the measurement of the temperature distribution in the liquid has been developed. This has led to an improvement in the design of the thermal diffusion cell such that the heat flow now appears to be essentially one-dimensional, free of turbulence and convection. This has increased the precision with which Soret coefficients may be determined and has permitted the study of salts at dilutions hitherto inaccessible to optical methods. Another advance has been the successful use of a cell in which the separation of the hot and cold plate is only 7 mm., a separation previously considered too small for precise measurements. The thin cell reduces the time required for the steady state to be established and thus permits the study of materials that diffuse less rapidly than most salts. Still thinner cells are contemplated, however, and will be essential for the investigation of the slowly diffusing proteins.

Interest in the thermal diffusion of salts centers on the relation
of this process to the electromotive force, emf, of the electrolytic thermal cell. This is a galvanic cell with identical electrodes except that one is held at a higher temperature than the other. At one time the emf of such a cell was thought to be a measure of the temperature coefficient of the electrode process but Onsager's principle of microscopic reversibility requires a small contribution to the cell emf from the effect of the temperature difference in the electrolyte between the electrodes. Thus the change in emf as thermal diffusion proceeds affords an independent measurement of the Soret coefficient. Recent results obtained by Agar and Breck in this manner at Cambridge are in agreement with the optical data for the only salt, cadmium sulfate, on which both types of measurement have been made.

Onsager's principle relates the Soret coefficient to the reversible entropy effect accompanying the motion of the particle through the liquid. In the case of water as solvent this effect is the order created by the orientation of the water dipoles in the region into which a particle moves and the relaxation of these dipoles after the particle has passed. The measurement of the thermal diffusion of both ions and non-electrolytes thus affords an interesting new insight into the submicroscopic structure of solutions. The role of thermal diffusion in biological systems will remain undetermined until thermal diffusion data on tissue components are available, virtually all work up to the present having been restricted to electrolytes.

Laboratory of Duncan A. MacInnes
Alfred R. Pray

It has been found necessary, in the development of physics and chemistry, to redetermine the fundamental constants of these sciences from time to time. This is due to the increasing demands for accuracy and also to the developments of new methods for obtain-
The constants. Thus the value of the charge, $e$, on an electron was first roughly found by J. J. Thompson, and more precisely by Millikin. These historic methods are, however, not accurate enough for present day purposes. Likewise, Perrin's original determination of the Avogadro constant, $N$, i.e., the number of molecules in a gram mol, has been superseded by determinations based on X-ray deflections and precision density measurements. The present method for obtaining the electronic charge, $e$, is from the relation

$$F = Ne$$

in which $F$ is the Faraday, the number of coulombs necessary to produce one gram equivalent of electrochemical reaction at a metal-electrolyte boundary. The interpretation of the results of many other physical measurements, such as the quantum limit of the X-ray spectrum, electron diffraction, photoelectric effects, etc., involves the electron charge, $e$, in ratios and products with the electron mass, $m$, the Planck constant, $h$, and in certain cases with the velocity of light, $c$. These considerations have led to a large volume of statistical computation, particularly by Birge, by DuMond and Cohen, and by Bearden and Thomsen, in order to arrive at the best values of the principal physical and chemical constants from the available numerical data. It is evident, therefore, that the Faraday, $F$, enters directly or implicitly into all these computations.

The Faraday is also involved in all computations including measurements of the potentials of galvanic cells, such as the determinations of activity coefficients of electrolytes, pH values, the free energies of electrochemical reactions, oxidation-reduction potentials, transference numbers, etc.

The value of the Faraday at present accepted depends upon researches on the silver coulometer, the oxidation of sodium oxalate, and on the iodine coulometer, but these methods do not
agree to the accuracy desired for present day purposes. The iodine coulometer has many advantages, the chief ones being that iodine occurs in only one stable isotopic form, and, as the electrochemical reaction is reversible, check values of the Faraday may be obtained by studying anode and cathode concentration changes. However, the work in this field, by Washburn and Bates, and by Bates and Vinal, is over forty years old. We are repeating the research using modern precision.

Experimentally, our research consists in the passage of a measured number of coulombs through the coulometer and determining the increase or decrease of the amount of iodine in the solution surrounding the electrodes.

The computations from the experimental results are based on the equation

\[ F = \frac{E}{R} \cdot \frac{t \cdot A}{m} \]

in which \( E \) is the potential of the standard cell, \( t \) the time of flow of the current, \( A \) the atomic weight of iodine, \( R \) the value of a standard resistance, and \( m \) the mass of the iodine liberated or absorbed. An apparatus maintaining current constant, at the value \( E/R \), to at least a part in a million has been developed. The time, \( t \), is determined to about this accuracy by means of radio broadcasts from station WWV of the Bureau of Standards. Fractions of seconds are interpolated from magnetic tape recordings. The analytical method for obtaining the masses, \( m \), of iodine is based on differential electrometric titrations. This determination has given us most of our experimental difficulties, due partly to the volatility of iodine, and to the necessity of obtaining that element, for calibration purposes, in a high state of purity. It is hoped, however, in the near future to present values of the Faraday, from data obtained from experiments in which the relevant variables have been adequately controlled.
Most of our knowledge of the mechanism of enzymatic action has been acquired through the study of such action in the homogeneous liquid phase. In biological processes, however, chemical reactions take place in a highly organized milieu. Electron microscopy has revealed for instance the richness of structure of the cytoplasm. As a result of the structure, numerous interphases are present at all levels of organization. The chemical reactions which take place at an interphase must be strongly influenced by the highly disymmetric field resulting from the interphase. Thus, there seems to be a fundamental gap between the conditions under which biochemical reactions take place in living entities and those under which the same reactions are commonly studied in the laboratory. For this reason, this laboratory has been engaged for a long time in the investigation of the mechanism of enzymatic action occurring at a liquid-solid interphase. Surface chemistry or two-dimensional chemistry is ordinarily thought of as a chemistry in which the reacting molecules are looked upon as balls running on a billiard table. Nothing could be further removed from the truth. The effective surface is not a geometrical surface, but is quite thick from a molecular point of view. The reactivities of the molecules located at the surface are influenced by molecules not taking a direct part in the reaction and located in some cases hundreds of angstroms from the surface.

