Review of

INTRAMURAL RESEARCH

NATIONAL INSTITUTES OF HEALTH

1959

U.S. DEPARTMENT OF

HEALTH, EDUCATION, AND WELFARE

Public Health Service

ISSUED IN A LIMITED EDITION FOR ADMINISTRATIVE USE

Review of

INTRAMURAL RESEARCH

NATIONAL INSTITUTES OF HEALTH

1959

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

PUBLIC HEALTH SERVICE

NATIONAL INSTITUTES OF HEALTH

INSTITUTE RESEARCH DIRECTORS

National Cancer Institute . . . . . G. Burroughs Mider, M.D.,
Scientific Director.

Charles G. Zubrod, M.D.,
Clinical Director.

National Heart Institute . . . . . Robert W. Berliner, M.D.,
Scientific Director.

National Institute of Arthritis
and Metabolic Diseases . . . . . DeWitt Stetten, M.D.,
Scientific Director.

Joseph J. Bunim, M.D.,
Clinical Director.

National Institute of Allergy
and Infectious Diseases . . . . . Dorland J. Davis, M.D.,
Scientific Director.

Vernon Knight, M.D.,
Clinical Director.

National Institute of Mental Health . . . Robert B. Livingston, M.D.,
Scientific Director.

Robert A. Cohen, M.D.,
Clinical Director.

National Institute of Neurological
Diseases and Blindness . . . . . Robert B. Livingston, M.D.,
Scientific Director.

G. Milton Shy, M.D.,
Clinical Director.

National Institute of Dental
Research . . . . . Seymour Kreshover, M.D., D.D.S.,
Scientific Director.

Division of Biologics Standards . . . . . Roderick Murray, M.D.,
Scientific Director.

Joseph E. Smadel, M.D.,
Associate Director for Intramural Research.

Eli M. Nadel, M.D.,
Executive Secretary, Scientific and Clinical Directors Group.

FOREWORD

The National Institutes of Health constitutes the principal research arm of the Public Health Service and is charged with the conduct and support of research, research training, and related activities. The research conducted by the National Institutes of Health is the subject of this publication. It is not, therefore, a comprehensive presentation of the program of the Public Health Service or of the National Institutes of Health. Rather, it is a series of reviews designed to provide acquaintance with what is familiarly known as NIH intramural research.

Glimpses of the extent to which the intramural research effort has contributed to the advance of medical research are provided in the eight Annual Reports comprising this compendium. These reports, which received only minor editing, provide the reader with the flavor and philosophy of the separate Institute research programs as presented by their scientific leaders. Yet, in such a presentation, one may miss the thread of mission that ties each of the operating research organizations into the program of the National Institutes of Health. It is appropriate, therefore, to pursue this aspect briefly here.

The mission of the National Institutes of Health as a whole and of its components is to develop the facilities, resources, and attitudes which would be most effective in acquiring new knowledge concerning disease processes, to the end of relieving suffering, bringing about cure and rehabilitation, and assuring the prevention, whenever possible, of disease. Broadly considered, this mission involves providing the wherewithal and cultivating suitable soil for a systematic study of man and his milieu with the ultimate objective of contributing to improved health. From this overall point of view, the NIH does not differentiate between what is done intramurally and extramurally. However, within these conceptions are contained more specific objectives only some of which can be sought for within an intramural research program, while the others may be searched out most expeditiously by support of work in other institutions through the extramural program.

This first Annual Review of NIH Intramural Research provides evidence of the magnitude of the effort—both in breadth and depth—and of the type of achievements that have placed this installation in the forefront of research in the medical sciences.

James Q. Shannon

JAMES A. SHANNON,
Director.

PREFACE

It has been customary in recent years for the intramural research activities of the National Institutes of Health to be summarized annually for the purpose of administrative review. The reports originated with senior investigators, were consolidated and commented on by section chiefs and laboratory chiefs, and assessed by the Clinical Directors and Scientific Directors before transmission to the Office of the Director, NIH. Since these annual program reviews were designed essentially to meet administrative needs, they were not reproduced in quantity and were distributed only among those scientists and scientist-administrators directly concerned.

The present volume represents an elaboration of the former practice in one respect. Although all the preliminary steps remain the same, and although the annual program reviews continue to serve their established purposes, it is now proposed to make available on a limited basis that material which represents the scientific contributions of the intramural staff of each Institute. The present undertaking stems from the consensus that a number of scientific and institutional needs would be met if there were an annual publication summarizing the work that has been done within NIH's own laboratory and clinical facilities.

Nineteen fifty-nine is the first experimental year for such a publication. Between these covers there have been brought together the 1959 annual reports of intramural research of the seven Institutes and the Division of Biologics Standards: Four sections summarize the total intramural programs of individual Institutes, one presents the combined basic laboratory research activity of the Mental Health and the Neurology Institutes, three separate reports describe the clinical work in programs of the Mental Health, Neurology, and Arthritis Institutes, and one represents the research activities that complement the regulatory activities of the Division of Biologics Standards.

It should be pointed out, without apology, that the volume is a compendium of individual reports that have been assembled and included without appreciable editorial change. Just as the administrative organizations of the Institutes vary, so these reports range from the simple summation of a group of intramural research projects to a broad philosophical treatise on the role of science in relation to the society it serves. The reports also vary in length and in the degree to which the Scientific and Clinical Directors wrote their own summations and interpretations, as contrasted with their use of materials submitted to them by the Laboratory and Branch Chiefs. It is anticipated that these annual program reviews in future years will be more uniform both in length and in general approach.

In their present form, however, the pertinent portions of the 1959 program reviews are deemed suitable for limited internal use among the senior administrative and scientific staff members of the Institutes and their Boards of Scientific Counselors, and our scientific colleagues in close association with NIH intramural research.

The reports focus attention on intramural research bearing directly on the missions of the several Institutes. Thus, they do not and cannot take into account the high quality and wide range of services that make it possible for the intramural research programs to be successfully carried forward.

One such group of services, for example, is found in the Division of Research Services. In addition to its housekeeping functions related to the maintenance of 40 buildings on a 300-acre installation where almost 8,000 persons devote their energy in one way or another to medical research, the Division provided innumerable supporting services to the thousand intramural scientists. Typical of these services are the provision of nearly a million small animals annually for use by the investigators at Bethesda; the design and construction of new laboratory equipment not available from commercial sources; the provision of computation and data processing equipment and of photographic and art services necessary for the documentation of experimental results and the

publication of scientific data; and the provision of a trained staff to assist the scientific community in analyzing its problems.

Similarly, the Office of Administrative Management provides a broad spectrum of central services related to the business operations of a large research establishment—personnel, budget and finance, purchase and supply, and so on.

The Clinical Center, too, is a central service of a highly specialized nature. It provides and maintains a 500-bed hospital environment, with the staff and equipment and the organization to permit the clinical investigators of the various Institutes to undertake their studies on selected patients.

While this document is devoted to the scientific product of the NIH laboratories and clinics, it must be remembered that the scientist not only draws upon but is in fact many times dependent upon others for the optimum conduct of his studies. In most instances the researcher may have under his immediate supervision only two or three technicians and other supporting personnel. On the average, however, if he is a laboratory scientist, he has four persons serving him in supporting capacities elsewhere on the grounds; and if he is a clinical investigator, the number is ten.

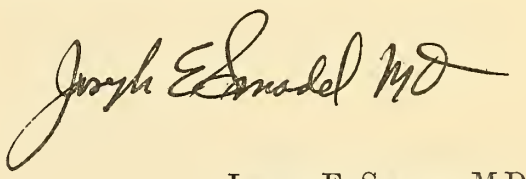
This preface to the 1959 annual program reviews of the Scientific Directors is an inappropriate place to attempt to describe the many cohesive factors of the intramural research environment which underlie the separate presentations. One thinks of the part played by NIH and its scientists in fostering national and international scientific meetings, of the contributions of NIH scientists to the scientific literature, of the role of NIH in fostering collaborative research—the number of items is legion.

The primary mission of the intramural program at NIH is to obtain new information of importance to biology and medicine. Because research and teaching go hand in glove, however, an appreciable portion of the effort of the NIH staff is devoted to the teaching and training of young men and women in the methods and philosophy of scientific research. Most of this educational activity is centered around the time-honored preceptor-student relationship, in the laboratory and on the ward. More formal instruction is given, however, to the Clinical Associates and the Research Associates: the former are assisted in qualifying for their Specialty Boards, and the latter are assisted in acquiring detailed knowledge in disciplines which are inadequately emphasized in the usual medical curriculum for those who would subsequently pursue a career in research. The course work, for example, includes 145 hours in organic chemistry, physical chemistry (including quantum mechanics, mathematics, statistics, tracer methods, and instrumentation), and seminars in psychology, biochemistry, physiology, microbiology, and genetics. In addition, a number of the NIH staff voluntarily assume some teaching responsibility in one or another of the neighboring universities, and many give individual lectures at academic institutions throughout the country.

It should be noted that during the year covered by these reports of the Scientific Directors, a great deal of thought was devoted to plans for the future intramural research program. In part this was a necessary concomitant of the construction of new buildings on the Bethesda campus and the receipt of authority to acquire a farm in nearby Maryland. Of a more elective nature was the preparation by the Scientific Directors of a careful and comprehensive projection of how and where NIH intramural research should develop over the next decade; this projection was submitted as a report to the Director, NIH, in May 1959. In brief, the availability during calendar years 1960 and 1961 of new buildings to house the Division of Biologics Standards, the National Institute of Dental Research, and a large portion of the extramural operations of the NIH will permit a moderate expansion of the intramural program. This is the first time such a possibility has approached actuality since the opening of the Clinical Center in 1951. The space to be acquired will be used to relieve the grave congestion of certain research groups, and to expand and to initiate research in selected fields not now adequately cultivated. One of the guiding principles in the allocation and utilization of the new space will be to move those nonclinical research groups who now occupy space in the Clinical Center to one of the laboratory buildings. This will allow an increasing emphasis to be given to clinical investigations at the NIH, which now provides an almost unique opportunity in the medical world for the

study of disease in man in an environment abundantly supplied with outstanding scientists in many basic disciplines.

The combination of facilities, equipment, staff, and stable operating funds for NIH's intramural research programs represents both a challenging opportunity in scientific terms and a major responsibility in social terms. During years of rapid growth and change in both the substance and the dimension of the sciences related to health, it has been a matter of pride that NIH has maintained the excellence, in terms of stature and productivity, that characterized those scientists and laboratories of which it is the lineal descendant. And it is a matter of conviction that the tradition of excellence shall be maintained.



JOSEPH E. SMADEL, M.D.
Associate Director.

556044—60——2

CONTENTS

NATIONAL CANCER INSTITUTE

INTRODUCTION

An increasingly vigorous and productive research program prosecuted by the intramural staff of the National Cancer Institute during 1959 makes this summary of selected accomplishments even less adequate than its predecessors. New approaches to critically important problems in the study of neoplastic diseases characterized the year's work. Group enterprises flourished to a greater extent than before, yet the talented individual who prefers to work largely by himself deservedly received equal encouragement. Ideas are born in individual minds and the communication of these ideas is probably the single most important stimulus to productive scientific research. Thus, creation of an environment conducive to intellectual give-and-take becomes management's most important function. There is no single or easy way to achieve this environment in a large organization which depends for its vitality on individual initiative and intellectual curiosity.

The virus oncology program, for example, commands respect for its consistently superior performance. Part of its effort is centered in personnel of the Virus Oncology Section of the Laboratory of Biology, but equally important are individuals in several different laboratories who are interested in developing significant segments of the total program by their own ingenuity and have only intermittent contacts with colleagues of similar interests.

The entire area of chemotherapeutic research, much larger at this point than the virus program, has been more strongly influenced by a few acknowledged leaders. While this area is compartmentalized to a degree, there is a greater tendency for the individual investigators to exchange information on a continuing basis.

These examples, which could be extended, simply indicate that research is people and that people differ in many important ways. The organizational chart, so dear to the heart of the professional administrator, can indicate channels for conducting ordinary business but can also stifle creative endeavor if taken as a blueprint for the conduct of research. Clearances, confirmations, and authorizations required by a mammoth Federal establishment can lead to personal frustration, impede progress, and cause the affected to seek employment elsewhere. Substantial decentralization of decision-making, through the wholehearted cooperation of the Laboratory and Branch chiefs and other senior staff members, has helped reduce a number of these petty but highly sensitive problems. The roles of the Administrative Officer and Administrative Assistant have been especially valuable in reducing causes of friction; yet much remains to be done.

Since the size of the working group centered about a scientific leader can be determined by the leader's ambitions, personality, and research competence, the section becomes the key part of the intramural organization. The long-range planning and business aspects of the operation require an individual actively engaged in a personal research program but able and willing to accept even broader responsibility—the laboratory and branch chief. The last few years have seen increasing concentration behind those with leadership capabilities. The head of a section has generally fared well. Some laboratory or branch chiefs, on the other hand, have been so overburdened with so many different responsibilities that their personal research, from which they derive a large degree of satisfaction, has been subordinated to the common good. It seems desirable, therefore, to reduce the size of some organizational segments of the Institute by creating one or more new laboratories or branches, probably during 1960, rather than to persist in stultifying research activity through imposition of related but essentially noncontributory duties on the Institute's leaders.

The death of Dr. Jesse Philip Greenstein, first Chief, Laboratory of Biochemistry, on February 12, closed the career of one of our most distinguished, imaginative, and productive scientists, a beloved friend and counselor, and a world leader in biochemistry and cancer research. Dr. Greenstein worked at a furious pace in 1939 when he

joined the group that became the National Cancer Institute, and that pace never slackened. His interests were extremely broad. His mature judgment, boundless energy, lucidity of expression, and his firm though understanding, even jovial, nature made a lasting impression on even the most casual acquaintance. He was completely loyal, and he had a profound effect on the development of the Institute's research program, which often extended into many areas of the Institute's expanding activities. Very few people realized the intense and benevolent interest he showed in the personal problems of his associates. Dr. Greenstein contributed most importantly to areas of amino-acid, peptide, and protein biochemistry, including enzymology. He defined the concept of anaplasia in biochemical terms, and left behind a monument, *Biochemistry of Cancer*, one of the most quoted works in the enormous literature of cancer.

Dr. Herbert A. Sober has succeeded to the position, Chief, Laboratory of Biochemistry.

Dr. Delbert Mauritz Bergenstal, Assistant Chief, Endocrinology Branch, died on September 12 after a long illness. A close associate of Dr. Charles Huggins, Dr. Bergenstal joined the staff of the National Cancer Institute in 1955 after a brilliant career at the University of Chicago. He was a superb clinician with great ability in clinical investigation. His warm magnetic personality, his tremendous drive, and infinite patience endeared him to his colleagues and his patients. His arguments were imaginative, his exposition stimulating. Among his important contributions to the endocrinology of cancer are studies on the metabolic influences of ACTH, cortisone and growth hormone, characterization of catabolic responses induced by adrenal and thyroid hormones, the effect of adrenalectomy on advanced prostatic and mammary cancers, and the effect of DDD on adrenal cancer.

Dr. Mortimer B. Lipsett has been appointed Assistant Chief, Endocrinology Branch.

Dr. Nathaniel I. Berlin was named Chief, General Medicine Branch, a duty most capably discharged for 5 years by Dr. Charles G. Zubrod, in addition to his many complex responsibilities as Clinical Director.

Two meetings of the Board of Scientific Counselors were held during 1959. Drs. Charles Huggins, Carl V. Moore, and Eugene P. Pendergrass retired on June 30 from the Board on completion of their appointments. Such events, though necessary, cause deep regret, especially when the relationships between the staff and the Board are as cordial as were those with the original Scientific Counselors. Continuing excellent relationships are in prospect, since the new members, Drs. Hugh R. Butt, J. Engelbert Dunphy, and Jacob Furth, are just as deeply interested, understanding, and cooperative as were their predecessors. Dr. Wendell M. Stanley relinquished his position as Chairman of the Board but consented to remain in active membership through June 30, 1961, with Drs. E. K. Marshall and Philip P. Cohen. Dr. Cohen is the new chairman.