Our most important finding has been the fact that protein multilayers deposited in an oriented fashion on a solid slide are acted upon by proteolytic enzymes even when the layers are protected by a thin plastic blanket. Three main possible explanations for this phenomenon have been advanced.

1. The enzyme molecules go through the blanket by an ordinary diffusion process under the influence of a gradient of concentration.
This explanation is untenable since the rate of the enzymatic reaction in the presence of a protective blanket runs an entirely different course from that which takes place when there is no protective blanket. The number of monolayers of fatty acid serving as anchorage and the length of the chain of the fatty acid molecules as well as the number of monolayers of protein play an important role when the enzymatic reaction is carried out in the presence of a blanket, a role which becomes insignificant in the absence of a blanket.

(2) The enzyme molecules are forced through the blanket on account of long-range specific or non-specific forces.

(3) The enzymatic action takes place across the blanket.

Until very recently the experimental evidence did not permit a decision between the last two hypotheses. New developments in the experimental technique now show the second assumption to be correct and that long-range forces of some specificity must be involved in the mechanism of action. These conclusions can be reached on the basis of the following experiments: It was found that if protein layers had been acted upon by trypsin, either directly or through an intervening blanket, some factor carrying tryptic activity could be recovered from the slide serving as support for the layers. This was accomplished by pressing down upon the slide coated with the fatty acid anchorage and whatever remained of the protein films partially altered by trypsin, a piece of scotch tape. Upon stripping the tape all the layers deposited on the slide were removed from it as a single unit, and transferred to the tape, with the exception of the first monolayer of fatty acid directly attached to the slide. If the strip was then deposited on another slide previously coated with multilayers of protein and with a thin film of water buffered at PH 7.5 separating the slide from the strip, it could subsequently be shown that some tryptic action had taken place on the multilayers of protein. However, if no tryptic action
had occurred on the first slide, no factor carrying trypsin activity could be recovered from the scotch tape.

In previous experiments it was shown that under certain conditions, trypsin action through a blanket had taken place if the protein layers had been deposited on five monolayers of fatty acid but did not take place when the anchorage consisted of three monolayers of the same acid. It can be said now that this difference in behavior does not result from a difference in the reactivity of the protein layers but from the fact that trypsin molecules have penetrated the blanket in one case and not in the other. A long-range mechanism is involved. So far, the experimental evidence favours the view that the material under the blanket must be of protein nature to permit the long range attraction to occur. In investigating the degree of specificity required for such an effect, experiments have been conducted with molecules of L-polylysine with a molecular weight of about 5000. It was demonstrated that L-polylysine molecules which can be hydrolized by trypsin, are capable of attracting trypsin molecules through a blanket. On the other hand no trypsin activity could be recovered from a strip if the substrate consisted of D-polylysine instead of L-polylysine. This would signify that the forces involved are stereospecific. However, it was discovered that D-polylysine molecules act as inhibitor for trypsin and consequently the failure to detect a factor carrying trypsin activity from a strip obtained in an experiment performed with D-polylysine does not permit the conclusion that trypsin molecules were not forced through in this case. If the trypsin molecules had been forced through their activity would have been inhibited by the D-polylysine. A series of experiments under way, with radioactive tritium-labeled trypsin should permit a decision between these two interpretations; that is whether trypsin molecules do not go through a blanket when there is a layer of D-polylysine under it or whether the trypsin molecules are attracted by the D-polylysine and become inactivated after they have gone through the blanket.
As in previous years, the interest of the laboratory was centered on muscle function and its regulation, with the ultimate aim of explaining the endocrine control of the uterus. During the past year, however, the experimental program led by Csapo was devoted largely to skeletal muscle.

The contraction cycle by which a muscle develops tension or performs work is a complex affair; the final event of contraction is preceded by at least three distinct processes: excitation, coupling of excitation to the contractile substance and activation. These events are themselves complex and can be understood only if isolated from one another and studied independently, under conditions which do not produce irreversible changes or artifacts.

Depolarization of the excitable membrane with excess potassium results in the loss of propagation but not of electrical “excitability” and contractility. Because upon repolarization the muscle recovers all the characteristic properties of intact muscle, it may be confidently asserted that the properties of the depolarized preparation are physiological. The loss of propagation (and, in the case of smooth muscle, loss of spontaneous activity) indicates that a partial and reversible loss of membrane function has occurred. This condition is in many respects intermediate between that of intact muscle and its “models” (e.g., glycerinated muscle, actomyosin threads), and it can be used to link information offered by the two main disciplines, biophysics and biochemistry.

The potassium-depolarized muscle, for example, does not contract if treated with various substances known to pharmacology as muscle stimulants. There was already reason to suppose that these substances do not stimulate the myoplasm directly, for
Csapo found in similar experiments that they do not cause contraction of the final contractile system: actomyosin + ATP + ions. The latter system might be thought to have lost substances necessary for pharmacological action. The similar lack of response, however, of the potassium-depolarized muscles, in which only membrane function is impaired, shows that these drugs act upon the intrinsic stimulatory system or on the “excess potential” of the excitable membrane, increasing the effectiveness of intrinsic stimulation or making it effective if latent.

Further evidence that potassium-depolarized muscle is a useful intermediate between living muscle and its models was given by the following observations: It is known that intact muscle is relatively insensitive to changes in pH and temperature. The muscle models, on the other hand, are very sensitive to such changes. Csapo found that potassium-depolarized frog muscle exhibits a very similar temperature and pH dependence to that of muscle models, indicating that in the intact muscle, the powerful regulation of the membrane obscures the intrinsic properties of the final contractile system.