The Board of Scientific Counselors continues to express confidence in the research staff and its program. Their discussions are most helpful. Differences of opinion, characteristic of a good and intimate working relationship, are resolved by free and frank discussion to everybody's ultimate satisfaction. The meetings to date have required unduly intense effort from the counselors, who meet throughout the day with various members of the staff and discuss program among themselves far into the night. Different types of meetings are being discussed with the chairman as a means of reducing the workload.

The increasing number of scientific meetings demanding the participation or attention of the staff, although gratifying in the sense of conferring some distinction solely for research accomplishment, is also disturbing because so much of our talented associates' efforts must be devoted to preparing papers. Program has not been seriously damaged as yet, but the saturation point has probably been reached. Foreign travel, though not yet as commonplace as domestic, has become the order of the day. The problem is almost impossible to control as long as the upper echelons of government are international-minded, and in some cases actually solicit the participation of staff members in planning foreign ventures. Every request for travel to foreign meetings has had obvious merit in relation to research, and the ability and desire of extramural organizations and groups to finance travel for National Cancer Institute staff have increased materially.

Tangible recognitions of leadership in cancer research on both national and international bases have been accorded to many persons in intramural

research. Dr. W. C. Hueper received the A.A.A.S-Anne Frankel Rosenthal Award for 1959 in recognition of his contributions to knowledge of the causation of cancer in man. Dr. Harold L. Stewart retired as President of the American Association for Cancer Research. Dr. Murray J. Shear is the Vice President of the Association, and Dr. Thelma B. Dunn was elected to the Board of Directors. Dr. Dunn is also President of the Washington Society of Pathologists. Dr. Roy Hertz was appointed to the Research Advisory Council of the American Cancer Society for a five-year term. Dr. L. W. Law has been named as delegate to the World Health Organization's Advisory Committee on Cancer. Dr. A. J. Dalton is President of the New York Society of Electron Microscopists.

An exhibit, "Tumor Cells in Blood," by Drs. J. F. Potter, R. F. Kaiser, R. A. Malmgren, A. W. Hilberg, and J. C. Pruitt received an Award of Merit at the annual meeting of the American Medical Association.

Causes of Cancer

Twenty years of experience in carcinogenesis research was highlighted late in the year through governmental actions required by amendments to the Federal Food, Drug, and Cosmetic Act enacted in 1958. Several Institute staff members were most helpful in furnishing advice to interested parties on general and specific aspects of carcinogenesis. A meeting of some 25 investigators experienced in research on cancer causation was held during December to determine the extent to which agreement could be reached on different facets of the complex carcinogenesis picture. A lively discussion elicited these important points:

1. Carcinogenic property is not comparable to a physical or chemical property.

2. Groups of compounds possessing common biological properties may be carcinogenic by virtue of those properties, specifically: ionizing radiations, estrogens, and goitrogens of the thiourea type.

3. Determination of carcinogenic property requires judgment as to the validity of the experimental design and its adequacy in relation to the known qualities of a compound and its relatives, to biological availability, to the reasonable interpretation of results and to the reproducibility of the responses. Many isolated experiments recorded in the older literature claiming carcinogenic properties for certain chemicals remain unsubstantiated and are of questionable validity.

4. The potential hazard of a chemical compound can be estimated only on the basis of currently known facts and each situation must be judged on its own merit.

Much more remains to be learned about carcinogenesis, and, indeed, substantial progress is being made in both practical and theoretical aspects. We conceive our duty to be the accumulation of additional facts and especially basic principles which may be applied broadly to general and special problems, rather than to embrace an extensive testing program of large numbers of compounds for purely pragmatic purposes. At the same time, however, we are glad within the limits of our knowledge to advise others interested in such testing, which may well assume increasing importance.

CHEMICALS

The ability of some viruses to induce cancers in mice only when injected shortly after birth has raised a question as to the relative sensitivity of infant and adult mice to the action of chemical carcinogens. Information advanced by Shubik suggests a heightened carcinogenic response to 9 : 10 dimethyl-1,2 benzanthracene. Drs. Margaret Kelly and R. W. O'Gara have injected microgram quantities of 3-methylcholanthrene or dibenz[a,h]-anthracene subcutaneously into newborn Swiss mice and mice of strain C3H. Both respond by increased frequency in pulmonary tumors of the characteristic murine type recognizable as early as 16 weeks and becoming progressively more numerous thereafter. The incidence of subcutaneous sarcomas, however, does not keep pace with that of the pulmonary neoplasms. The same procedure elicits fewer tumors of the lung in mice of strain C57B1. Other types of cancer have appeared sporadically. This research is being extended to other chemical carcinogens, since the differences already observed suggest both qualitative and quantitative importance.

Drs. W. C. Hueper and W. W. Payne have been cooperating with Messrs. A. C. Stern and E. C. Tabor (Taft Sanitary Engineering Center) and with Dr. Paul Kotin (University of Southern

California) in a study of cancer production in mice by dusts collected from several American cities. The benz[a]pyrene content of each sample has been analyzed so that the cancerous response, which can confidently be expected from the experience of Drs. Joseph Leiter, M. B. Shimkin, and M. J. Shear reported in 1942, may be related to a reference standard. Aliquots of each sample have also been divided into several fractions representing different types of aromatic or aliphatic compounds. Preliminary results indicate differences in the carcinogenic potency of the fractions or extracts relating both to the source of the starting material and to the chemical nature of the fraction tested. In at least two cases, the aliphatic fractions appear to be more carcinogenic than those composed of aromatic compounds.

Further experience with certain chromium compounds now permits Dr. Hueper to evaluate their relative carcinogenicity for rats, when injected intramuscularly or intrapleurally in this descending order: calcium chromate, sintered calcium chromate, sintered chromium trioxide, zinc chromate, strontium chromate, barium chromate (weak), and lead chromate (noncarcinogenic). Dr. Payne reports calcium chromate and sintered calcium chromate carcinogenic for mice when sheep fat is used as the vehicle for subcutaneous injection.

Fractionation by particle size and chemical analysis of the residue from leached chromite ore reveal that the smaller particles contain higher proportions of hexavalent chromium, thus increasing their acute toxicity when inhaled. Removal of water soluble compounds from the residue does not alter the degree of carcinogenic potency, according to Drs. Payne and Hueper. Evidently both the discarded part of the roast as well as the desired mixture of chromium compounds may be carcinogenic hazards in industry.

Earlier reports have recorded the production of neoplasms by subcutaneous injection of a variety of water insoluble polymers studied by Dr. Hueper. He now has completed a study of water insoluble polyvinyl pyrrolidones, polyvinyl alcohol, and dextrans. Sarcomas develop in those organs in which the injected material is deposited, especially in the reticulo-endothelial system. Polymers of the same type, but produced by different processes, possess different carcinogenic potencies and neither chemical nor physical factors of the molecules themselves can be recognized to account for the differences. These results seem almost identical with those obtained with water-soluble compounds of the same general classes. The deposits are easily recognized in the tissues into which they were originally introduced or subsequently transported. Possibly the physical state within the cells may be partially responsible for the carcinogenic effect.

Silicone gum and powdered silica are inert, but silicone rubber, introduced into the subcutaneous tissues of rats, produces neoplasms when the molecule contains many cross linkages. Linear molecules of silicone rubber are weakly carcinogenic at best. Polyethylene is moderately carcinogenic when implanted subcutaneously as films or discs but is inactive as a powder, a principle established earlier by the Oppenheimers for other plastics. Polyurethane as a sponge also produces connective-tissue tumors.

Heated fats and charred foods are known to contain substances that can elicit a cancerous response in mice. Some years ago Dr. Hueper became interested in the potential carcinogenic hazard from coffee consumption. After Dr. M. Kuratsune identified benz[a]pyrene in coffee soots, a limited study was initiated with the cooperation of various members of the coffee industry. Drs. Kuratsune and Hueper now conclude that aromatic hydrocarbons are present in most roasted coffee beans in extremely low concentration. Only certain "dark roasts," which form an extremely small fraction of the total production of coffee and are characteristically made by family concerns, contain any detectable amount of known carcinogenic hydrocarbons. Coffee consumption, then, plays no significant role in human carcinogenesis.

Soots from coffee-roasting plants are an important commercial source of caffeine, in which benz[a]pyrene is quite soluble. Drs. Kuratsune and Hueper made use of this fact to study distribution of the carcinogen in the stomach of the mouse. Intense fluorescence develops in the rumen of the stomach, where benz[a]pyrene is known to elicit tumors when fed, but does not appear in the glandular mucosa in which benz[a]-pyrene evokes no cancerous response. Earlier experiments reported by Dr. H. L. Stewart have

clearly shown that some carcinogenic hydrocarbons elicit some adenocarcinomas when injected directly into the glandular gastric mucosa. The entire experience reemphasizes the need for study of gastric secretions in relation to cancerigenesis in the stomach.

Study of causation of gastric cancer has been hampered by lack of reliable and reproducible methods for producing adenocarcinomas of the stomach. There has been some progress following the discovery by Drs. H. P. Morris and H. L. Stewart of a few lesions in the glandular stomach of rats fed 2,7 fluorenylenebisacetamide. The same lesions seen in human material would unquestionably be diagnosed as adenocarcinomas, but experience over the years has revealed a number of gastric lesions in mice that simulate gastric cancer but have proved to be non-neoplastic. It is essential, therefore, to have a clear picture of the biological behavior of gastric lesions when interpreting significance of morphological changes. While some of the lesions have invaded the muscularis in the rats fed 2,7 fluorenylenebisacetamide, no metastases have been recognized. All of the subjects develop metastasizing carcinomas of the small intestine, and some also have malignant tumors of other sites including the salivary glands.

Dr. W. D. Conway and Miss E. J. Lethco have examined the purity of several commercial preparations of the dyes yellow AB and yellow OB. The Food and Drug Administration limits the quantity of β-naphthylamine in those products to 500 parts per million and only 1 of the 10 samples contained more than the permissible quantity.

Four melanomas have appeared among 51 guinea pigs painted with 9,10-dimethyl-1,2 benzanthracene by Dr. J. H. Edgcomb.

Drs. M. L. Hesselbach (NIAID) and R. W. O'Gara report production of subcutaneous fibrosarcomas in rats by multiple injections of fast green or light green. The earliest tumors appear at 7 months. The sarcomas are readily transplantable and frequently metastasize.

MECHANISMS OF CARCINOGENESIS

Study of the mechanism by which N-2-fluorenylacetamide produces cancers in rats has required identification of excretion products, their synthesis and bioassay, chemical modification of the parent molecule, and evaluation of carcinogenic potency of the new compounds. Much has been learned from this approach and several new carcinogens with interesting types of activity have been described, such as 2,7-fluorenylenebisacetamide. The experience to date indicates that hydroxylation of N-2-fluorenylacetamide in positions 1, 3, 5, or 7 reduces its carcinogenic potency, which Dr. Morris interprets as a detoxification mechanism or a means of solubilization to facilitate excretion. Fluorination in the omega position of the acetyl group enhances carcinogenic potency, and two fluorinated compounds produce intestinal tumors when fed to rats, but hepatomas when fed to dogs.

The influence of dietary factors on N-2-fluorenylacetamide has also been studied by Dr. Morris. A high dietary intake of pyridoxine results in many mammary tumors among rats eating N-2-fluorenylacetamide. If the mammary lesions are removed surgically, the rats ultimately develop hepatic neoplasms. Conversely, rats with low dietary intakes of vitamin B_6 develop tumors of the liver but not of the breast.

Detailed studies by Dr. H. M. Dyer of the metabolism of N-2-fluorenylacetamide in Buffalo rats which ate a tryptophan-rich diet were recorded in last year's report. The objective (as reported by Dunning) had been the production of tumors of the urinary bladder; yet none occurred. The same procedure does produce cancers of the bladder in Fischer rats, however, and Dr. Dyer reports no chemical differences in the excretion products of N-2-fluorenylacetamide or of tryptophan in the two strains. Addition of tryptophan is not essential to cancer production. Once again, the genetic constitution of the host becomes a most important factor in carcinogenesis.

Dr. Dyer finds differences in tryptophan metabolism in rats fed hepatic carcinogens and chemically related but noncarcinogenic compounds; she ascribes this to differences in the extent of damage produced in the liver.

Greater attention is now being directed towards the fate of N-2-fluorenylacetamide in organs and tissues where the compound is bound to proteins. The Doctors Weisburger report that binding of N-2-fluorenylacetamide labeled with C^{14} cannot be explained on the basis of artifacts created by *in vitro* manipulations. Furthermore, orthohydroxylated compounds form stable complexes with a variety of metal ions. Introduction of a hydroxyl

group at a greater distance in the molecule does not confer the same property.

Dr. H. F. Blum, reviewing his vast experience in the quantitative study of ultraviolet carcinogenesis, writes in his most recent book:

". . . By treating the quantitative description as empirical, one may arrive at certain conclusions regarding this kind of carcinogenesis.

"It seems clear that whatever the process of cancerization, it is continuous throughout the whole of the development of the cancer and cannot be separated into distinct periods.

"Cancerization is in some way cumulative—if dosage is stopped, development continues at a retarded pace but speeds up with renewal of dosage.

"Although there is evidence of a small degree of recovery, this is slight in overall effect, and carcinogenesis may be regarded as essentially irreversible.

"Since there is recovery—and we take particular account of the evidence of this in the failure of reciprocity at very low dose-rates—it seems necessary to conclude that there is a threshold dosage at which recovery just balances cancerization. But this threshold is certainly very low and is not directly measurable because of the limited lifetime of the animals. Thus, in any practical sense, carcinogenesis by ultraviolet light may be considered as essentially nonthreshold."

Dr. Blum has examined the possibility of similar cumulative effects among chemical carcinogens, but finds the quantitative data now available inadequate to decide the point.

With Mr. G. A. Soffen (Princeton University), Dr. Blum is studying the effects of single doses of ultraviolet irradiation on mouse skin. The original insult renders cells incapable of dividing, but five different doses result in normal mitotic counts at 1.5 days. A strong hyperplastic response follows in the epidermis with higher proliferation rates than are found in cancers resulting from repeated doses of ultraviolet. The hyperplastic response falls off with time. The higher the dose, the greater the number of cells destroyed and the greater the initial rate of cell proliferation as measured by mitotic indices.

VIRUSES

The problem of identifying viruses that may cause cancers in man is extremely complex. One group of investigators and 3 individual scientists working with different techniques have screened more than 100 clinical cancers for viruses. Only one of these people has found a clearly identifiable virus. The recovery of a number of viruses from transplanted experimental tumors such as sarcoma 37 is quite common and includes two new varieties of murine hepatitis virus found recently by Dr. R. A. Manaker in experimental leukemias. Either the cancers that affect man are basically different in their ability to harbor passenger viruses or we are working toward isolation with comparatively insensitive tools. Continuing reevaluation of the techniques employed in the search for viruses in human cancers proceeds, together with encouragement of individual initiative in modifying techniques of tissue extraction and tissue culture, in the belief that passenger viruses inhabit some proportion of cancers that affect man.

Dr. S. E. Stewart has observed a peculiar reaction in monolayer cultures of freshly isolated human amnion or embryonic cells produced by concentrates of two different human cancers and the urines of three children with neoplastic diseases. Focal areas of increased growth appear in the affected cultures leading to the formation of discrete dense, raised patches, easily visible to the naked eye. Although serial passage in tissue culture of the proliferative effect is readily accomplished by use of supernatant fluids, the cell-free nature of such fluids has not been established. Filtration in bacteriaproof systems or centrifugation at high speed destroys capacity of the fluid to transmit the proliferative effect. The sediment obtained from these procedures retains the ability to cause a focal proliferative response in new cultures, and this property is maintained after repeated freezing at the temperature of dry ice and intermittent thawing. The nature of the agent responsible is not known.

Injections of many tissue-culture preparations or tissue extracts into newborn hamsters and mice, and observations of the animals for long periods, have reemphasized the need for better information on diseases occurring naturally among laboratory species. Attention has been drawn to a curious but reasonably common lesion of the placenta in hamsters often resulting in fetal death *in utero*. Hamsters also develop ulcerating and/or polypoid lesions of the intestines which bleed profusely.

Polyoma virus or parotid tumor agent

Some confusion may be caused by use of the synonyms polyoma virus and parotid tumor agent for an agent which produces multiple primary neoplasms in mice and tumors in hamsters, rats, and rabbits.