Lundsgaard, in an experiment now classical, observed that muscle poisoned with IAA + N₂ develops only a limited number of contractions; when contraction ceases the ~ph compounds of the muscle are exhausted without formation of lactic acid. Csapo, in cooperation with Dr. J. Gergely of the Massachusetts General Hospital, found that in this condition phosphocreatin disappears but there is little change in the amount of ATP. Why should the muscle cease to contract when ATP is still available? Csapo found that although the muscle becomes inexcitable by conventional electrical stimulation of moderate intensity, it can be repeatedly tetanized in a strong electrical field, as can the non-propagating, potassium-depolarized muscle. When the muscle, so stimulated, finally ceases to contract its ATP stores are largely exhausted.

Kernan and Csapo, using the toe muscle of the frog, observed that the duration of tetanus may increase five-fold if the muscle is
soaked for ten minutes in calcium deficient Ringer’s solution. Recording action potentials from such a preparation during and after a half-second tetanus, it appears that action potentials persist several seconds after cessation of the stimulus, disappearing as relaxation becomes complete. Although such a muscle does not develop significant tension in spontaneous activity, it is nevertheless in a state of potential activity as shown by the repetitive firing of its membrane once the muscle is fully activated. This condition is comparable with the spontaneous activity of uterine muscle, which is known to be regulated by hormones.

The effect of the ovarian steroid on the calcium balance of uterine muscle in relation to the polarized state of the membrane is being investigated by Lowenhaupt and Csapo. Development of a method of calcium analysis sufficiently sensitive for a quantitative study of the correlation is under way.

Nagy and Csapo succeeded in isolating myosin from uterine muscle with only traces of contamination with actin. The fact that uterine myosin thus prepared combines readily with actin derived from skeletal muscle, indicates that these substances are chemically similar in muscles of quite different character. An attempt is being made to isolate myosin-free actin from the uterus for the reverse experiment. Further comparative studies along these lines are in progress using the ultra-centrifuge and electrophoresis.

Mashima and Csapo continued studies on excitation-contraction coupling. The question was: is it true, as generally believed, that internal currents have no role in the activation process, the essential event being depolarization of the excitable membrane? Instrumental in the success of these experiments was a new method in which the muscle is marked into segments with a non-toxic fluorescent dye* and the displacement of these marks as the muscle shortens is photographed with a constant-speed camera. Frog and turtle muscles so marked and then depolarized with excess potas-

*We are grateful to Mr. C. Berkley who developed and supplied us with the dye.
Physiology of Reproduction

sium, as in previous experiments, were stimulated electrically and the photographs were examined to see whether such a nonpropagating muscle shortens most where the further depolarizing effect of the current is greatest or where current density is known to be greatest. The records show a convincing correlation with current and not with depolarization. The evidence is the following: in muscles which are only slightly depolarized, a longitudinal d.c. field results in shortening both at the cathode and in the middle portion. It appears that at the cathode effective currents are generated by effective depolarization, but in the middle portion by the applied field. The fact that the anodal half of the muscle shortens (with the exception of the extreme anodal end) is sufficient evidence that depolarization is not directly linked to activation. Increasing the extent of depolarization by increasing the external potassium gradually depresses the cathodal effect. There is a critical potassium concentration at which only the middle portion of the muscle shortens. In this condition, the transverse d.c. field also becomes ineffective. This suggests that the loss of propagation does not coincide with the loss of ability of the membrane to set off effective currents. The effect of the transverse field seems to be mediated by effective depolarization, which results in effective internal currents. Under conditions in which the cathodal effect is still present, it can be reduced by increasing the applied electrical field slowly rather than abruptly. The effect of a.c. is that of two direct currents of the opposite sign; shortening occurs symmetrically from the middle toward the two ends. In a longitudinal field, shortening is greatest in the middle portion. The convexity of the curve plotted from those observations gradually straightens out as the field is rotated from the longitudinal towards the transverse direction. Our conclusion that internal currents are more directly linked to the activation process than is depolarization should be further developed in the attempt to explain in molecular terms the change from rest to activity.

In cooperation with Dr. L. Bengtsson of the Department of Ob-
stetrics, University of Lund, Sweden and Professor T. Suzuki of Fukushima Medical College, Japan, both former associates of this laboratory, Csapo is collecting evidence that the effect of progesterone in maintaining pregnancy in humans is the same as in the laboratory animals.

PLANT PATHOLOGY

Laboratory of Armin C. Braun
Karl Maramorosch, Ross B. Pringle, Thomas D. C. Grace, Virginia C. Littau, Ulrich Näf, Tom T. Stonier

A Physiological Basis for Autonomous Growth in Crown Gall.
(Dr. Braun) Oncologists have long asserted that the uncontrolled growth of tumor cells reflects an independence of those cells from the morphogenetic restraints that govern the growth of normal cells in higher animals and plants. What precisely constitutes the morphogenetic restraints placed upon a normal cell and what is entailed in overcoming those restraints under conditions of neoplasia are not well understood at present. Studies are therefore being continued in this laboratory in an attempt to gain insight into these questions.

Crown gall, a true transplantable tumor of plants, was used as the test object in these studies. It has been found that the alteration of normal to tumor cells in this disease takes place gradually. This process can, moreover, be accurately controlled experimentally. Thus, very rapid-growing potentially malignant tumors, moderately fast-growing tumors, and very slow-growing benign tumors can be induced in the same plant or plant species at will. Cells isolated from the three types of tumors described above and planted on a basic tissue culture medium maintain indefinitely their characteristic growth patterns. Since the three clones of tumor tissue, which showed varying degrees of neoplastic change, were obtained from the same plant species, they were admirably
suited for a characterization of the factors that are limiting for rapid autonomous growth. In these studies the rapid-growing fully transformed tumor cell was used as the standard. This cell type synthesized in optimal or near optimal amounts all of the growth factors needed for its continued rapid proliferation from the mineral salts and sucrose present in the basic culture medium. The moderately fast-growing tumor cell required that the basic medium be supplemented with the plant hormone auxin, the vitamin meso inositol, as well as L-glutamine to achieve a growth rate comparable to that of the fully transformed tumor cell. The nutritional requirements necessary to raise the very slow-growing benign tumor to that of the fully altered tumor cell were more exacting. In addition to the three compounds described above, two amino acids, proline and histidine, are required in this instance. These studies clearly indicate that, as the crown-gall tumor cell becomes more autonomous, its growth requirements in terms of externally supplied growth factors become less exacting. It thus appears that the transition from the slow-growing benign to the rapid-growing potentially malignant tumor cell is a gradual one involving the progressive but permanent activation of a series of growth-substance-synthesizing systems.

Normal cells of the type from which the three clones of tumor cells described above were derived do not grow on the basic culture medium. Although, as indicated, the difference between the tumor cells showing varying grades of neoplastic change is of a quantitative nature since all can grow continuously but at different rates on the basic medium, the difference between the three types of tumor cells and the normal cell is qualitative. While such a growth factor as 6-furfurylamino-purine is not essential as a supplement for the rapid growth of any of the three clones of tumor tissue studied, this factor is essential for the continued growth in culture of the normal cells. The absolute requirement of 6-furfurylamino-purine for the continued growth of normal cells represents, then, a basic difference between a normal cell and a tumor cell. The nor-
mal cell, unlike the tumor cells, also possesses an absolute requirement for an auxin for its continued growth in culture. There is, in addition, a third not as yet completely characterized requirement for the growth of normal cells which appears to be largely satisfied by a combination of guanylic and cytidylic acids. Although the minimal requirements for the continued rapid growth of normal cells has not as yet been completely defined, these studies have progressed sufficiently to indicate that it is possible for a cell to acquire the capacity for autonomous growth as a result of the permanent activation of several growth-substance-synthesizing systems, the products of which are concerned specifically with growth accompanied by cell division. These systems are precisely regulated in all normal plant cells.

**Studies on the Nature of the Tumor-Inducing Principle in Crown Gall.** (Dr. Stonier) One of the important unsolved problems in the crown-gall disease is concerned with the nature of the tumor-inducing principle that is responsible for the alteration of normal plant cells to tumor cells. The characterization of this principle might help to explain how its presence within the cell accomplishes the permanent unblocking of several growth-substance-synthesizing systems, the products of which are known to be concerned with the continued abnormal proliferation of the crown-gall tumor cell. The tumor-inducing principle is presumably elaborated by the inciting bacterium of the crown-gall disease and a search for this agent is presently being carried out. It had been found previously that crown-gall bacteria labeled with radioactive phosphorus (P\textsuperscript{32}) when grown in nonradioactive media release large amounts of P\textsuperscript{32}. The chemical nature of the P\textsuperscript{32} involves a large spectrum of compounds. A search is underway to isolate and characterize agents showing biological activity. Among the agents presently under investigation is a highly unstable substance released by one strain of the bacteria which specifically affects certain other strains of the same organism. This substance appears to
be neither a bacteriophage nor a bacteriocin. The search for other agents possessing biological activity continues.

**Genetic Studies on Plant Tumors.** (Dr. Naf) Certain interspecific hybrids within the genus *Nicotiana* are perfectly organized both morphologically and histologically during the period of their active growth and in the absence of irritation. When such plants reach maturity and terminal growth ceases, a profusion of tumors invariably appear on all parts of the plant. The genetic constitution of the cell appears to be critical in this instance. Only such a nonspecific stimulus as irritation is required to transform the potential tumor cells, of which these hybrid plants are composed, into actively proliferating autonomous plant cell types.

It has been found in this laboratory that the parents of the tumorous hybrids can be divided into two groups which have arbitrarily been designated as “plus” and “minus.” The former comprises the Alatae and perhaps the Noctiflorae sections of the genus, the latter all other species involved in tumor formation. Information is now available on 47 such hybrids. If an intragroup cross is made either between two “plus” species or two “minus” species, the resulting hybrid will not develop tumors. On the other hand, crosses between a “plus” species and a “minus” species produce, with very few exceptions, tumor-bearing offspring. These findings suggest that the development of tumors in certain hybrid crosses is a function of two factors or factor complexes, one being dependent on the genome of the “minus” species and the other on the genome of the “plus” species. While primarily genic in nature, the critical contributions of the parents should be reflected in parental metabolism. Experiments are now underway to identify these different parental contributions at a physiological level.

**A Specific Inducer of the Male Sex Organ in Certain Vascular Plants.** A naturally occurring substance has been found which induces parenchymatous cells of the gametophytic generation of certain vascular plants to differentiate and organize the male
sex organ or antheridium. The controlled induction of the antheridium is a unique example of a simple chemical substance directing morphogenesis in a highly specific way. Physiological and chemical studies on the nature and mode of action of this substance are continuing.

Physiological Aspects. (Dr. Näf) The activity spectrum of the biologically active substance has been determined in the Filicinae. An attempt was next made to learn the relationship of the antheridium-inducing factors isolated from several distinct fern species to the morphological structure of the antheridium that those factors induce. The morphological structure of the antheridium in different species of ferns may be different. The antheridium found in *Woodsia obtusa*, for example, differs in structure from that found in *Onoclea sensibilis* in that the cap cell of the former is divided. The antheridium of *Blechnum gibbum* differs from that of *Onoclea* and *Woodsia* in the shape of its basal cell. Antheridia induced in gametophytes of *Woodsia obtusa* in response to the antheridial factor elaborated by either *Pteridium aquilinum* or *Blechnum gibbum* show a structure characteristic of the *Woodsia* antheridium with the divided cap cell. Similar results were obtained with other combinations. Thus, the specific structure of the antheridium that is initiated is a function of the protoplasmic substrate on which this factor acts rather than of the antheridium-inducing factor itself.