The chemical nature of polyoma virus has been studied by scientists of the Sloan-Kettering Institute in collaboration with Drs. Stewart and Eddy. Infective nucleic acids prepared after the techniques of Gierer and Schramm or of Kirby are inactivated by deoxyribonuclease, but by ribonuclease as measured by effects on cytopathic changes in tissue culture, hemagglutination of guinea pig erythrocytes, and tumor induction in mice and hamsters. The infectivity of intact viruses is not influenced by either of the enzymes. The infective component of polyoma, therefore, would appear to be a deoxyribonucleic acid.

Early in the year Dr. W. G. Banfield demonstrated intranuclear, intracytoplasmic, and extracellular, relatively uniform, spherical particles 27 to 35 millimicrons in diameter in lymphoma cells grown in tissue culture by Dr. C. J. Dawe for propagation of the parotid tumor agent. Uninoculated cultures contained no similar bodies. The descriptions provided by Drs. Banfield and Dawe are comparable to the measurements reported last year by Dr. H. Kahler and colleagues on isolated particles possessing polyoma activity when allowance is made for the different methods of preparation. Drs. Banfield and Dawe consider that propagation of the virus is predominantly intranuclear. The intranuclear localization of polyoma demonstrated by fluorescent antibody techniques in tissue cultures of a lymphoma, as reported by Drs. R. A. Malmgren, A. S. Rabson, and Giancarlo Rabotti (Visiting Scientist), supports that view. These workers, however, have not been able to demonstrate polyoma virus in parotid-gland tumors by fluorescent antibody techniques. Others have shown that not all tumors produced by polyoma contain detectable amounts of the virus when tissue-culture methods are employed.

Dr. Robert Love has applied his sensitive toluidine blue molybdate methods for the demonstration of intracellular ribonucleoproteins to the study of lymphoma cells infected *in vitro* with polyoma. Neither nuclear parachromatin nor the nucleolinus can be demonstrated. The nucleolus is enlarged, and vacuoles containing a diffuse and condensed, abnormally staining, form of ribonucleic acid appear in the nucleus. The nucleus first enlarges, then shrinks and eventually disintegrates by karyorrhexis. Dr. Love has not been able to identify a deoxyribonucleic acid in association with the newly formed intranuclear ribonucleoprotein by histochemical procedures.

Dr. Rabson and Dr. Ruth Kirschtein (DBS) have produced intracranial sarcomas through the intracerebral inoculation of polyoma virus in hamsters. These neoplasms appear to arise from the pia mater and the adventitia of the meningeal and cerebral blood vessels.

Drs. Stanton, Stewart, and Eddy report that injection of polyoma virus into Swiss mouse fetuses *in utero* late in fetal life results in earlier death and a higher incidence of tumors than injection on the first day of extrauterine life. Mice infected as fetuses develop no new histological types of neoplasms. Some of the mice, however, born to mothers reared in close proximity to adult mice known to harbor parotid-tumor agent had a significantly lower incidence of salivary-gland tumors and "renal tubular lesions." A comprehensive analysis of histological changes by Dr. Stanton reveals that some cells in the infected mice hypertrophy, form intranuclear inclusions, and degenerate. The neoplasms in the series are composed of hyperplastic epithelial elements and proliferating or differentiated cells, limited in their capacity to invade surrounding tissues or to metastasize. Some tumors regress; others kill their hosts by local growth in vital organs.

In another study of polyoma virus infection, Dr. Sarah Stewart, Dr. Kahler, and Dr. Stanton have studied the tumors occurring with limiting dilutions of virus culture preparations subjected to differential high-speed centrifugation. All fractions produce tumors, but the greatest activity is found in sediments produced with forces designed to contain the greatest quantity of the agent. Again, the variety of neoplasms produced by the fractions is similar. Analysis of the tissue reactions that follow minimal doses after long-term study reveals many nonspecific lesions in older mice, especially in the kidneys and salivary glands, resembling either abortive attempts at cell

proliferation or regression of minimal lesions. Older mice do develop a significant number of neoplasms, particularly bone tumors that show clear evidence of the capacity to invade and metastasize. Dr. Stanton comments:

"The polyoma virus-tumors serve as exceptional experimental tools. The wide range of species that respond and the convenience in working with small rodents constitute an advantage over chemically induced tumors both in the short, latent period and opportunity of following the progress and behavior of the tumors in relation to virus as detected by new virological and morphological techniques . . . There is good evidence to indicate that induction and progression proceed in a slow, stepwise fashion which may allow separate study of the two most important attributes of tumors, namely, cell proliferation and the acquisition of aggressive behavior patterns by the proliferating cells."

Perhaps the most significant part of this statement is the recognition of phases in the evolution of the cancerous state among neoplasms not associated with known endocrine functions.

Especially important to the search for viruses that may cause cancer in man is the ability to recognize a cancerous change in some foreign species or semiartificial system such as tissue culture. Last year's report referred to the most interesting changes produced by parotid-tumor agent in salivary gland anlagen grown *in vitro* by Dr. Dawe. He now reports that increasing chronological age to as much as 15 months does not diminish the proliferative response of submaxillary glands of mice to polyoma virus in tissue culture. If anything, the reaction, which mimics neoplasia in its morphological characteristics, is more pronounced. The altered cells still fail to produce local neoplasms when transplanted to genetically appropriate hosts, although evidence for virus transfer is clear. On the other hand, infection *in vitro* of salivary gland rudiments in the earliest stages of morphogenesis produces little in the way of a neoplastic response.

Dr. Dawe also reports that growth of adult parotid gland in sponge matrix culture results in digestion of the sponge by a protease secreted by the cells.

While Dr. K. K. Sanford has been able to propagate polyoma virus in a clone from normal C3H mouse parotid tissue and in a long-term strain of C3H embryonic fibroblasts, two strains of C3H adult fibroblasts and one strain of Chinese hamster cells, although supporting limited maintenance, show no conspicuous effects of infections.

The failure of certain newborn mice to develop tumors when injected with polyoma virus may, in part, be due to the presence of antipolyoma antibodies in mothers' milk transmitted to the nurslings. Antibody titers as measured by inhibition of hemagglutination are as high in milk as in sera, according to Drs. Stewart and Eddy. Similar reasoning would account for the failure of naturally infected stocks of mice to develop salivary-gland tumors so characteristic of polyoma-virus action. Dr. L. W. Law reports that mothers whose blood contained hemagglutination inhibiting antibodies in titers greater than 200 delivered young which resisted the oncogenic action of the parotid-tumor agent. Foster nursing experiments conducted by Dr. Law point to the transfer of antibodies in the milk of infected mothers and also suggest that antibodies may be transmitted transplacentally.

Leukemia virus (Moloney)

The murine leukemia virus isolated by Dr. J. B. Moloney from transplantable sarcoma 37 is being studied intensively. Standardized techniques based on differential centrifugation at alternating high and low speeds or on bacteriological filtrations combined with differential ultracentrifugation yield equally potent preparations from sarcoma 37 or from transplantable leukemias produced by the virus. Leukemic spleens or lymph nodes are excellent sources of the agent, but the liver is a relatively poor one.

BALB/c strain mice have been used by Dr. Moloney as a reference standard in studies of viral leukemogenesis. Susceptibility is retained for at least 5 months, but the latent period is somewhat longer among older mice. Mice of strains C3H, C3Hf, A/LN, I, RIII, DBA/2, C57BL and randomly bred Swiss mice all develop leukemia from virus infections. The incidence approximates 100 percent and the latent intervals of leukemia production do not vary significantly except for strain C57/BL, which develops the disease in only about 30 percent of infected subjects. The F_1

hybrids obtained by mating each of the strains noted above with BALB/c mice respond to leukemogenesis as promptly as do mice of the reference strain.

Potency of the virus has been increased by selective virus passage *in vivo*. After eight passages the latent interval has decreased from 6.4 months to 10–12 weeks in all mice tested.

Dr. T. B. Dunn reports that this leukemia is morphologically indistinguishable from spontaneously occurring lymphocytic leukemias in several mouse strains. The lymph nodes, spleen, liver, and thymus are characteristically enlarged and infiltrated with typically lymphoid cells that also involve the salivary glands, kidneys, lungs, and meninges in the advanced stage of the disease. The same histologic pattern is seen in all mice examined whether the leukemia is transmitted by cell-free preparations or by leukemic cells. Blood from the leukemic mice presents moderate leucocytosis and anemia. No neoplasms other than those of the lymphocytic type have as yet been observed in any of the test animals. Leukemia can be produced by Dr. Moloney's virus by subcutaneous, intravenous, intracerebral, or intraperitoneal inoculation.

The leukemia virus remains stable at temperatures below −50° C. for long periods of time when prepared in citrate buffer and withstands lyophilization without apparent loss of activity. Biological activity is retained when the virus is exposed to 37° C. for 90 minutes but is lost at exposure to 56° C. within 30 minutes.

Dr. A. J. Dalton has examined leukemias, produced by Dr. Moloney's virus, under the electron microscope. Particles representing the agent belong to morphological class Type A (Bernhard), have an external diameter averaging 100 millimicrons, and contain an electron dense nucleoid of 48 millimicrons. They are not particularly abundant, but may be found in spleens, lymph nodes, and thymuses of infected mice and are found most frequently in the intercellular spaces and only occasionally within intracytoplasmic vacuoles.

The Moloney virus is difficult to propagate in tissue culture. Dr. R. A. Manaker presents evidence that the agent can be maintained *in vitro*, but its behavior is not entirely clear at this time. Ameboid cells found in cultures of liver, thymus, spleen, or lymph nodes of leukemic mice seem to maintain the virus best, while cells clearly recognizable as lymphomatous support it little, if at all. Although many other types of cells have been used to propagate leukemia virus, activity has usually died out within four or five serial passages *in vitro*.

Preliminary evidence obtained by Dr. Moloney suggests that immune sera prepared from rabbits receiving multiple injections of the leukemia virus do contain neutralizing antibodies. A diligent search by Dr. M. A. Fink for other antigenic properties which might be used in epidemiological studies has been unsuccessful. The agent does not fix complement, nor does it inhibit hemagglutination. As a matter of fact, most of the oncogenic viruses are rather weakly antigenic. Polyoma is an exception.

Mammary tumor agent

Dr. H. B. Andervont recently described the disappearance of the mammary tumor agent from two female mice of strain RIII. Experiments designed to analyze the respective roles of the virus and the host in this phenomenon have included production of both C3H and RIII mice known respectively to carry and to be free of the agent. These stocks have been interbred. Ninety-five percent of the agent-free C3H mice exposed to their own mammary tumor inciter develop tumors of the breast. Breeding decreases the latent interval but has no effect on the incidence. RIII milk in C3Hf mice produces a smaller number of mammary carcinomas but is transmitted serially through the strain. It is already apparent, however, that some RIII mice resist the carcinogenic effects of the C3H agent. While the RIII agent is much less potent than the C3H mammary tumor inciter, the constitution of the host is also important in determining carcinogenesis. Dr. Andervont's earlier observations on the enhanced activity of the mammary-tumor agent obtained from wild mice and propagated through 20 generations in BALB/C, coupled with his other studies on the disappearance of C3H agent from mice of strains C57B1 and I, take on added significance.

Further work along these lines has been conducted by Dr. W. E. Heston in his studies on genic influences associated with the propagation of the mammary tumor inciter. Segregation data obtained from the matings of strains C3H and C57B1 indicate that the agent can be propagated

in the presence of one or several genes, rather than being under the control of a single gene. A female mouse lacking any of the genes required to propagate the agent does not eliminate it, but the virus does not multiply and is lost in subsequent generations. The quality and quantity of mammary-tumor agent vary then according to the number of genes required for its propagation and their availability to the individual concerned.

Dr. Sanford, with Drs. Dunn and Andervont, reports the persistence of mammary tumor inciter in cultures of certain cells for more than a year. Activity is lost if the cells take on a sarcomatous appearance. The usual structural characteristics of mouse mammary carcinomas are maintained when the cells grow in serum-supplemented, chemically defined, media but are lost when the medium contains serum and chick embryo extract.

Rous sarcoma virus

Examination of physical, chemical, and immunologic properties of the Rous sarcoma virus requires a highly potent source of material. Dr. W. R. Byran reports a four-fold increase in activity during the past year in viral preparations obtained by selective serial passage. This represents an increase of 100 times the potency of the best material available two years ago and makes purification studies practical. The intranuclear and intracytoplasmic location of Rous virus antigen in Rous sarcomas has been demonstrated by Drs. Malmgren and Fink using fluorescent antibody techniques.

Through the cooperation of Dr. J. A. Reyniers (Germ-Free Life Research Center, Tampa, Fla.), Dr. Bryan has acquired a number of Japanese quail (Coturnix japonica) for use in Rous virus research. These birds prove to be as susceptible to tumor production as the most sensitive chickens yet investigated. Yields of virus are fully as high from quail tumors as from chicken tumors. The small space required to house the Japanese quail, which are no larger than 5-day-old chicks, is a considerable advantage.

OTHER CARCINOGENS

While much is known about the quality of carcinogenic responses to ionizing radiations, much remains to be learned concerning the mechanism of their action which can be approached through manipulations that increase or reduce the frequency of the response. Dr. Law, particularly interested in X-ray leukemogenesis, described a reduction in the incidence of X-ray-induced lymphocytic leukemias in thymectomized C57BL mice some years ago. The normal response could be restored by subcutaneous transplantation of thymic fragments in which the leukemias apparently originated. Last year's report referred to the failure of thymic tissue placed intraperitoneally within millipore diffusion chambers to restore the capacity of thymectomized mice to develop leukemias after X-irradiation. The thymic tissue within the chamber does not become leukemic in either C57BL mice or in hybrids produced by mating C57BL to strain A. Nevertheless, 20 to 30 percent of these subjects develop reticular neoplasms of Dr. Dunn's type A and B. No plasma cell neoplasms have been found under these conditions.

Dr. Law now reports the prevention of neoplastic changes in thymectomized mice bearing subcutaneous thymic grafts when isologous bone marrow is given immediately following the last of four weekly doses of total body X-irradiation. The incidence of reticular neoplasms other than lymphocytic leukemia is not affected. Chimeras produced by injection of AKR marrow into irradiated C3Hf mice, of Law or Bittner subline, less than 12 hours after birth develop the same incidence of leukemias as do the controls, nor is the leukemia incidence influenced by transplantation of AKR thymic tissue. A most interesting observation, however, is a frequency of unilateral parotid tumors, approximating 10 percent among C3H/Bi mice receiving tissue from AKR donors known to be infected with polyoma virus as demonstrated by HI antibodies.

Miss D. E. Uphoff and Dr. Law have extended their attempts at altering the high incidence of leukemia in irradiated AKR mice to include the use of marrow from four strains that are compatible at the H–2^k locus. The results are incomplete, but most of the leukemias arising to date in mice irradiated and protected when young have proved to originate in donor cells even though they come from strains with low frequency of spontaneous leukemia, Marrow treatment in AKR

mice irradiated at six months of age has little effect on the leukemia incidence.

Dr. R. L. Swarm reports hemangioendotheliomas of the liver and spleen in two of three rabbits receiving two injections each of 3 ml. of thorotrast (colloidal thorium dioxide). The lesions appeared at 36–37 months and the other noncancerous animal died at 19 months. A single intravenous dose of 3 ml. produced no neoplasms in any of nine rabbits, four of which survived at least 38 months.

HOST FACTORS IN CARCINOGENESIS

As better knowledge of constitutional factors concerned with the carcinogenic process is acquired, the interrelationships are more properly discussed in association with specific responses which they modify under controlled conditions. The principal identifiable genetic determinant of the cancerous response seems to reside in specific organs or tissues as shown in the work already reported by Heston, Gardner, Kirschbaum, and others. Two strains of mice may be almost completely resistant on the one hand, and exquisitely susceptible on the other, to production of a given anatomical variety of cancer under readily controllable conditions. In what way do these organs differ? The morphologist, well equipped to study this problem, arrives at no definite conclusions. The biochemist is confronted with large numbers of cells of which an almost insignificant fraction is destined to become cancerous. The problem does not seem insuperable, however, when one considers the availability of microchemical techniques as developed by Linderstrom-Lang, Lowry, and others.

Dr. Heston has observed an increased frequency of hepatomas in C3H mice during recent years. He ascribes this in part to genetic change in the strain, but also to change in the diet in his animal colony, since mice eating a standard commercial laboratory ration develop significantly fewer tumors of the liver than those eating a formula especially compounded by Dr. Morris for use at NCI. The presence or absence of the mammary tumor agent in the stock has no influence on the incidence of hepatomas.

The influence of the lethal yellow (Y) gene on mammary tumor development studied by Dr. Heston is manifested by a prolonged latent period in virgin mice of agouti color as compared with those having yellow coats. Both color types are obtained by mating C3H and Y strains since the Y gene can be propagated only in heterozygotes. The difference in latent period would seem to be related to some hormonal influence because it does not occur in breeding females. The Y gene also affects hepatoma incidence which is greater in yellow mice. An even larger difference is seen when the number of hepatomas per mouse is compared. Positive correlations exist between frequency of tumors of the liver and body weight, body height, and length of the femora.

Dr. M. K. Deringer has previously reported a reduction in the incidence of mammary tumors in mice of strain C3He which she produced by transplanting fertilized C3H ova to the pregnant uteri of C57 black mice. It now appears that DBA/2e mice, comparable to C3He, have even fewer breast carcinomas.

Mice of the hairless strain HR develop a considerable number of skin tumors but principally those arising in dermal appendages rather than from epidermis. At any rate, they exhibit a genetically determined tendency towards tumor formation. Ability to enhance chemical carcinogenesis in the skin by painting with ethyl carbamate is well-established, though urethane produces few if any epidermal tumors by itself. Dr. Deringer has not been able to influence the production of skin tumors in HR mice by topical administration of ethyl carbamate.

Cancers and Their Properties

Knowledge of neoplastic diseases has profited enormously from search by imaginative, diligent, and talented scientists for common denominators that would permit development of unifying concepts relating all cancers to one another. At the present time everyone would probably agree that the transformation from normal to neoplastic is basically a heritable and irreversible change in cellular behavior—and probably the general acceptance of the somatic mutation theory of cancer production means no more than that. Associated with the cancerous change or consequent upon it are varying degrees of taxonomic abnormalities in the affected population, and the loss to greater or less extent of specialized cellular function. The normal tissue of any adult organ

has certain definable chemical attributes which vary somewhat among individuals of the species. Neoplasms arising from these same organs may reflect widely different biochemical patterns of activity, and when one compares tumors of different histogenesis among themselves the complexity is compounded.

Borst in Germany and Ewing in the United States emphasized the diversity of structural patterns and biological behaviors among cancers arising in the same organ. The thesis has been accepted by clinicians, and recent experience in chemotherapeutic excursions has intensified the need for better and different classifications of malignant neoplasms than now exist. It seems desirable to restudy the biochemistry of cancer to:

1. Delineate certain qualities measurable by simple techniques with a view towards correlating rather gross metabolic characteristics with biological behavior,

2. Establish the degree of change of certain specific enzyme activities in neoplasms arising from a given organ or tissue, and,

3. Search for progressive changes in metabolic attributes or enzymic patterns as the recognizably malignant tumor progresses to an increasingly autonomous state.

A part of this is already in progress. The orientation of much biochemical activity in cancer is concerned with the description of drug action; yet equal concern with the natural history of selected cancers as definable in chemical terms might well expedite progress in therapeutic research. It is unreasonable to expect the biochemist to solve all the problems of cancer by himself, and, indeed, a better familiarity with neoplastic diseases will require development of new techniques and concepts, perhaps even new disciplines of science.

The search for common denominators should continue, but there is every reason to focus attention on cancers of common origin or different behavior within a histogenetically homogeneous group. The final result may reveal entities as discrete as mumps, chicken pox, and measles within one anatomical type of cancer, though one would not expect such differences to be associated primarily with qualitatively different etiologies.

NEW TUMORS

The colony of mastomys established by Dr. H. L. Stewart is developing a few carcinomas of the glandular stomach as expected from experience in South Africa. Hepatomas are the most frequently occurring neoplasms at this time. Attempts to transplant tumors within the species have been unsuccessful.

Dr. Dunn is studying several unusual uterine neoplasms with markedly organoid structure which arise in strain BALB/C mice. One of these after seven months' growth as a transplant produces a cystic cavity lined by endometrium with a well defined stroma and an outer layer of smooth muscle.

A transplantable osteogenic sarcoma studied by Drs. Dunn and Dawe contains many large multinucleated osteoclastic cells which disappear when the lesion is grown *in vitro* but reappear when the tissue culture cells are transplanted back to mice.

Some interesting lesions have been found in Dr. Deringer's colony of strain BL mice. The majority of the females develop large ovaries due to deposition of amyloid in corpora lutea. The ovaries are also one site of a peculiar necrotizing arteritis which affects other tissues including the kidneys, though its exact distribution and frequency are not yet determined. Strain BL, which also develops some mammary and pulmonary tumors, has been reported by Drs. Leon Sokoloff (NIAMD) and R. T. Habermann (DRS) to have ideopathic necrosis of bone as well.

The Institute maintains about 175 different transplantable tumors, and specimens have been sent to all parts of the world. Each represents special characteristics, and every new neoplasm that can be propagated becomes a useful tool. In some cases new tumors generate entirely new programs. Research on plasma cell myelomas is a case in point.

PLASMA CELLS AND PLASMACYTOMAS

Dr. Dunn described a plasma cell tumor arising in a C3H mouse during 1954, and maintained it in serial transplantation. Dr. Michael Potter in 1956 commenced an experimental program based on availability of this myeloma to which others have been added subsequently. His infectious enthusiasm focused the attention of colleagues on the value of experimental myelomas as research tools, and some information was summarized in earlier annual reports.

Plasma cells first appear in the mesenteric lymphatic tissue of mice at about the 10th day of life, according to Dr. R. C. MacCardle. Their most distinctive structural feature is a juxtanuclear, clear, cytoplasmic "hof" which contains the Golgi apparatus and the centrosome. Plasma cells in the 10-day-old mouse have distinctly smaller Golgi than those in adult mice. Examination of plasma-cell neoplasms leads Dr. MacCardle to speculate that the myeloma cells are derived from lymphoma-like cells which in turn probably originate from undifferentiated lymphocytogenic cells in the germinal centers of lymph nodes.

Dr. Emma Shelton, seeking concrete information on the behavior of normal cells in the presence of tumor cells, studied the behavior of normal cells when grown in double diffusion chambers placed in the peritoneal cavities of mice. Cells cannot traverse the walls of these chambers, but fluids can. Cells of the peritoneal fluid placed within chambers ultimately form an organized tissue containing typical fibroblasts. Detailed study of the sequential events leads Dr. Shelton to conclude that lymphocytes grown in this manner modulate into an indifferent cell type which has the capacity to become either a fibroblast or a plasma cell. Macrophages appear to have the same capabilities. Granulocytopoiesis occurs in the chambers, and most cells persist beyond 140 days. Collagen can be identified at 2 weeks and its content then increases with time.

Thus two different approaches complement one another insofar as the origin of plasma cells is concerned, and Dr. Shelton has also awakened a long-standing controversy.

Substantial impetus was given to plasmacytoma research through the occurrence of several new plasma cell myelomas in mice into which diffusion chambers containing mouse mammary carcinoma cells had been implanted by the late Dr. G. H. Algire and Dr. Ruth Merwin as described in the report for 1958. The exact reason for this phenomenon is still not clear, as some few myelomas have now been obtained through implantation within the abdominal cavity of empty chambers containing no cells, as reported by Dr. Merwin. Experiments testing the capacity of various parts of the diffusion chambers to initiate the plasma-cell tumors have not yet been completed. These myelomas have a distinct advantage over the earlier plasmacytomas available for study since they grow more rapidly. A series of individualistic plasmacytomas is now available which reproduces most of the features of their human counterpart, including circulating myeloma proteins characterized by Dr. J. L. Fahey.

Morphological studies of these neoplasms by Dr. MacCardle correlated with biological and biochemical attributes described by Drs. Potter and Fahey reveal three types of Golgi apparatus: a few small strands, a diffuse network, or a large, thick, and hypertrophied ball-type network. Cells of two tumors which produce β-globulins have the third variety, but the type of Golgi seems to relate better to the speed of production of myeloma proteins than to their electrophoretic properties.

Dr. A. J. Dalton has examined the ultrastructure of 10 transplantable plasma-cell neoplasms. Differences within the group relate principally to the extent and complexity of the ergastoplasm, but these do not correlate well with the physicochemical characteristics of the myeloma globulins. A feature common to all of the tumors is the presence of minute viruslike intracytoplasmic bodies. The particles are formed on the membranes of the endoplasmic reticulum, and the membrane itself forms the outer shell. Similar bodies are absent from normal plasma cells of mice. The significance of the particles is not known, but they may well be related to the neoplastic state. They are not the mammary tumor agent, as some of the myelomas have arisen in agent-free mice and bioassay has failed to reveal presence of that virus. An attempt to examine cells from human plasmacytomas under the electron microscope has been beset with a series of technical difficulties which are yet to be surmounted.

Evidence that the myeloma cells secrete the myeloma proteins into the serum was published in 1958 by Drs. Nathan, Fahey, and Potter. Attention then focused on the relation of these proteins to those normally occurring in the serum. Dr. Fahey has characterized the physicochemical properties of human gamma globulins which are clearly a large family of related but not identical protein molecules. Two major groups with ultracentrifugal sedimentation coefficients of 18S and 6.6S respectively can be separated by anion-exchange cellulose chromatography. The 6.6S group can then be further subdivided into 4 or

more fractions. Detailed characterization of these fractions reveals a family of molecules sharing similar ultracentrifugal and immunochemical properties but differing in electric charge, hexose content, and antibody activity.

A similar family of molecules is found when myeloma proteins from sera of patients with plasmacytomas are examined in detail. These myeloma proteins appear to represent proteins normally present in serum in small quantities. Similar observations have been made on the serum myeloma proteins associated with eight transplantable plasma cell neoplasms.

Dr. Fahey argues that since the variety of myeloma proteins represents a variety of plasma cell neoplasms, the spectrum of normal 6.6S gamma globulins probably represents a spectrum of individual normal but distinctly different plasma cells.

Immune response of myelomatous mice to sheep erythrocytes and bovine serum albumin has been studied by Dr. Falconer Smith. Capacity to form antibodies to either particulate or soluble antigens decreases as the tumor grows larger. The secondary response remains unaltered and the half-life of passively transferred antibodies does not change. The results suggest a factory so busy forming myeloma proteins that it gives an increasingly lower priority to antibody production as the factory expands.

Dr. Michael Potter is studying the effect of chemotherapeusis on the transplantable myelomas. Some of them will regress completely when treated with 5-fluorouracil even though initial treatment is delayed until the lesions are 2 cm. in diameter; others fail to respond. The optimum dose of 5-fluorouracil is 20 mg./kg. six times weekly. Toxicity occurring within 2 to 3 weeks requires 50 percent reduction in the dose, and the tumors recur. Some mice apparently cured of one myeloma have accepted a second graft of the same myeloma in contrast to Dr. Abraham Goldin's experience with mice cured of leukema L1210 by treatment with 3′, 5′-dichloroamethopterin (*vide infra*).

New plasmacytomas will doubtless appear in the course of time and be added to the fine collection now available. The progress made to date already permits an aggressive attack on specific problems concerned with causation and treatment, and also emphasizes the usefulness of these tools in pursuing basic problems in protein chemistry and metabolism. All logical reasoning requires a considerable expansion of the chemotherapeutic effort. The individual tumors faithfully reproduce the manifestations of multiple myeloma as seen in the clinic, including some variety of individualistic behavior patterns.

While plasmacytoma is rather well-defined in biological and chemical terms, macroglobulinemia occurs also under more poorly understood circumstances. Dr. Fahey has studied gamma macroglobulinemia in patients with a variety of diseases, including the syndrome described by Professor Jan Waldenstrom of Malmö, Sweden, who visited the Institute during February, and the Bing-Neel syndrome. Correlative studies by Dr. T. F. Dutcher of lesions among those patients with cancer and macroglobulinemia have revealed characteristic lymphocytoid plasma cells with intranuclear inclusions. Drs. Dutcher and Fahey suggest that the intranuclear periodic-acid-Schiff positive material and intranuclear vacuoles seen on phase contrast microscopy are chemically identical to the circulating 18S, hexose-rich, γ-macroglobulin. Sequential cellular changes among the lymphocytoid plasma cells suggest those cells as the site of macrogobulin formation. The investigators regard macroglobulinemia as a neoplasm of reticuloendothelial origin, a variant of multiple myeloma, or a type of lymphoma.

Blood viscosity may be markedly elevated in macroglobulinemia and was the immediate cause of cardiac failure in one of Dr. Fahey's patients. He has designed a therapeutic regimen for maintaining the blood viscosity below the critical level.

DISSEMINATION OF CANCER

The ability of cancerous cells to invade surrounding tissues and colonize distant parts of the body through access to vascular channels is the most important factor limiting the effectiveness of surgical or radiological therapeusis. While the routes of dissemination are reasonably well understood, the basic biological facts responsible for metastasis are obscure, especially since metastasizability may be an attribute acquired after the transformation to the cancerous state has occurred.

Dr. Shelton is studying experimental lymphoid neoplasms which, on transplantation, remain localized or disseminate rapidly. Difference in the capacities of these neoplasms to penetrate the walls of diffusion chambers has been described in earlier reports. Normal cells of the peritoneum and invasive lymphomas L1210 or L2 grow independently of one another when placed in diffusion chambers. The growth of such non-invasive lymphoma cells as L1 and P353, however, tends to be associated with growth of the peritoneal cells, since the tumor cells grow in definite clumps, surrounded and enclosed by normal cells. Fibroblasts from subcutaneous fat pads of the mouse grow luxuriantly in diffusion chambers, and Dr. Shelton has introduced neoplastic cells into the fibroplast population. Again, cells from L1210 grow as free-floating independent entities. Growth of L1 cells is intimately related to proliferating fibroblasts, and these tumor cells never grow beyond the advancing edge of the fibroblast colony.

Study of circulating tumor cells in cancer patients proceeds as a joint effort of the Surgery Branch, the Laboratory of Pathology, and the PHS Hospital in Baltimore, led by Drs. R. R. Smith, R. A. Malmgren, and J. F. Potter. Cancer cells are found more often in the efferent blood from a malignant neoplasm than in blood taken from the antecubital vein. This was to have been expected and could relate almost solely to dilution effects, though the filtering action of the liver, lungs, and other organs cannot be excluded at this time. Patients whose cancers are apparently amenable to definitive treatment have tumor cells in peripheral blood about half as often as do those whose disease is recognizably disseminated.

Circulating tumor cells have been found in peripheral venous blood in about 75 percent of patients with malignant melanoma. The cells are seen sporadically in serial specimens even though the melanoma is widespread. Only two of six patients whose melanomas were surgically resectable had recognizable cells in the peripheral blood preoperatively. Both of them rapidly developed recurrent disease and died.

The significance of circulating tumor cells will become apparent only after a long comprehensive study of specific anatomical types of cancer in individual patients. Correlated animal studies are in progress, but the same methods by which one prepares specimens of human blood cannot be applied to mouse blood for some obscure reason, and, of course, the small size of most laboratory animals that develop cancer in a reasonably predictable fashion or will support the growth of transplanted neoplasms militates against the study of serial specimens from the same individual.

Other studies of metastasis in experimental animals have been especially rewarding. Housing tumor-bearing mice individually has produced a marked reduction in the variability of results as far as metastases studies are concerned. The ability to identify and count minute deposits of nonpigmented tumor cells in the lungs has been greatly improved by Miss Hilda Wexler, who introduces a solution of india ink into the trachea, thereby increasing the contrast between normal and cancerous tissue.

A much larger experience in the study of pulmonary metastases produced from transplanted tumors growing in the thighs of genetically appropriate mice points to a uniform type of behavior among the neoplasms investigated. Dr. A. S. Ketcham reports definite reduction in the number of metastases when the tumor-bearing extremity is amputated as compared with control mice whose tumors are either undisturbed or partially excised. The mean size of the pulmonary metastases in unit time is greater among the mice with amputated legs than among controls. The previous divergent results obtained by Dr. W. E. Schatten seem to relate to experimental design.