It has been found, further, that the cells of gametophytes of *Onoclea* and *Pteridium* become resistant to induction quite early in their development. The cells of gametophytes of these two species can be induced to form antheridia only during the first 14 days after the germination of the spore. The loss of sensitivity first becomes apparent in cells behind the meristem which lies in the notch of the heart-shaped prothallus. This observation suggests that the loss of sensitivity and perhaps its maintenance is a function of the meristem. Excision of the meristem actually leads
to a restoration of sensitivity of certain cells to the antheridium-
inducing factor.

**Chemical Aspects.** (Dr. Pringle) As is often the case with natu­
 rally occurring compounds possessing great biological activity, the
antheridium-inducing factor has been found to be present in ex­
tremely low concentration. One liter of culture filtrate yields about
one milligram of a concentrate which will initiate antheridia at
$4 \times 10^{-4}$ micrograms per milliliter. Thus, to obtain one gram of
concentrate for chemical study, 1,000 liters of filtrate from 30,000
flasks must be processed. Since cultures require 6 weeks to produce
maximum activity, the accumulation of material is necessarily a
slow process. A large volume of active culture filtrate has now
been processed and studied. The highly active concentrate so ob­
tained has been subjected to various separation procedures includ­
ing solvent fractionation at various pH's, chromatography on
alumina and paper, and fractional precipitation. It has been found
that the concentrate consists of a mixture of at least six compounds
with very similar chemical and physical properties, only one mem­
ber of which has antheridium-inducing activity. The physical and
chemical properties of the purified antheridium-inducing sub­
stance are being studied but complete characterization must await
the production of more material.

**Studies on the Helminthosporium victoriae Toxin.** (Dr. Pringle)
During 1956-57, studies on the biochemistry of infectious diseases
of plants have continued. The *Helminthosporium* blight of oats
continues to serve as the experimental model for this work. This
disease is characterized by the most potent and most specific toxin
known. *Helminthosporium victoriae*, the causal agent of this
disease, remains localized in the host but produces a soluble toxin
that diffuses throughout an affected plant and is responsible for
the destructiveness of the disease. This toxin is of particular in­
terest because of its highly selective action. Resistant oat varieties
tolerate, without injury, concentrations of toxin one thousand
times greater than those necessary to kill susceptible varieties.

During the past year a large-scale procedure was developed which makes possible the isolation of this extremely unstable biologically active material from 100-liter quantities of culture filtrate. Since the material is only stable in acid solution, the processing must be done without raising the pH or drying the material. The toxin was isolated by growing the fungus in shallow layers in modified Fries' No. 3 basal medium at 25° C for 28 days. After removal of the fungal mycelium, the filtrate was acidified and concentrated in vacuo to one-tenth its original volume. Inert material was removed from the concentrate by precipitation with methanol.

After further evaporation in vacuo to remove methanol, the toxin was extracted into butanol and purified by chromatography on columns of alumina and starch. The purity of the toxin was checked by further chromatography on a variety of adsorbents, by paper chromatography and by ionophoresis. The isolated toxin inhibits the growth of susceptible oat roots at a concentration of $2 \times 10^{-4}$ micrograms per milliliter.

The toxin appears to be polypeptide in nature. Although fresh biologically active preparations did not react with ninhydrin, this reaction was noticed as the preparations lost activity. When the toxin was hydrolyzed by strong acid or alkali, the ninhydrin reaction became progressively stronger. Completely hydrolyzed samples of the pure toxin show at least seven ninhydrin-reacting substances.

The inactivation of the toxin by sodium bicarbonate solution is being studied. The degradation products produced by this inactivation are being separated and characterized chemically.

**Acquired Immunity to a Strain of Corn-Stunt Virus in Insect Vectors.** (Dr. Maramorosch) It was found that corn-stunt virus occurs in two different forms, designated Rio Grande and Mesa Central. Studies on interactions between these viruses showed that they represent closely related strains. Cross protection studies between these two strains were carried out in corn-leafhopper transmitters. Individual insects acquired Rio Grande virus by feed-
ing 14 days on diseased plants; later the insects were fed for 14 days on plants infected with the Mesa Central strain. All of these inoculated insects invariably transmitted only Rio Grande virus, thus showing protection by this strain against infection with Mesa Central. However, when the order of virus acquisition was reversed, no protection against Rio Grande took place. Leafhoppers fed first on plants with Mesa Central for 14 days and afterwards on Rio Grande for 14 days transmitted first Mesa Central virus, later both strains, and finally only Rio Grande. This experiment demonstrated that protection of related strains of virus in arthropod transmitters may vary in degree and may not be reciprocal. It seems that the Rio Grande strain either multiplied faster or invaded insect tissues more rapidly than did the Mesa Central virus.

**Studies of Vector Specificity.** (Dr. Maramorosch) Two closely related species of aster leafhoppers, *Macrosteles fascifrons* and *M. laevis*, known to transmit strains of viruses causing the aster-yellows disease, were tested as transmitters of the eastern strain of aster-yellows virus. Only *M. fascifrons* acted as a transmitter. Although the two species of leafhoppers could not be separated on the basis of morphological characteristics, attempts to cross them resulted in no progeny. Studies on the possible causes of this high degree of specificity are being continued.

**Reversal of Virus-Caused Stunting by Gibberellic Acid.** (Dr. Maramorosch) Reports on the use of gibberellic acid in overcoming genetic and physiologic dwarfism in plants suggested testing whether this substance could induce growth of plants stunted by certain virus diseases. It was found that gibberellic acid influenced significantly the growth of plants stunted by three different virus diseases. Although stunting could be overcome to a considerable degree by three applications of a water solution of 100 ppm of gibberellic acid, diseased plants retained other signs of virus infection. Insect transmitters were able to recover virus from treated plants as readily as from untreated controls.