Transplantable mouse melanoma S–91 has been a most useful tool in these studies. Several different specimens of the tumor have been obtained from Dr. M. W. Woods who propagates the neoplasm by trocar transplantation, and pulmonary metastases seem to have been rather infrequent. Dr. Ketcham and associates find it necessary to transplant cytosieved preparations of S–91 melanoma through 7 to 9 serial passages before a reproducible pattern of metastasis develops which can be quantified. This tantalizing experience may be extremely important since it suggests that some factor of selection may be involved in the production of metastasizing melanomas and that only some of the cellular population of this particular neoplasm are capable of metastasizing. This phase of the program will be extended.

Equally fascinating is an observation on factors involved in the localization of metastases as reported by Dr. D. L. Kinsey. Last year's report referred to the predilection of the lungs for cells of melanoma S–91 introduced into either arteries or veins despite the ability of the same cells to grow when introduced directly into various organs and tissues. (Metastases can be produced in the liver when large, approximately lethal, doses of S–91 cells are introduced into tributaries of the portal vein.) Dr. Kinsey transplanted pieces of lungs to the thighs of genetically suitable mice and observed growth of melanoma cells in both the ectopic and normal pulmonary tissue when the tumor cells were injected into the blood stream. Transplants of other organs and tissues to the thigh would not support growth of intravascularly injected cells of melanoma S–91. This seems to be the first experimental confirmation of the thesis ascribing certain bizarre patterns of metastasis to a *locus minoris resistentiae*. In this particular example the basic mechanism may well relate to the known capacity of the lungs to filter cells from the circulating blood.

Dr. A. H. Harris' attempts to standardize the growth of a transplantable hamster melanoma provided by Dr. J. H. Edgcomb have not been successful.

Experiments concerned with chemotherapeutic prevention of metastasis formation or treatment of artificially produced pulmonary metastases have produced no significant new findings. On the other hand, Dr. R. C. Hoye finds that treatment of three out of four different experimental mouse tumors with 715r X-irradiation reduces the incidence of pulmonary metastases by 70 percent and a dose of 2,000r effects 81–100 percent reduction.

POLYSACCHARIDE RESEARCH

A long-term interest in polysaccharides at the National Cancer Institute has stemmed from the observation by Dr. M. J. Shear and his colleagues that this class of macromolecule can damage both clinical and experimental neoplasms. Certain problems arising repeatedly over the years required some expansion of this research area during 1956, designed especially to broaden its scope, especially at a fundamental level. Substantial information has been obtained regarding many important aspects of lipopolysaccharides.

Bacterial endotoxins are important members of this chemical species. During the year, Dr. Maurice Landy and Dr. Edgar Ribi (NIAID) have reduced the lipid content of lipopolysaccharides from 19.2 to 1.2 percent without reduction in their toxicity and without detectable effect on endotoxic properties. Dr. Woods reports that bacterial endotoxins in concentrations of 0.003 to 0.3 p.p.m. stimulate aerobic glycolysis of cells from melanoma S–91, and correlates the degree of stimulation with the degree of activity *in vivo*, as determined by Dr. Landy.

The endotoxin detoxifying component (EDC) discovered by Dr. Landy, which inactivates polysaccharides with respect to tumor necrosis and stimulation of specific antibody formation, also acts in the same way against killed *S. typhosa* but not against the viable organisms. The latter can be inactivated by sera from several species by a different bactericidal system. Normal antibody, all four recognized components of complement, and divalent cations are required for the bactericidal systems. The concomitant elimination of endotoxic attributes of typhoid bacilli in the bactericidal process of normal serum has obvious important implications for the host-parasite relationship.

Plasma of febrile rabbits that have been treated with bacterial lipopolysaccharides has been reported to contain "endogenous pyrogen," a substance with fever-producing properties quite different from those of the polysaccharide injected originally. Since reduction in the pyrogenicity of endotoxin following incubation *in vitro* with normal human serum was reported years ago, Dr. Landy undertook a study to compare the properties of "endogenous pyrogen" with endotoxins incubated with serum. The very properties used to distinguish "endogenous pyrogen" from polysaccharide are those which appear after incubation of the macromolecules in serum with respect to:

1. Fever production in endotoxin-tolerant animals.

2. Dissociation of leukopenia from the febrile response.

3. Loss of tolerance to daily injections of altered endotoxins.

4. Failure to develop acute refractoriness to multiple injections given over a short period of time.

Strains of *E. coli* vary in their susceptibility to the bactericidal system in serum. Study of two widely divergent strains by Dr. Landy and Dr. J. G. Michael (Visiting Scientist) reveals that the concentration of polysaccharide is inversely related to susceptibility of the organism to the bactericidal system. Cells and extracts of the resistant strain exhibit greater endotoxic potency as measured by ability to necrose sarcoma S37. Normal human and rabbit sera contain low concentrations of natural antibodies specific for bacteria of several genera of the Enterobacteriaceae. Their bactericidal action is not increased for *E. coli* by addition of a specific immune antibody.

These observations have led Dr. Landy to search for substances in normal serum which affect the viability of tumor cells. Incubation of cells from sarcoma S37 with normal human serum abolishes their capacity to grow in mice. Metabolic activity, as measured in Warburg flasks by Dr. Woods, is lost. The cells no longer reduce neotetrazolium. They stain readily with eosin, and blebbing of the cell membranes occurs. In short, they are dead. The effect of the tumoricidal action can be eliminated by incubation of 52° C. for 30 minutes or by adsorption with washed sarcoma cells at 4° C. Original activity is restored by combining the heat-inactivated serum with the adsorbed one, indicating that two factors are concerned with the tumoricidal activity. All four components of complement and divalent cations are needed. Tumoricidal effect is undiminished at dilutions of 1 : 8 but is decreased at 1 : 16. One milliliter of serum will destroy 30 million cells in two minutes at 37° C. Although this factor is widespread in nature, it is not detectable in the sera of all species, nor has it any noticeable effect when studied *in vivo*. Cells of several different mouse tumors are killed by the tumoricidal factor, but the only normal cells which exhibit similar sensitivity are reticuloendothelial.

A cytolytic factor in human serum has been described by Dr. B. Björklund (State Bacteriological Laboratory, Stockholm, Sweden). The agent is normally coupled with an inhibitor from which it can be dissociated by dialysis and obtained in a reasonably pure state by electrophoresis. The isolated factor lyses four established strains of human cells *in vitro*. Dr. Björklund visited the National Cancer Institute late in the year to compare his agent, which also produces marked changes in a variety of experimental ascites tumors, with the agent described by Dr. Landy. They appear to be quite different.

The chemical component of the polysaccharide research program is led by Dr. P. T. Mora. Polyglucoses and their derivatives are being used to study fundamental aspects of enzyme-substrate interactions (*vide infra*) and are in great demand by extramural scientists. The majority of the derivatives are negatively charged, but during the year Dr. Mora and Mr. J. W. Wood have prepared positively charged synthetic polyglucose derivatives to study the inhibition of biologically active anionic macromolecules. The synthetic method of preparing polysaccharides by chemical polycondensation procedures is being extended to numerous other sugars. High molecular weight polymers of 2-deoxyglucose, ribose, rhamnose, galactose, maltose, mannose, arabinose, *etc.*, have been obtained in good yield.

A new, large-scale polysaccharide preparation designated P–45, made by Mr. Adrian Perrault from *S. marcescens*, resembles the earlier P–25 material obtained from the same organisms. Dr. Ezio Merler (Visiting Scientist) finds P–45 electrophoretically homogeneous, although two components with different sedimentation coefficients appear in the ultracentrifuge. The heavier component seems to be an aggregate of the lighter one. P–45 is a strongly negative macromolecule. Titration of negative groups, in collaboration with Dr. H. A. Saroff (NIAMD), reveals a pKa of about 2.7, most likely due to carboxyl groups of the neuraminic acid type. The great bulk of the polysaccharide is a polymer of glucose containing 0.3 percent phosphorus in some organically bound form and an extremely small amount of amino acids.

The ability to bind certain molecules to synthetic substituted polyglucoses by electrostatic forces and to release the bound molecule by adding a second macromolecule with a stronger charge seems to apply to many different enzymes. For example, the most important factor in inhibiting ribonuclease activity with the macromolecules of

interest is the charge density as measured by degree of sulfation. The maximum sulfation is 3 per anhydroglucose unit which produces more than 1,000 times the inhibition possible with heparin, the best agent previously described possessing similar activity. Charge density seems to be the most important factor in enzyme inhibition also with carboxyl derivatives of polyglucose. The molecular weight of the inhibiting substance must be at least 2,000, but the internal configuration of the molecule appears to be less important.

Other physicochemical studies carried out by Dr. Mora have provided further information on enzyme-substrate interaction involving two oppositely charged macromolecules. Titration of polycations with polyanions follows stoichiometric equivalence. Such interactions differ from those involving low molecular weight ions, since a firm electrostatic complex forms under certain conditions, leading to precipitation of the macromolecular components. The precipitation phenomenon can be used to fractionate macromolecular substances and the principle has been used in concentrating EDC activity from human serum by Dr. Mora and Mr. B. G. Young.

Administration of polymyxin, neomycin, streptomycin, or protamine to mice in doses which cause death within 25 to 40 minutes can be counteracted by a neutralizing equivalent of anionic synthetic polysaccharide derivative administered by the same intraperitoneal route if given 10 minutes after the toxic dose of the cationic antibiotics as reported by Dr. Mora, Mr. Young, and Dr. Shear. Prior subcutaneous injection of the antidote prevents death from large intraperitoneal doses of cationic antibiotics. Again, the most highly charged polyglucose derivatives are the most effective antidotes in this system. The same type of approach has been used to inhibit the activity of T2 phage.

These studies of macromolecular interactions which block important biological activities are extremely important not only in relation to the nature and mechanism of nonspecific resistance as well as immunologic factors concerned with cancer, but also in providing a new method for separating biologically active compounds.

Inactivation of a polysaccharide by electron bombardment has been studied by Drs. J. W. Preiss and Morris Belkin. Progressive inactivation occurs with increasing dose as measured by decreasing ability of the molecule to produce vacuolation in tumor cells growing *in vitro*. Inactivation of this polysaccharide takes 16 times the dose required to attenuate the sucrose-hydrolyzing activity of invertase. Since the densities of these molecules are similar, the molecular weight of the active site of the polysaccharide is of the order of 7,500.

PROTEIN AND AMINO-ACID CHEMISTRY

The chromatographic separation of soluble proteins as initiated by Drs. H. A. Sober and E. A. Peterson has had a profound effect on the development of biochemistry throughout the world. Isolation of antibodies from serum is readily accomplished by a simple, rapid procedure, described by Dr. Sober, Dr. Peterson, and Dr. H. B. Levy (NIAID), which recovers about 90 percent of the gamma globulins free of macroglobulins. The macroglobulins can be obtained by zone electrophoresis of the remaining proteins.

Chromatography is also being used in collaboration with Dr. B. L. Vallee (Peter Bent Brigham Hospital, Boston), to isolate metal-containing proteins. The same metal may occur in distinctly different fractions.

Drs. Peterson and Sober can produce extensively resolved and reproducible chromatograms of serum or plasma in 6 hours. Limitation of flow rate is hydrodynamic, but resolution is not greatly affected by the highest flow rates permitted by the mechanical properties of the adsorbents used. Buffer exchange on Sephadex provides some advantages over dialysis as a means of equilibrating protein and buffer required at the start of a chromatographic experiment. Buffer exchange permits salt displacement chromatography which increases adsorptive capacity of the column and permits new approaches to stepwise displacement to overcome objections inherent in ordinary stepwise elution.

The principal problem created by protein chromatography is the considerable variety of physicochemically distinct entities with the same or similar biological or immunological properties (see Annual Report 1958). Drs. Peterson and Sober report an increase in the heterogeneity of serum proteins when treated with 8 M urea as

measured by electrophoretic analysis of the chromatographically separated peaks. The urea treatment, however, does not alter the chromatographic elution profile significantly. Further study of complexing of human serum mercaptalbumin with lipids reveals a markedly increased affinity of the protein for DEAE-cellulose. Addition of a cationic lipid, however, can abolish affinity of the protein complex for this adsorbent but increases its adsorption to CM-cellulose, indicating that the lipid-protein complex is highly basic.

Treatment of alkaline solutions of an amino acid with an aldehyde in the presence of copper ions as described by Drs. T. T. Otani, Nobuo Izumiya (Visiting Scientist), S. M. Birnbaum, and Milton Winitz proceeds with the formation of the corresponding α-amino-β-hydroxy acid. The fundamental reaction has permitted single-step preparation of:

1. β-hydroxy-β-methylaspartic acid from pyruvic acid and glycine.
2. β-hydroxyaspartic acid from glyoxylic acid and glycine.
3. β-hydroxyleucine from isobutyraldehyde and glycine.
4. Serine from formaldehyde and glycine.

Evidently the method is a general one providing a simple and economical route to the production of α-amino-β-hydroxy amino acids useful in biochemical research.

Dr. Marco Rabinovitz, interested in the inhibition of protein synthesis by amino acid analogs, has studied several in which the methylene group in the side chain has been replaced by sulfur. Methionine sulfoxamine reduces by 50 percent the rate of protein synthesis in washed Ehrlich ascites cells through prevention of glutamine synthesis. The inhibition of protein synthesis by the mixed disulfide of methylmercaptan and cysteine in low concentrations is prevented by glutathione, but not by methionine. Dr. Rabinovitz reports interference of incorporation *in vivo* of isotopically labeled lysine into the tumors and livers but not the spleens of cancerous mice by administration of the lysine antagonist 4-thialysine.

Drs. K. Michi (Visiting Scientist), Birnbaum, and Winitz have separated diastereomeric mixtures of amino acids such as isoleucine-alloisoleucine, hydroxypropoline-allohydroxyproline, and threonine-allothreonine by chromatography on Amberlite columns in amounts as great as 20 grams. Recovery is 90–95 percent. The program initiated by the late Dr. Greenstein for the production of optically active, pure amino acids in adequate amount and at reasonable cost to provide powerful tools for research in the chemistry and metabolism of amino acids and proteins has been completely successful.

Drs. Winitz and Birnbaum, with Mr. M. C. Otey and Drs. T. Sugimura and V. Mitbander (Visiting Scientists), have commenced to use chemically defined diets to study specific enzymes and metabolic processes in the intact rat. Earlier workers having shown that the *D*-isomers of the essential amino acids methionine, tryptophan, and leucine can replace, in whole or in part, *L*-isomers of the same compounds in rats, the subject was restudied with the chemically defined rations. Methionine was the only one of the group in which weight increment in weanling rats was equally good on *D* and *L* isomers. *D*-tryptophan was only partially effective in replacing its *L* antipode, and 1.4 times the amount of *D*-leucine was required before any growth occurred. A series of diets was designed to discriminate between the relative capacities of *D*-amino acid oxidase to convert the *D*-amino acids to corresponding α-keto acids and of transaminases to accomplish the transformation of α-keto acids to corresponding *L*-amino acids. All possible combinations of the isomers of alanine and methionine were employed, but only the diet using *D* forms of both amino acids produced marked retardation in weight increment.

Other investigators had shown sodium benzoate to be a potent inhibitor of *D*-amino acid oxidase activity *in vitro*. Synthetic diets containing 2 percent sodium benzoate and *D*-methionine produced strikingly diminished rates of weight gain, according to Dr. Winitz and associates. These data point to oxidative deamination as rate limiting, and studies on the effects of the α-keto analog of methionine are in progress. One additional point of interest is the sparing effect of dietary glycine on the inhibition of *D*-amino acid oxidase by sodium benzoate through its removal as hippuric acid.

A long study designed to produce a chemically defined diet suitable for human use has been com-

556044—60——3

pleted as a joint effort of the Laboratory of Biochemistry and the Surgery and General Medicine Branches, with Dr. R. B. Couch serving as the coordinator. The earlier diets were semisynthetic and contained racemic mixtures of amino acids as the only source of nitrogen, and the general composition conformed to recommendations published by the Food and Nutrition Board of the National Research Council insofar as they were available. Substantial progress was made, however, from the experience obtained by nutritional studies in rats by Drs. Greenstein, Winitz, Birnbaum, and Mr. Otey. The source of carbohydrate gave considerable difficulty because excessive sweetness produced nausea. Dextrose finally proved to be the best carbohydrate and, in fact, it was found advisable to add a flavoring material if a high dextrose diet is to be taken by mouth. The problem of frequent small watery stools with any of the soluble diets was solved by addition of carboxymethylcellulose. The best diet, which contains *L* rather than *D*-amino acids, can be stored in dry form and dissolved as needed; fat-soluble vitamins and ethyl lineolate mixed with polysorbate 80 to form a reasonably stable emulsion are added and mixed.