**Cytopathogenic Effects of the Aster-Yellows Virus on its Insect**
Vector. (Dr. Littau) The earlier report that the fat-body cells of the vector of aster-yellows virus undergo changes after the vector has acquired virus by feeding on diseased plants has been confirmed. The nuclei become stellate; basophilic cytoplasmic material becomes stringy and sparse and apparently is easily torn. Cell boundaries are not clearly seen (Carnoy fixation, azure B staining). In contrast, nuclei of the fat body of nonviruliferous insects tend to be round or to have smooth contours; the cytoplasm is generally homogeneous, with a large number of vacuoles of varying size enclosed within a definite cell membrane. Virus-induced changes are seen best in males. Cytopathogenic changes occur after the insects have fed on diseased plants for between 18 and 28 days, that is, long after the completion of the incubation period of the virus in the insect. In addition to insects inoculated by feeding, others that had been injected with the juice of viruliferous insects and allowed to feed on immune rye plants and healthy asters for 28 days also developed typical changes in the fat body.

Tissue Culture and Organ Culture of Insects. (Dr. Grace) Attempts over the past 40 years to obtain the continuous growth and survival of insect tissues in vitro have met with little success. It has not been possible until recently to maintain the tissues in culture for longer than 4 weeks. During the year the organs and tissues from three species of moths and the aster leafhopper have been cultured in a partially synthetic medium containing insect plasma. The ovarian tissue from the promethea moth, *Callosamia promethea*, has survived and grown for 26 weeks. The other tissues have been maintained alive for periods ranging from 4 weeks to 14 weeks. Subculturing of actively multiplying cells and growing tissues has also been achieved.

An attempt is being made to inject cultivated insect cells with viruses of plants and animals, and to develop a quantitative method of measuring insect and plant-virus concentration by a plaque technique.
Between July 1, 1956 and June 30, 1957 investigations were continued or initiated as follows:

1. Tomatoes recently developed to breed true for resistance to infection by tobacco-mosaic virus produced normal segregation ratios this year after rehybridization with susceptible stocks. In second generations of the new hybrid progenies, ratios were essentially three resistant plants to one susceptible, instead of the deficient two to one ratios formerly encountered exclusively. This is taken to mean that the chromosomes of resistant plants now pair in a regular manner with normal tomato chromosomes. First-generation hybrids between the resistant line and an ordinary susceptible variety have shown resistance and hybrid vigor. Seeds of the resistant line have been deposited for permanent storage at the U. S. Department of Agriculture's Regional Plant Introduction Station at Ames, Iowa, under the Plant Introduction number P. I. 235673. Investigators in all parts of the world may obtain samples of seeds representing the resistant tomatoes by requesting them under that number.

2. In cooperation with the staff of the Citrus Experiment Station of the University of Florida, an effort was made to transfer one or more of the viruses that produce disease in Citrus trees to potential test plants in families other than the Rutaceae. Like all earlier efforts to find useful test plants in this way, the current attempt failed. A direct attack on this and related problems is difficult because nothing is known, on the one hand, about the suitability of selected test plants or, on the other, about techniques that would be necessary for successful inoculation if the test plants were potentially adequate. An attempt is now being made to introduce viruses from other plants into some Citrus species, in the hope that the use of recognized test plants for these viruses may permit a study of the techniques of inoculation needed for transmission
of viruses from rutaceous plants. The problem is of fundamental importance because our knowledge of viruses in trees in general still suffers from a dearth of methods that would permit study of tree viruses in fast-growing test plants such as have facilitated research on the viruses of many herbaceous species.

(3) A survey of the past decade’s work on spotted-wilt disease led to an examination of the existing data on single-gene resistances in phytopathological literature. A generalization emerged to the effect that no single-gene resistance has ever been proved applicable to more than one phytopathogenic virus. Hence an effective gene is capable of identifying a virus or a viral strain. The applicability of this general rule seems not to have been recognized previously. Exceptions to the rule may come to light eventually but none has appeared to date. This rule does not imply that all strains of a virus will be resisted by a single gene, although they may be. Nor does it imply that a resistant-type plant may not resist two or more viruses; independent genes for resistance may be present within the same individual or even within a single chromosome. It merely means that single-gene resistances, as such, can be used to identify diseases caused by single viruses or strains of viruses. When no specific antisera are available, use of this technique will tend to obviate the necessity for importing or exporting virus strains for comparative study and identification.

Laboratory of L. O. Kunkel

Work on Slow Development of Resistance to Yellows-Type Viruses in Vinca rosea. During the past year aster yellows, the California strain of aster yellows, and Texas carrot yellows have been maintained and transmitted by grafting in Vinca rosea. The purpose has been to determine whether or not development of resistance in Vinca is affected by frequency of transmission through grafting. Some lines of the viruses were graft-transmitted several times dur-
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ing the year, while others were graft-transmitted only once. Up to the present time frequency of transmission has not affected rate of development of resistance in *Vinca*. However, only a small degree of resistance is developed against these viruses in the course of one year. The experiments are being continued.

**Study on honeysuckle yellows.** A vein-clearing disease of *Lonicera japonica* (Japanese honeysuckle) has been known in this country and England for many years. Since the disease could not be transmitted manually by means of juices from affected plants, it was for some time thought to be a variegation. However, transmission by grafting proved that it was caused by a virus. The disease was not known in other families of plants and symptoms in honeysuckle did not disclose whether it belonged in the mosaic or yellows-type group of plant virus maladies. In hope of learning more about the nature of the disease and of classifying it more precisely than had previously been possible, an effort was made to transmit it to plants outside the honeysuckle family. The dodder parasite *Cuscuta campestris* was used.