Seven patients have received 12 separate trials on the chemically defined diets, five by indwelling esophagostomy tubes, and two orally. After it had become possible to feed a diet for 7 to 12 days without weight loss in an asymptomatic individual whose weight had been stable for some time, the critically important experiments were done by Drs. D. M. Watkin and L. E. Rosenberg on two patients under complete metabolic control. These studies revealed a need for approximately 25 percent increase in caloric intake over that provided by an isonitrogenous diet of natural foodstuffs in order to maintain weight and permit nitrogen retention after the period of nitrogen loss induced by a nitrogen-free diet. The experience in patients coincides with the findings of Dr. Birnbaum that the best chemically defined diet is inferior to diets made of whole foods and supports the contention of Rose that diets containing only pure amino acids as the source of nitrogen require a greater caloric intake for efficiency comparable to isonitrogenous diets of whole foods.

NUCLEIC ACID RESEARCH

Complex interrelationships among the purines, pyrimidines, and chemical intermediates involved in the production of polynucleotides are slowly being defined, and the contributions by scientists of the National Cancer Institute are steadily increasing. Drs. W. C. Schneider and Jean Rotherham, continuing their studies of deoxyribosidic compounds, have defined factors concerned with urinary excretion of these substances. Deoxycytidine is the major deoxyribosidic compound found in the urine of rats; deoxyuridine and an unidentified compound are also present. These materials are not derived from dietary sources, since neither fasting nor the use of chemically defined diets, through the cooperation of Dr. Winitz, has much influence on the level of excretion. The germ-free state also has no pronounced effect; hence, bacterial synthesis in the gastrointestinal tract cannot be a major factor in their production. Excretion of urinary deoxyribosidic compounds usually declines for 2 or 3 days following partial hepatectomy and also decreases during the most rapid growth phase of the Novikoff hepatoma. These two responses, however, are not entirely constant.

Incubation of hepatoma cells with isotopically labeled deoxycytidine results in progressive labeling of deoxyribonucleic acid. Dr. Schneider also finds an amount of radioactivity in the nucleotide fraction sufficient to suggest the presence of 14 or more deoxynucleotides of which some contain deoxycytidine. Administration of labeled deoxycytidine to the rat results in uniform distribution of C^{14} throughout the body; the amount of radioactivity in any given tissue is so low as to discourage attempts at isolation of the labeled compounds. Dr. J. H. Weisburger has collaborated with Dr. Schneider in the synthesis of phosphoethanolamine-1,2-C^{14}, and synthesis of the phosphocholine analog is in progress. Deoxycytidine, containing analogs of these compounds, was isolated from the Novikoff hepatoma, and a study of their function seems desirable.

Dr. R. K. Kielley has encountered difficult technical problems in her studies of enzymes involved in synthesis of deoxyribonucleic acids by normal and neoplastic cells. Older techniques for isolation of nuclei have required substantial modifica-

tion for use with certain hepatoma cells, and separation of diesterase and nucleotidase activities in snake venom has been accomplished.

Reports from several sources of major quantitative changes in urinary excretion of purines and pyrimidines during chemotherapeutic treatment of acute leukemia encouraged a collaborative program, led by Dr. J. C. Reid of the Laboratory of Physiology with colleagues in the General Medicine Branch, to isolate and identify purines, pyrimidines, and intermediates presumably associated with nucleic acid synthesis and/or degradation. Chromatographic procedures have been used extensively by Dr. Reid in devising analytical methods for detection and quantification of nucleotides, nucleosides, purines, and pyrimidines in human urine. Most difficulties have been obviated during the past two years, but xanthosine recovery is still low and the ammonium acetate gradient used for elution of the major nucleoside fraction does not yet give good reproducibility. Quantification of all individual components at the present time is an arduous and complicated task that would seriously limit the usefulness of the method for study of disease. Dr. A. W. Pratt is working with Dr. Reid to automate the process to the fullest possible extent. One approach now being pursued is a computational analysis of multicomponent spectra applying several mathematical techniques made practical by the digital computer.

Methods for isolation of nucleic acids and the determination of their chemical and physical properties are improving. These large molecules are sometimes rather fragile, as are proteins, and comparatively small insults during isolation may denature the molecule. Dr. Joseph Shack finds the binding of deoxyribonucleic acids by magnesium ions essentially independent of pH in the range where no titration of nucleate occurs. The quantity of magnesium bound is proportional to the concentration of deoxyribonucleic acid at a given concentration of magnesium ions.

Evidence presented last year by Dr. Shack and Dr. Kilham (DBS) suggested that the transforming agent prepared from rabbit myxoma virus contained deoxyribonucleic acid. Attempts to isolate an active deproteinized nucleic acid by the phenol procedures have been unsuccessful. The transforming factor prepared from myxoma virus by heat alone is active in such various tissue cultures as squirrel and monkey kidney, rat embryo, and cells from the kidneys of either cottontail or domestic rabbits. It can enter the cell in the absence of fibroma virus where its activity can be detected for as long as 3 days. Transforming factor prepared by a procedure using both heat and urea is active only in tissue cultures of rabbit kidney, the natural host of both fibroma and myxoma viruses, but this preparation is destroyed in the cell within three hours of its entry.

Fractionation of the Novikoff hepatoma by Dr. E. L. Kuff using gentler physical procedures in conjunction with electron microscope studies by Dr. R. F. Zeigel has permitted isolation of free nucleoprotein particles representing 50 percent of the total ribonucleoprotein in that neoplasm. The spherical particles occur in several size groups, the most abundant of which measure 24 mμ in diameter and have a molecular weight of 4.5 million. Addition of adenosinetriphosphate in concentrations comparable to those found in tissues causes dissocation of the particles into smaller subunits. Adenosinediphosphate is less effective and treatment with deoxycholate, the detergent usually used for isolating ribonucleoprotein particles, removes one-third of the protein without changing the spherical form. Dr. Kuff suggests that the physical form of ribonucleoprotein may relate to the metabolic state of the cell.

Dr. M. E. Maver has previously described the hydrolysis of cyclic adenylic acid to 2′ adenylic acid by a ribonuclease preparation obtained from spleen. This type of activity has been separated by chromatography from acid ribonuclease activity. The ribonuclease activities of rat lymphosarcoma closely resemble those of calf spleen. The acid ribonucleases of rat liver and hepatoma hydrolyze only cyclic pyrimidine nucleotides to give the 3′ nucleotides. The corresponding acid ribonucleases obtained from spleen or lymphosarcoma hydrolyze both purine and pyrimidine cyclic nucleotides to yield 2′ derivatives before purification by chromatography.

Dr. Maver also describes some differences in deoxyribonucleases isolated by the same procedure from different tissues. Those obtained from calf spleen and liver have a sharp pH optimum at 4.8, whereas the deoxyribonuclease from rat lymphosarcoma acts equally well from pH 4 to pH 5.8.

Trypsin destroys a substance obtained from human leucocytes which inhibits the activity of deoxyribonuclease I but does not affect the enzyme. Destructive action of trypsin is prevented by soybean trypsin inhibitor which does not prevent destruction of the inhibitor by a factor present in normal human serum. These findings have provided Dr. Shack a basis for assaying the activity of both deoxyribonuclease I and the inhibitor and to show that normal human urine contains large quantities of the enzyme but no soluble inhibitor.

Dr. E. P. Anderson, working with Dr. L. A. Heppel (NIAMD), has studied a new phosphodiesterase, which hydrolyzes certain 5′-phosphate-ended ribopolynucleotides to mononucleoside-5′-phosphates. Individual nucleotides are released serially from the end of the polymer. The enzyme is active against ribo-oligonucleotides but not against simple esters of nucleoside-5′-phosphates, because of a need for a nucleoside moiety on each side of the susceptible phosphodiester bond. Enzymes with similar hydrolytic activity are found in tumors.

Drs. Sober and Peterson and Dr. Matthias Staehelin (Visiting Scientist) report that oligonucleotides prepared by digestion of ribonucleic acids with pancreatic ribonuclease can be fractionated on cellulose anion adsorbents using volatile salts for elution. Fractionation is based primarily on differences in size of the molecules, but the order of emergence from the column also relates to basic composition and sequence. Whereas other methods permit separation of tetra- or pentanucleotides, column chromatography permits fractionation of decanucleotides and even larger molecules, encouraging further study of specificity of interactions of nucleic acids and enzymes.

ENDOCRINOLOGY

Extensive metabolic studies conducted by Drs. D. M. Bergenstal and M. B. Lipsett with hypophyseal growth hormone from beef, whale, and sheep treated with chymotrypsin have revealed no characteristic activity in man. Purified and untreated growth hormone from these species is similarly inactive. Human growth hormone itself is a potent anabolic agent and increases the daily excretion of urinary calcium from 20 mg. to 150 mg. daily.

Administration of growth hormone to three patients with acromegaly, reported by Drs. Bergenstal and Lipsett, caused no retention of nitrogen, though the excretion of calcium in the urine increased. Testosterone did produce an anabolic effect in the same patients; hence, different mechanisms must be concerned with nitrogen retention on administration of growth hormone and testosterone, respectively.

Drs. Bergenstal and Lipsett describe significant catabolic effects of small doses of cortisol and triiodothyronine in patients with panhypopituitarism. They emphasize the necessity for defining physiological effects of hormones in relation to endocrine status of the patients treated.

A study of the initial stages of stimulation of the adrenal cortex with ACTH has been completed by Dr. Lipsett and Dr. H. N. Wilson (NIAMD). Biosynthesis of adrenal androgens depends less on ACTH than does the biosynthesis of cortisol. Study of the precursors of dehydroepiandrosterone by these scientists has encountered difficulty because of problems involved in identifying a metabolite.

Dr. Lipsett has completed a study of hirsutism in women without establishing any correlation of extent or severity of hirsutism with the excretion of $C_{19}O_2$ or $C_{19}O_3$ metabolites or of pregnanetriol.

The availability of progestational compounds free of estrogenic activity which act on oral administration has permitted examination of the effects of large doses of progestin by Drs. Bergenstal and Lipsett. Provera (6-methyl 17-acetoxyprogesterone), in contrast to progesterone, has caused negative nitrogen balance only once in seven trials. Provera has no antialdosterone activity, nor does it suppress gonadotropin excretion in postmenopausal patients or in girls with precocious puberty. Administration of this progestogen causes large increases in the excretion of Porter-Silber chromogens; hence, the urinary metabolites were partially characterized. Known functional groups concerned with the Porter-Silber reaction have been ruled out as a source of the increased chromogen excretion. The findings suggest a previously unrecognized phenylhydrazine-reacting group among metabolites of Provera.

Optimal progestational response of the endometrium usually requires "priming" with estro-

gen. Hisaw, *et al.*, however, report that large doses of progesterone alone can induce proliferation in the rabbit endometrium. This conceivably could be related to production of estrogenic substance by the adrenal cortex. Dr. W. W. Tullner has observed this progestational response in adrenalectomized rabbits, thus eliminating the necessity of an adrenal factor.

Dr. Hertz finds that prepubertal growth and differentiation of the ovary proceed independently of pituitary stimuli, because growth and differentiation progress normally when ovarian grafts are transplanted to hypophysectomized rats of either sex. Extensive destruction of the hypothalamus fails to influence the process. Ectopic pituitary glands continue to produce ACTH, growth hormone, and gonadotropin.

Clinical experience in inhibiting the activity of the adrenal cortex with amphenone or with o,p′-DDD has pointed to the need for better drugs with similar primary effects but fewer undesirable reactions. Administration of o,p′-DDD to dogs not only suppresses excretion of adrenal hormones or their urinary metabolites but will actually destroy the adrenal cortex in 90 days. While Dr. Tullner has not observed evidence of regeneration of the cortical tissue, the period of observation has not yet been adequately long to be decisive. Despite this drastic effect in the dog, and evidence for comparable qualitative effects in some human situations, o,p′-DDD does not influence adrenal cortical activity in either the rat or the monkey.

Studies of the pharmacology of o,p′-DDD have been hampered by its incomplete absorption on oral administration, so Dr. Tullner and Dr. Hertz have developed a suitable vehicle for intravenous injection of this fat-soluble compound. Secretion of cortical 17-hydroxysteroids decreases markedly within 48 hours with concomitant profound reduction in secretion of cortisone and aldosterone equivalent to the depression produced by hypophysectomy.

Dr. Tullner is now studying a number of analogs of DDD. Difluorodinitrodiphenyldichloroethane inhibits production of 17-hydroxycorticosteroids. Diaminodiphenylmethane has an action similar to amphenone but produces no anesthetic effects and has pressor activity. This indicates that the methylketone structure is not essential to an antiadrenal effect, but the compound is too toxic to warrant further investigation.

Transplantable rat adrenal carcinoma 494 is being studied by Drs. H. L. Stewart and K. C. Snell. Biochemical studies of this neoplasm by Dr. D. F. Johnson (NIAMD) reveal an excess of steroid hormones similar to those found in the adrenal cortex. A substrain, 494H, derived by passage in hypophysectomized rats, is morphologically distinct, and its characteristic histology is maintained on transplantation to intact rats. It produces polyuria, polydypsia, degeneration of renal tubules, and causes hyperplasia of the mammary glands, which become filled with milky secretion in both sexes. The uterus enlarges and the vaginal epithelium is mucified in female rats; the testes and secondary sex organs of the male atrophy. Growth of this tumor in hypophysectomized rats, however, induces development of the secondary sex organs but not of the gonads.

Thyroid neoplasms in rats and mice represent a wide variety of structural and biological types. Dr. H. P. Morris also finds their behavior inconstant with respect to changes in morphology or growth rate when stimulated by administration of thyroid hormone or pituitary thyrotropin.

Dr. S. H. Wollman and Dr. Leonard Warren (NIAMD) found sialic acid, a 9′-carbon amino sugar, in normal thyroid gland. A specimen of pure thyroglobulin, provided through the courtesy of Dr. Harold Edelhoch (NIAID), contained one percent sialic acid. Hence, a broader study of sialic acid in relation to thyroid function has been initiated. The highest concentration is found in the thyroid glands of hypophysectomized rats, the lowest in glands from rats fed thiouracil; thyroids from euthyroid rats have concentrations intermediate between those two extremes. Administration of thiouracil reduces sialic acid concentrations progressively, with a minimum being reached at about 9 days. This may be due to the known resorption of thyroglobulin caused by thiouracil. Since some hold that thyroglobulin must be changed by enzymes before thyroxin is liberated, Drs. Wollman and Warren have measured the concentration of free sialic acid which should be liberated by the same proteolytic process and find it to be highest at the time when concentration of total sialic acid is declining most

rapidly. Free sialic acid content is lower in thyroids from hypophysectomized than from normal rats.

These basic observations have been extended to a study of experimental thyroid tumors in rats by Dr. Wollman, and Drs. Warren and S. S. Spicer of NIAMD. Colloid in some of the neoplasms is stained by the periodic acid-Schiff (PAS) reaction. Homogenates of these tumors are viscous, suggesting the presence of mucins. Other thyroid tumors contain no PAS-positive material. Many of the tumors contain acidic mucins in which the acid group proves to be sialic acid rather than sulfate. Growth of one neoplasm in which sialic acid can be demonstrated intracellularly is associated with increased levels of sialic acid in blood serum and urine. Some of the more functionally active thyroid tumors contain neutral mucins, as does the thyroid. Autoradiographic studies of a tumor with neutral mucins reveal that all follicles form organic iodine compounds, whereas another functional tumor containing acid mucins in the colloid contained some follicles in which no radioiodine was detected. The relation of acid mucins to thyroid function is being clarified.