During the past year the virus was transmitted to tomato, *Nicotiana glutinosa*, and *Datura stramonium* in the Solanaceae, to carrot in the Umbelliferae, and to *Vinca rosea* in the Apocynaceae. Symptoms in these plants showed clearly that it belonged in the yellows-type group. In *Vinca rosea* and carrot it could not be distinguished symptomatically from aster yellows. In tomato and *Nicotiana glutinosa* it caused rosette-type symptoms, quite different from those characteristic of aster yellows. Since, however, it was thought that it might be due to a variant strain of aster-yellows virus, efforts were made to take it from *Vinca* and carrot to aster plants by means of the aster leafhopper *Macrosteles fascifrons*. Up to the present time these efforts have not been successful.

Experiments now in progress will show whether or not the aster-yellows virus can be transmitted to Japanese honeysuckle and, if so, whether symptoms in this plant are like those caused by the
honesuckle virus. Also, experiments are underway to determine whether the honeysuckle disease can be cured in honeysuckle or Vinca plants by means of heat treatment and, if it can be so cured, whether it can be cured by treatments comparable to those that cure aster yellows in Vinca. Present evidence suggests that honeysuckle yellows is different from any yellows disease previously described.

Field evidence of natural spread of honeysuckle yellows has been sought. In a ten-acre field fairly well overgrown by healthy honeysuckle plants, a single plant with the disease in one of many tips was found. This plant brings the only evidence of spread in the field that has been found up to the present time.

Since all yellows diseases that have been studied carefully have been found to be spread by leafhoppers, this one also may have a leafhopper vector. Two species of unidentified leafhoppers found feeding on honeysuckle have been tested for ability to transmit. Neither has proved capable in this respect. The search for a leafhopper vector is being continued.
PROFESSIONAL ACTIVITIES
OF THE FACULTY

ACADEMIC HONORS

Detlev W. Bronk
——. LL.D., Reed College.
——. Sc.D., Brandeis University.
Warren O. Nelson. M.D. (Hon.), University of Giessen.

ACADEMIC APPOINTMENTS

Armin C. Braun. Visiting Professor and Lecturer, University of Nebraska.
——. Visiting Professor and Lecturer, Cornell University.
Karl Maramorosch. Visiting Professor, Department of Plant Pathology, Cornell University.
Paul A. Weiss. Visiting Professor, Massachusetts Institute of Technology.

SOCIETY ELECTIONS

Detlev W. Bronk. Council, Society of the Sigma Xi.
——. Council, American Association for the Advancement of Science.
Vernon Brooks. Member, American Physiological Society.
——. Treasurer, New York State Society for Medical Research.
Vincent P. Dole. Member, Association of American Physicians.
H. K. Hartline. Fellow, American Academy of Arts and Sciences.
KARL MARAMOROSCH. Secretary, Division of Mycology, New York Academy of Sciences.
FLOYD RATLIFF. Member, Society of Experimental Psychologists.
ROY E. RITTS, Jr. Member, American Rheumatism Association.
——. Member American Federation for Clinical Research.
——. Member, Society of Nuclear Medicine.
HOWARD A. SCHNEIDER. Associate Editor, American Journal of Nutrition.
RICHARD E. SHOPE. Member, American Epidemiological Society.
IGOR TAMM. Associate Editor, Virology.
EDWARD L. TATUM. Member, Editorial Board, Science.
——. Member, American Philosophical Society.
——. Member, Harvey Society.
WILLIAM TRAGER. Editor, Journal of Protozoology.
PAUL A. WEISS. Fellow, American Academy of Neurology.
VICTOR J. WILSON. Member, American Physiological Society.

PRINCIPAL ADDRESSES

ARMIN C. BRAUN. Eleventh Annual Symposium, Society for Experimental Biology, Aberystwyth, Wales.
——. Tissue Culture Association, Woodstock, Vermont.
——. New York Academy of Medicine.
DETVW W. BRONK. Address on Manpower and Education, Air Force Association Annual Conference.
——. Address at Midwest Conference on Graduate Study and Research.
——. Presidential Inaugural Address, Reed College.
——. American Institute of Chemists.
——. Phi Beta Kappa Address, University of South Carolina.
——. Centennial Convocation, American Institute of Architects.
——. Electrochemical Society.
——. Commencement Address, University of Texas.
Faculty Activities

---. Commencement Address, Pennsylvania State University.
---. Dedication of new Laboratory, New York Botanical Garden.
---. Dedication of Medical Sciences Building, University of Florida.
---. Ground Breaking Ceremonies, Bronx High School of Science.
---. 63rd Annual Convention of the Association of Military Surgeons.
---. Phi Beta Kappa Address, University of Virginia.
---. Dedication, Hospital for Joint Diseases.
---. Dedication of International Business Machines, “World of Numbers” Exhibit, Chicago.


GEORGE W. CORNER. Honors Convocation, Hunter College.
---. Dedication of Bardeen Medical Laboratories, University of Wisconsin.


RENE DUBOS. Gehrmann Lecturer, College of Medicine, University of Illinois.

HERBERT S. GASSER. Hughlings Jackson Memorial Lecture, Montreal Neurological Institute.


H. K. HARTLINE. Symposium, International Congress of Photobiology, Turin, Italy.

FRANCIS O. HOLMES. Invited Lectures, School of Hygiene and Public Health, The Johns Hopkins University.
---. Citrus Experiment Station, Lake Alfred, Florida.
Frank L. Horsfall, Jr. Cornell University Medical College.

----- Conference on Immunology and Cancer, New York Academy of Sciences.

----- Diamond Jubilee Convocation, New York University-Bellevue Medical Center.


----- Conference on Program of Medical Research, Department of Health, New York City.

Rollin D. Hotchkiss. Conference on Recombination, Association of Microbial Geneticists, Glasgow.


----- Danish Association for the Natural Sciences, Copenhagen.


----- Symposium on Hemoglobins, American Society of Biological Chemists.

----- Panel on Liver Disease, National Closed-circuit Television.


David P. C. Lloyd. Twentieth International Physiological Congress, Brussels.

----- Dedication. Instituto Venezolano de Neurologia e Investigaciones Cerebrales, Caracas, Venezuela.