RADIATION BIOLOGY

From a precise and detailed study of the deleterious effects of visible light on a strain of haploid yeast cells, Dr. M. M. Elkind has turned to investigation of X-ray damage and recovery in mammalian cells in culture. Initial studies have utilized two strains of cells from the Chinese hamster, *Cricetulus griseus*, propagated in tissue cultures of the type originally designed by Puck. This permits the growth of individual cells as essentially clonal colonies whose morphological and biological properties can be compared. Experience to date includes detailed analysis of damage produced by a single dose or fractionated doses of X-rays generated at 55 KV. and delivered at the rate of 720 rads per minute.

These results are especially significant:

1. The vast majority of the surviving cells completely repair their accumulated damage before their first division post-irradiation.

2. The kinetics of recovery depend on the physiological state of the cells and/or can be caused to appear to undergo large oscillations depending on the recovery medium. These apparent oscillations may result from combined effects of changes in sensitivity and repair of inactivated sites.

3. Although there are important quantitative differences, log-phase cells respond similarly.

4. A cell can undergo repeated cycles of damage and repair with no apparent attenuation of the repair process.

As Dr. Elkind and Miss Harriet Sutton state in their paper (Nature 184: 1293–1295, October 24, 1959):

"There are several contexts in which these findings are of interest. If the chromosomes are the X-ray sensitive sites and chromosome breaks are the hits leading to lethality, then some new properties of restitution must be considered. First, restitution goes to completion in surviving cells. Secondly, the cell's ability to restitute breaks remains unimpaired after repeated doses. In view of the preceding, Puck's report of a high yield of mutant characteristics in the progeny of cells surviving five to seven mean lethal doses may be applicable to the material he was using; may be evidence of a radiation-induced chromosomal lability which is expressed after recovery and during clonal growth; may imply that mutation production and lethality are not, in general, closely connected; or may indicate that the chromosomes are not the primary sensitive sites related to viability.

"Another area in which these results may apply is in connection with tumour therapy. Treatment protocols involving fractionation are common, permitting in general, ample time between treatments for considerable if not complete recovery. . . . If recovery is not duly accounted for, the survival using fractionation can be higher than expected by several orders of magnitude. Of course, tissue recovery in a general sense has been recognized by radiation therapists for a long time. These results, however, provide a cellular basis for this phenomenon and lend specific direction to the research that should be undertaken both to take advantage of, as well as to control, this effect."

Similar experiments conducted by Dr. R. Z. Lockhart, Jr., and Dr. Elkind indicate that HeLa cells respond in a manner similar to those described for cultured cells of the Chinese hamster, but the HeLa line studied is not as stable.

Dr. R. E. Bases has exploited the availability of single-cell culture techniques to study the in-

fluence of actinomycin D on the response of HeLa cells to *in vitro* X-irradiation. He reports enhanced lethal effects of X-rays on single cells exposed to the drug just before or after irradiation or during clone formation. Cells are more sensitive to X-irradiation when 85 percent of the water in the medium is replaced by heavy water, and, indeed, the cells can not survive indefinitely in the heavy-water medium. Cells surviving prolonged exposure to heavy water are abnormally sensitive to subsequent irradiation.

A radioactive cobalt source is now being installed to replace machine-generated X-rays in the study of radiation chemistry by Dr. C. R. Maxwell. Use of gas chromatography permits isolation, concentration, and quantification of acetic acid resulting from irradiation of glycine. Like ammonia and formaldehyde, but unlike glyoxylic-acid formation, acetic-acid production depends on the concentration of glycine in the radiated solution as do most organic solutes. Examination by mass spectrography of acetic acid formed by irradiation of glycine in heavy water suggests that the mechanism of acetic acid proposed by Weeks and Garrison is only partially correct. Only one-third of the acetic acid contains deuterium. On the other hand, Dr. Maxwell has confirmed quantitatively the finding by Weeks and Garrison of substantial yields of aspartic and diaminosuccinic acids. One unknown compound resulting from the irradiation of glycine remains to be identified.

Information on the abstraction reactions of hydrogen atoms in the radiolysis of aqueous organic solutions is being obtained by Dr. Peter Riesz, who joined Dr. Maxwell during the year. When aqueous organic solutions are exposed to ionizing radiation, hydrogen is produced by two distinct processes. The primary molecular hydrogen yield arises from the recombination of H atoms in a series of small regions of high radical concentration and is independent of added solute under certain conditions. Additional hydrogen is formed from the reactions of H atoms which diffuse out of the small regions and react with the organic solute. Analogous processes occur when organic solutes dissolved in heavy water are irradiated, and the primary molecular yield of deuterium is known from studies such as those reported by Dainton.

Dr. Riesz has studied abstraction reactions of certain amines, amino acids, amides, peptides, and thiols. Deuterium atoms react with glycine, acetamide, and glycyl glycine at neutral pH by abstracting from carbon but not from nitrogen. In strongly basic solutions, an appreciable fraction of deuterium atoms abstract from the amino groups of glycine and n-butylamine. The rate of hydrogen abstraction for substances such as n-butylthiol and cysteine is higher from sulfur than from carbon. In neutral solutions abstraction reactions account for no more than one-half of the deuterium atoms produced by X-irradiation. Results for glycylglycine are particularly interesting in relation to radiation chemistry of proteins, since the reactions of H atoms with the peptide bond will not produce free radicals with the odd electron on the nitrogen of the peptide bond.

Interest in effects of total-body X-irradiation continues. Dr. Falconer Smith reports that recovery of immune responses in mice after two doses of X-ray given at varying intervals demonstrates relationships similar to those associated with lethality studies. Multiple exposures are cumulative in injuring the immune response even though no effect may be noted on the leucocyte count. The recovery of transplantation immunity of cells from DBA-strain mice in LAF_1 hybrids following sublethal irradiation and various treatments which accelerate hematopoietic recovery are also unrelated to recovery of the blood cells. Dr. W. W. Smith, Mrs. Joanne Hollcroft, and Prof. Jerome Cornfield (Johns Hopkins University) find that treatment with cells from immature mouse spleens hastens recovery; treatment with colchicine delays it.

Dr. W. W. Smith finds important differences in the protective effects against lethal total-body irradiation of bacterial endotoxin on the one hand, and colchicine and its derivatives on the other. Endotoxins are effective in protecting young mice and will protect mice five weeks old, though hematopoietic recovery is delayed. They also protect irradiated guinea pigs. The endotoxins produce their effects in guinea pigs only after the radiation has been given. Colchicine and the derivatives studied so far by Dr. Smith do not protect guinea pigs or very young mice but are active in mice five weeks old. They act when given prior

to total-body irradiation but not during the post-treatment period. The protective effects of both endotoxins and colchicine are associated with more rapid recovery of the bone marrow among the treated subjects.

"Secondary disease" is a condition occurring in mice at inconstant, long intervals after exposure to lethal doses of X-irradiation and treatment with protecting doses of nonisologous bone marrow. The disease, which is basically an immunological phenomenon, may be fatal. Miss Uphoff has previously associated incompatibility at gene locus H–2 as a major factor in production of secondary disease, and now reports histoincompatibility at loci H–1 and H–3 to be unimportant in the pathogenesis of the condition. Other factors influence the long-term survival of the lethally irradiated and protected mice. Miss Uphoff has investigated the efficacy of marrow from parent-strain mice in the protection of F_1 hybrids produced in seven different combinations. Early fetal marrow is effective in protecting irradiated hybrids in most of the combinations studied. Marrow obtained from neonatal mice produces less severe secondary disease than is obtained with adult marrow. When a gene difference is present at H–2, only 50 percent of the recipients of fetal marrow survive secondary disease.

NATURAL HISTORY OF LEUKEMIAS

Earlier annual reports have included a number of precise clinical and pathological studies on phenomena associated with the leukemic state, such as lesions of bone, spontaneous bleeding and its basis, intracranial lesions associated with hemorrhage, intercurrent infection, and the immunologic response to specific antigens. These have been achieved through the continuing studies of leukemia patients by clinicians and pathologists working together on common problems.

Drs. E. J. Freireich and E. Frei, III, have recognized a syndrome due to increased intracranial pressure in 25 of 150 patients, primarily children with acute lymphocytic leukemia. Eight of the twenty-five developed their first symptoms while in drug-induced remissions. Studies by Dr. L. B. Thomas and colleagues of the Pathologic Anatomy Branch associate the symptoms with an internal communicating hydrocephalus and extensive infiltration of the pia-arachnoid by leukemic cells which in extreme cases obliterates the subarachnoid space over the spinal cord and brain. This is not a completely new lesion, but review of the literature reveals a sharp increase in the occurrence of the syndrome since the advent of chemotherapy.

All this emphasizes the need for more intensive investigation of the blood-brain barrier, as approached by Drs. D. P. Rall and C. G. Zubrod. This physiological or biochemical barrier develops early in life since it is already present in puppies 5 to 20 hours old. Neither hypotension nor administration of cortisone increases the entry of drugs into the cerebrospinal fluid. Sulfanilic acid, a strong acid, fails to enter the cerebrospinal fluid in the dogfish at 96 hours in more than traces. Para-aminohippuric acid, a weak electrolyte of which only one part in 6,000 is dissociated at body pH, will achieve equilibrium in time by diffusion. Comparison of the ability of these two compounds to pass the blood-brain barrier further indicates that undissociated rather than dissociated drugs will penetrate into the cerebrospinal fluid and strengthens the lipoidal concept of the barrier.

Comparison by Drs. Rall and Zubrod of the entry of antipyrine, sulfadiazine, and paraaminohippurate into the cerebrospinal fluid of man and dog shows the same basic phenomena in both species. The human, however, approaches equilibrium at a slower rate. While methotrexate does not readily enter the cerebrospinal fluid, Dr. T. L. Loo has detected 6-mercaptopurine in cerebrospinal fluid of dogs receiving intravenous injections of the drug. The spectrophotometric identification of the compound has been confirmed by paper chromatography.

Dr. George Brecher (NIAMD) and Dr. L. R. Schroeder, Jr., are studying synthesis of deoxyribonucleic acids in human leukemic cells by an *in vitro* technique, utilizing tritiated thymidine and radioautography in such a way that no new cells enter the synthetic phase of the generation cycle and none divides during the period of study. They observe no correlation between deoxyribonucleic acid synthetic activity and the usual morphological criteria of cellular immaturity. Although a wide range of rates of synthesis is observed, they do not exceed the range exhibited

by leucocytes from patients with infectious mononucleosis.

The Rebuck skin-window technique is being employed by Dr. Frei in a study of experimental inflammation in patients with acute leukemia. Frequently these patients fail to mobilize polymorphonuclear leucocytes in response to injury. This abnormal response correlates well with increased susceptibility to infection and in some degree is related to the number of polymorphonuclear leucocytes in the circulating blood.

Drs. Frei and Fahey report frequent and progressively intense hypogammaglobulinemia in patients with chronic lymphocytic leukemia. This disease is accompanied by depression of the patient's formation of antibodies to specific antigenic stimulation. The failure of antibody production seems to relate more closely to increased susceptibility of infection than to the concentration of γ globulins in the serum. Dr. J. P. Utz (NIAID)) has collaborated most effectively in the immunological studies.

Dr. Frei continually reviews the infections occurring among leukemic patients. Pseudomonas sepsis and clostridial infections continue to be troublesome. A prospective study of moniliasis reveals thrush in 17 percent of 148 patients. Almost all of the episodes have commenced during the last 2 months of life and duration of the episode has been significantly diminished by a nystatin mouth wash. While antibiotic and corticosteroid therapy contributes to the leukemic patient's susceptibility to moniliasis, they are relatively less important than the severity of the leukemia. Disseminated cryptococcus infection with extensive hepatic involvement has been seen in three lymphoma patients.

Drs. D. M. Watkin and I. B. Weinstein report that the seromucoid fraction of human plasma binds vitamin B_{12}. Anion-exchange cellulose chromatography permits further separation of the seromucoid into three fractions. There is a marked increase in one of these fractions among patients with chronic myelocytic leukemia.

ANEMIA AND RELATED DISORDERS

A patient with cerebellar hemangioblastoma and polycythemia admitted during the year was studied by Dr. T. A. Waldmann, who described marked stimulation of erythropoiesis in the rat by a nondialyzable factor obtained from the contents of the cystic portion of the neoplasm. This appears to be the first demonstration of erythropoietinlike activity in any biologic material other than urine or plasma and supports the concept that polycythemia, not infrequently associated with cerebellar hemangioblastoma, may be caused by production of an erythropoietinlike factor by the neoplasm.

Dr. R. E. Greenfield, Jr., has extended his studies of erythrocyte fragility in relation to age of the red-blood-cell population. Erythrocytes more than 12 days old hemolyze at 0.4 percent sodium chloride while younger forms which hemolyze at a concentration of 0.2 percent can be further separated by exposure to gradients of salt content approaching 0.3 percent. The absolute values vary somewhat among species. Systematic study of red-blood-cell repletion in rats rendered anemic by repeated bleeding reveals fragility values for immature red-blood cells and reticulocytes of 0.1 percent, and 3-day-old erythrocytes of 0.2 percent, while hemolysis occurs at 0.3–0.4 percent concentration when the red blood cells are 12 days old.

Dr. Greenfield and Dr. V. E. Price have also shown the slow disappearance from the site of deposition of erythrocytes labeled with radioactive iron when injected into a transplantable tumor or into subcutaneous tissue. The results are comparable to those reported last year for studies of labeled erythrocytes injected into skeletal muscle.

Having previously described the loss of catalase activity by parenteral injection of 3-amino-1,2,4-triazole which, nevertheless, does not interfere with synthesis of the enzyme, Drs. V. E. Price and Miloslav Rechcigl (Research Fellow) report kinetic studies, showing that, although the rate of catalase destruction by aminotriazole is the same in both liver and kidney, the rate of enzyme synthesis is fourfold greater in the liver. Eventually an equilibrium is attained at which destruction balances replacement.

Dr. Waldmann is attempting to produce hypersplenism in dogs by intravenous infusion of methylcellulose. Moderate splenomegaly is obtained together with an anemia in which life span of the erythrocytes is reduced. The picture is complicated by severe renal damage and uremia. Ad-

556044—60——4

ministration of large doses of desiccated thyroid increases the dog's blood volume as much as 25 percent. Increased synthesis of erythrocytes can be demonstrated, but their life span remains within the normal range.

WEIGHT LOSS

An increased requirement for sodium ions was reported last year by Dr. J. White as characteristic of rats bearing progressively growing Walker carcinosarcoma 256. He, with Dr. F. K. Millar and Mrs. J. N. Toal, has studied sodium metabolism in normal and cancerous rats by balance techniques. The normal subject excretes sodium in relation to the amount provided in the diet, but the cancerous rat reduces its sodium excretion 15 to 20 days after the Walker tumor has been implanted even though the animal is storing nitrogen. Chloride and water follow the same pattern as sodium, but potassium metabolism is not affected. When the diet contains adequate amounts of salt, and this in considerable excess over the requirement of the normal rat, the cancerous subjects lose little weight despite their large tumors and the adrenal glands do not enlarge. The cachectic tumor-bearing rats whose sodium intake is restricted have marked enlargement of the adrenals affecting particularly the zona glomerulosa. In rats eating a diet in which lyophilized tumor forms the only source of protein, the excretion of allantoin is inversely related to the excretion of sodium, though such a diet is rich in salt and nucleic acids.

Dr. White and colleagues have also studied sodium excretion of rats bearing the Murphy-Sturm lymphoma. The diet with restricted salt content results in initial fall in sodium excretion as the neoplasm grows, but the level then rises and is maintained as in normal rats, and the adrenals do not enlarge. Some tumors may have so great an extracellular space that the ordinary diet sufficient for growth of the normal rat cannot fulfill the needs of a greatly expanded extracellular space. It seems quite likely that a need for additional building blocks to manufacture proteins or nucleic acids for a rapidly growing protoplasmic mass might have equally deleterious consequences on the host. This general subject will be explored further.

At any rate, the characteristic growth pattern of the Walker tumor and similar consequences have been described in modified form for rat lymphosarcoma R2788 and rat hepatoma 3683 by Drs. Rechcigl and Greenfield.

Dr. Pratt finds it possible to determine the gross body composition of cancerous rats *in vivo* through serial calculations of ratios of nitrogen stored or lost to caloric expenditure. Preliminary data indicate severe gross changes as would be anticipated. The direct calorimeter for the rat, designed by Mr. W. C. White, has required additional modification, but should be in use in the near future.