----- University of Pennsylvania, School of Medicine.

D. A. MacInnes. Lecture before the Electrochemical Society.

Karl Maramorosch. Tenth International Congress of Entomology, Montreal.

----- Annual Address, Biology Club, Connecticut College.

----- McCollum-Pratt Institute and Biology Department, The Johns Hopkins University.
Faculty Activities

—. Annual Meeting, National Academy of Sciences.

STANFORD Moore. Harvey Society Lecture.

ULRICH NAFL. Torrey Botanical Club, Columbia University.

GERTRUDE E. PERLMANN. Enzyme Institute, University of Wisconsin.

ALFRED R. PRAY. Lecture before the Electrochemical Society.

PEYTON ROUS. Address on Presentation of the Kober Medal to Richard E. Shope, Association of American Physicians.

MARCIA A. Rudzinska. Round Table Discussion on Nutrition and Aging.
—. First Pan-American Gerontological Congress, Mexico City.

HOWARD A. SCHNEIDER. Annual Convention, Medical Library Association.
—. American Advisory Council of the National Multiple Sclerosis Society.

IRVING L. SCHWARTZ. Cornell University College of Medicine.
—. American Academy of General Practice.

—. Society of the Sigma Xi, State College of Washington.
—. School of Medicine, University of Washington.

IRA SINGER. Rutgers University.

WILLIAM H. STEIN. Harvey Society Lecture.

IGOR TAMM. New York University College of Medicine.
—. Conference on Chemoprophylaxis and Chemotherapy of Virus Infections.
—. The National Foundation for Infantile Paralysis.

VICTOR J. WILSON. University of Utah School of Medicine.

—. WESCON, San Francisco.
R. M. ARCHIBALD. Member, American Board of Clinical Chemists.
———. Consultant in Clinical Chemistry, American Board of Pathology.
———. Member, Advisory Board of Analytical Chemistry.

A. G. BEARN. Board of Editors, *Genetica Medica*.

DETLEV W. BRONK. Member, Editorial Planning Board, McGraw-Hill Encyclopaedia of Science and Technology.
———. Member, Trinity College, Cambridge.
———. Member, Visiting Committee, National Bureau of Standards.
———. Vice Chairman of Executive Committee, National Advisory Committee for Aeronautics.
———. Member, President’s Committee on Civilian National Honors.
———. Member, Committee on the Magellanic Prize, American Philosophical Society.
———. Member and Trustee, General Education Board.
———. Member, Trustees’ Educational Policies Committee and Medical and Hospital Affairs Committee, University of Pennsylvania.
———. Member, Advisory Board, *Perspectives in Biology and Medicine*.

MERRILL W. CHASE. Chairman of Advisory Consultants, 8th International Symposium on Mechanisms of Hypersensitivity, Henry Ford Hospital.
———. Vice Chairman, Program Committee, American Society of Bacteriologists.

LYMAN C. CRAIG. Chairman, Gordon Research Conference on Separation and Purification.

SAM GRANICK. Member, Visiting Advisory Committee to the Trustees of Brookhaven National Laboratory on the Department of Biology.
Faculty Activities

FRANCIS O. HOLMES. Consultant on Virus Diseases and Related Problems, University of Florida.

FRANK L. HORSFALL, JR. Member, Defense Science Board, Department of Defense.

—. Member, Scientific Advisory Committee, Institute of Microbiology, Rutgers University.

—. Member, National Advisory Committee, Oklahoma Medical Research Foundation.

—. Member, Committee on Virus Research and Epidemiology, The National Foundation for Infantile Paralysis.

—. Chairman, Research Council, The Public Health Research Institute of New York.

—. Member, Board of Directors, The Public Health Research Institute of New York.

—. Chairman, Visiting Committee for Medical Department, Brookhaven National Laboratory.

—. Member, Executive Committee, Committee on Public Health, New York Academy of Medicine.

ROLLIN D. HOTCHKISS. Biology Development Committee, Adelphi College.

—. Member, Board of Directors, Long Island Biological Association.

—. Member, Advisory Committee for Personnel, American Cancer Society.

MOSES KUNITZ. Carl Neuberg Medal for 1957, American Society of European Chemists and Pharmacists.

L. O. KUNKEL. Member, Board of Managers, The New York Botanical Garden.


KARL MARAMOROSCH. Lalor Foundation Senior Summer Research Fellowship.

PEYTON ROUS. Award for Distinguished Service, American Cancer Society.
RICHARD E. SHOPE. George M. Kober Medal, Association of American Physicians.
— Member, Board of Scientific Consultants, Sloan-Kettering Institute.
— Member, Board of Scientific Advisers, Merck Institute.

NORMAN R. STOLL. Member, Scientific Advisory Committee of the American Foundation for Tropical Medicine, Inc., and the Liberian Institute.

EDWARD L. TATUM. Member, Advisory Committee to the U. S. Department of State on the Living Cell Class, Brussels World’s Fair.

WILLIAM TRAGER. Member, Scientific Advisory Committee of the American Foundation for Tropical Medicine, Inc., and the Liberian Institute.
— Consultant, University of Florida.
— Chairman, Symposium on Insect Nutrition, Tenth International Congress of Entomology, Montreal.

DONALD D. VANSLYKE. First Van Slyke Award of American Association of Clinical Chemists.

PAUL A. WEISS. Chairman, Scientific Advisory Board to the United States Commissioner General for the Brussels World’s Fair and Chairman, Advisory Committee to the U. S. Department of State on the Living Cell Class, Brussels World’s Fair.

DOUGLAS M. WHITAKER. Consultant to the United Fruit Company on research organization and activities in Central America.
— Member, Advisory Committee to the U. S. Department of State on the Living Cell Class, Brussels World’s Fair.
— Chairman, Advisory Committee on Institutional Research Grants, American Cancer Society.


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III. Manometric arginase determination of arginine. 1044.

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