Measurement of changes in body composition seems a reasonable approach to the study of cachexia in cancerous patients and may lead to some means of describing the changing mass of a tumor which cannot be seen or measured by more conventional means. Additional experience in the measurement of changes in body fat in the Siri apparatus by Drs. Berlin and Watkin, correlated with metabolic balance techniques and independent measurement of body water, reveals excellent agreement among the values obtained by the different methods.

Dr. Watkin has characterized a "weight-loss syndrome," which includes negative caloric balance, low respiratory quotient, and increase in unesterified-plasma fatty acids, which he associates with actively growing cancers. Study of the effect of fasting reveals that the patient with rapidly advancing malignancy either exhibits this syndrome at the beginning of the experiment or changes to that general pattern within 12 hours. Such persons whose basal metabolic rate is high initially, continue to manifest the same high rate despite fasting. Normal individuals, on the other hand, show little evidence of a "weight-loss syndrome" until 16 to 36 hours after the fast begins.

TISSUE CULTURE

Measurement of several important biochemical qualities by Dr. B. B. Westfall points again to the high degree of chemical variation among cells grown *in vitro* for extended periods. All of the cell strains studied form α-keto acids. Concentrations of nucleic acids, glycogen, lipids, and cholesterol vary widely from strain to strain. No

glycogen can be demonstrated in cultured fibroblasts; yet after 7 years *in vitro* a line of skin epithelium stores glycogen, as do two strains of hepatic parenchymal cells; cells of a hepatoma strain derived from one of these hepatic parenchymal strains, on the other hand, have lost that capacity. In certain cell strains, enzyme activities have been lost; in one cell strain a very great increase in arginase activity was demonstrated. Certainly cells in rapid proliferation over long term in tissue culture show numerous divergent variations.

A strain of monkey kidney cells obtained originally from Eli Lilly Research Laboratories has been adapted to grow in a chemically defined medium by Dr. V. J. Evans. This strain supports growth of poliomyelitis virus very well. Dr. Evans has adapted a continuously cultured line of human skin epithelium to growth in chemically defined medium by slowly reducing the concentration of serum in the original mixture. A new medium NCTC 117, derived from the familiar NCTC 109, omits coenzymes, sodium glucuronate, deoxyguanosine, deoxyadenosine, 5-methylcytosine and "essential" unsaturated fatty acids. The only nucleic acid derivatives required by strain L are thymidine and deoxycytidine.

Dr. Sanford, studying the effects of vitamin requirements of cells growing in the chemically defined medium NCTC 117, reports pantothenate, choline chloride, niacinamide, thiamin, and riboflavin essential for cell survival. Folic acid, pyridoxal or pyridoxine, and possibly biotin, while not essential for cell survival, increase the rate of cell proliferation.

The effect of methylcellulose in improving cell growth in a chemically defined medium in agitated fluid suspension cultures is not related to its viscosity. Mr. J. C. Bryant, Dr. Evans, Mr. E. L. Schilling, and Dr. W. R. Earle find no notable growth-promoting effect, in such cultures, of widely different concentrations of methylcellulose on cells of a monkey kidney strain.

Study of the characteristics of cell populations growing *in vitro* is made possible by comparative time-lapse cinematography. The first study by Mr. W. T. McQuilkin and Dr. Earle concerns changes during adaptation of one clone of strain L to growth in a protein-free medium. The average generation time is prolonged and the migration rate on the glass surface is greatly decreased, as is the cytokinetic phase of growth. The cells are less compact, refractile, and granular. They round up only for prophase. Monkey kidney cells, studied under the same conditions, demonstrate a generation time similar to that of fibroblasts, and the population increases 24-fold in a week.

Lymphoma P388 grows well on Eagle's medium plus 5-percent calf serum. Dialysis of the serum destroys its capacity to support growth of these tumor cells when added to Eagle's mixture. Dr. Robert Roosa (Research Fellow) finds that addition of pyruvate or *L*-serine to the dialyzed serum restores its growth promoting properties.

CYTOCHEMISTRY AND HISTOCHEMISTRY

A number of chemicals cause blebbing of cells under *in vitro* conditions. Dr. M. K. Belkin has extended his study of blebbing to the extent that he considers this to be a common property of neoplastic cells. Experience with cells from five normal tissues fails to reveal bleb formation by the same agents that produce the change so readily in cancer cells. Most compounds producing blebs form mercaptide linkages, but a few oxidizing and alkylating agents also display the same property.

Dr. Robert Love can recognize nine morphologically distinct forms of ribonucleoprotein in cells stained by his toluidine blue molybdate technique. Electron-dense molybdate is deposited at sites of metachromasia. Some hepatic ribonucleoproteins are affected by starvation; some disappear altogether and reappear on feeding. Ribonuclease in concentrations of 40 mg. per ml. has no effect on the staining capacities of cells.

Dr. Love can demonstrate a nucleolinus in a number of normal and neoplastic cells. Although it is usually large in cancerous tissues, a variety of other unrelated conditions may also cause it to enlarge. Availability of the toluidine blue molybdate technique has permitted a study by Dr. Love of the effect of colchicine on ribonucleic acids in cells. Metaphase arrest, characteristic of colchicine action, is associated with failure of nuclear parachromatin to diffuse into the spindle zone at the end of prophase. Some doses of the drug result in complete inhibition of mitosis with increase of parachromatin during interphase and enlargement of the nucleus and nucleolinus.

Parachromatin may play some role in the origin of spindle fibers.

The variation in the reaction of ascites tumor cells to tetrazolium salts varies so widely that Dr. G. Z. Williams has turned his attention to study of mouse and rat hepatic cells, which he has learned to separate and to count electronically. Isolated mitochondria possess a variety of dehydrogenases demonstrable by tetrazolium techniques, but mitochondria in whole cells react quite differently, as Dr. MacCardle has been saying for years. The differences in reaction rates observed in whole cells, as compared with isolated mitochondria, are probably not due to differences in permeability, because addition of a rapid reducing agent after 15 to 30 minutes of exposure of intact cells to one of several tetrazolium salts causes rapid reduction of the intracellular dyes.

Dr. Williams has calculated reduction rates for several tetrazolium-substrate combinations. Concentrations of 0.0012 M tetrazolium and 0.034 M succinate result in rapid, intense reduction to formazan in cytoplasm of hepatic parenchymal cells near the mitochondria with general increase in cytoplasmic density. Lower concentrations lead to accumulation of formazan in lipid droplets.

DERMATOLOGY

Dr. E. J. Van Scott reports that autotransplantation of epidermal tissue free of stroma from the donor site is either unsuccessful or the transplanted cells grow and assume the characteristics of the epithelium of the new site. The cells will not grow in the dermis. Cells from a basal-cell carcinoma will grow in a new site only if donor stroma is available in the transplanted fragment.

His continuing studies of psoriasis include the description of a number of agents which cause prompt clearing of the local lesions on topical application with recurrence in one to several weeks. Such drugs as methotrexate, 5-fluorouracil, actinomycin D and colchicine, all active against some neoplasms, will clear up the psoriatic lesions when administered systemically. Antimetabolites are inactive when applied locally. Serial histological studies of the lesions have indicated that psoriasis is basically a hyperplastic process, and that cytotoxic agents retard its development and permit the cells to form keratin.

Nine protein fractions, three fractions each isolated by identical methods from human epidermis, psoriasis scale, and ichthyosis scale, have been shown by Dr. Simon Rothberg to display major similarities when their peptide patterns obtained by enzymatic hydrolysis are compared. Solubilization of the proteins does not require cleavage of disulfide bonds. Some peptide differences have been observed among proteins obtained from normal epidermis, psoriasis, and ichthyosis.

Additional work on the relation of hair growth to the dermal papilla is reported by Dr. Van Scott and Dr. R. G. Crounse (Research Fellow). The mitotic activity of the germinal matrix is proportional to the number of cells in the papilla, normally 1 mitosis per eight papilla cells. Mitotic activity decreases in successively higher levels of the hair bulb but is a function of the area of the papilla to which the matrix cells are exposed and the transverse thickness of the matrix at each level. Permanent baldness results from destruction of the papilla. Drs. Crounse and Rothberg find arginase in the hair sheaths but not in the bulbar portion of epilated roots. This suggests that arginase is not directly concerned with synthesis of keratin of the hair shaft. While arginase is known to exist in the epidermis, other enzymes of the Krebs-Henseleit urea cycle can not be demonstrated in this tissue.

Detailed study by Drs. M. K. Barrett and E. J. Breyere (Research Fellow) of tumor transplantability among several combinations of instrain and outcross matings provides some unexpected results. Among females of a given or first strain of mice, those impregnated by a male of a different or second strain exhibit tolerance towards a tumor originating in the second strain. This is exhibited by successful transplantations of the neoplasm in immunized females mated to males of the strain from which the tumor originated, but not in those mated to other males. The effect increases with increasing multiparity.

Dr. O'Gara is transplanting thymic tissues of newborn mice to the spleens of adult mice. The thymus regenerates in this location. Adrenalectomy and orchiectomy enhance the growth of the transplants, but ovariectomy and thymectomy produce no effect.

Some cancers clear glucose from the blood quite rapidly. Dr. H. A. Kahler, studying the effects of glucose administration on pH of an experimental neoplasm, has used both intraperitoneal and intravenous routes of administration. The former causes flow of fluid from the blood into the peritoneal cavity with dehydration of the tissues; the latter increases the blood volume including blood flow through the tumor, thus producing a more rapid drop in pH of the neoplasm and a more rapid return to normal values. The reduction in pH following intravenous injection of glucose is 0.65 pH in the viable part of the neoplasm, but only 0.16 pH in its necrotic center.

Dr. E. D. McLaughlin has standardized study of the rat liver regenerating after partial hepatectomy so that reproducibility is adequate to permit quantification of some aspects of the process. Preliminary results reported by Dr. McLaughlin suggest that normal human serum contains something that retards orderly regeneration, whereas sera from cancer patients have no such effect.

Surgical and Virus Treatment of Cancer

Independent measurements of circulating plasma and red-blood-cell volume by modern isotopic techniques have been applied to study of the postoperative state by Dr. A. S. Ketcham and other members of the Surgery Branch. Once more the unreliability of the hemoglobin, hematocrit, and erythrocyte enumeration, as determined by conventional methods, to reflect changes in the effective circulating blood volume is emphasized. During the first 2 weeks after a major surgical operation, a patient may lose 25 percent of his volume of circulating erythrocytes without any remarkable change in hemoglobin concentration or in hematocrit. Tachycardia occurs when the effective circulating volume of red blood cells is reduced by 30 to 40 percent, and these patients respond dramatically to transfusion of two units of blood.

Review of surgical experience at the National Cancer Institute includes 245 major operations and 214 minor surgical procedures. Only seven patients have died within 30 days of operation. There have been 44 postoperative infections of which 20 have been associated with avascular operative wounds caused either by extensive X-irradiation of the site at varying periods before surgery or by the creation of large skin flaps as part of the operative procedure. Antibiotic-resistant, coagulase-positive, hemolytic staphylococci have been major factors in 29 of the 44 cases, but an additional 17 patients known to harbor such organisms have not developed wound infections postoperatively. Nineteen of the forty-four patients had contaminated wounds that involved lesions of the mouth, pharynx, trachea, or the gastrointestinal tract before any operation was performed. This experience led Dr. R. R. Smith some time ago to design a prospective study of postoperative infection, and it is already clear that 23 of 30 patients harbored, at the time of operation, the organisms which subsequently caused postoperative infection.

Continuing study by Drs. Smith, J. F. Potter, and Malmgren reveals that the contamination of wounds by cancer cells occurs most often after operations that involve excision of a primary cancer. Neck dissection for metastatic cancer alone seldom results in the recovery of recognizable cancer cells from the wound washings. The frequency of contaminated wounds in operations for epidermoid carcinoma continues at 26 percent and no correlation between contamination and local recurrence is possible. Use of dilute solutions of formaldehyde has been ineffective in reducing the frequency of local recurrences. No new leads have developed in the experimental study conducted by Dr. Ketcham in a search for agents which may kill the residual cells. Of the several chemicals tried, all promote the growth of cells when the wound is treated before introduction of the brie, and thiotepa has a similar though less pronounced effect when given by intraperitoneal injection. Any one of the agents studied will kill cells of experimental tumors when suspended in the same concentration used to wash the wound. The wound is simply a different and more complex environment.

Cancers of the head and neck are commonly said to metastasize to the organs and tissues below the clavicles in only 12 or 13 percent of patients. Quite a different picture is afforded from limited experience with this type of malignant disease at the National Cancer Institute. Dr. Smith has reviewed the 25 post-mortem examinations performed by the Pathologic Anatomy Branch on patients who have succumbed to cancers originat-

ing in 12 different sites of the head and neck. Almost all of the lesions were epidermoid carcinomas, and one-half of them had not metastasized at the time of operation. Thirteen, a little more than half, of these 25 patients had distant metastases involving 17 different locations, and in 12 cases the lungs were involved. Three of the patients with disseminated disease never had metastases in the cervical lymph nodes. The average interval between operation and death in this small group is only 10 months for those patients who had no clinically demonstrable evidence of metastases at the time of operation and 19 months for those whose cancers had spread beyond the site of origin when surgery was performed. Each of the latter series of patients was treated at least once for local recurrence. The prolongation of life among these people can hardly be attributed to the surgeon's skill, since they would seem to represent a somewhat more indolent type of cancer than that which characterized the patients whose life expectancy was materially shorter.

Urinary diversion via an ideal conduit in radical pelvic exenteration for cancer of the uterus continues to give satisfactory results. Infection of the upper urinary tract and hyperchloremic acidosis are distinctly unusual in this group of patients, as contrasted with their frequency in patients with wet colostomies. Dr. Smith and colleagues observe a ureteral reflux when radiopaque material is introduced into the ileal pouch, and bacterial contamination of the ureters is relatively common.

VIRUS TREATMENT

The use of a strain of Coxsackie virus B_3, trained by Suskind, *et al*, to destroy HeLa cell tumors growing in rats, in the treatment of advanced human epidermoid carcinomas of the cervix (Annual Report, 1957), has been studied for several years. The enterprise has involved many people, including Drs. R. R. Smith, R. B. Couch, Manaker and Love, NCI, and Drs. R. J. Huebner and W. P. Rowe (NIAID). Although this smoldering effort is not likely to break into flame at this time, experience with 25 patients is interesting.

1. Among nine patients whose serum contained no antibodies against Coxsackie B_3 no difficulty was experienced in recovering virus during the first 4 days after its injection *per vaginam* into the cancerous mass. Titers dropped precipitously on days 4 through 8 as did ability to recover virus. Virus was recovered from only three patients after the 8th day and persisted beyond the 11th day in only one case. Despite this experience, the total value calculated for recovered virus exceeds the amount injected originally. Among six patients whose sera contained antibodies against the virus, recovery of Coxsackie B_3 was impossible beyond the first 5 days following injection.

Cultures taken from the throat and anus were intermittently positive for the virus up to day 6 in some patients.

2. Antibody titers were increased or antibodies appeared in all patients by the fourth day. Subsequently the titers rose to between 256 and 2,048 about 2 weeks after treatment.

3. Fever was the only sign of infection observed in 12 of the 25 patients. It exceeded 39° C. in two of them and occurred 12 to 72 hours after injection. Five of seventeen patients had leukopenia and relative lymphocytosis.

Injection of both adeno and Coxsackie viruses into the cancers of four patients produced general malaise and fever of 39–40° C. in 12 hours; the fever slowly returned to normal. All blood cultures were negative for bacteria.

4. The oncolytic response was not striking, though a significant change was observed twice as frequently in the nonimmune patients as in those whose sera contained antibodies before treatment was given. No specific cytologic or histologic changes were observed.

5. Tumor-to-tumor passage in patients did not enhance the oncolytic effect of Coxsackie virus B_3.

Dr. Love's studies of Newcastle disease virus adapted to infect and destroy Ehrlich ascites tumors in mice have also yielded important results. This strain of the virus grows poorly *in vivo* in cells of the Krebs ascites tumor and originally produced no oncolysis. Dr. Love was able to adapt the virus to grow in this tumor after 18 passages *in vivo*, but he could not adapt original chick-embryo Newcastle disease virus to the Krebs carcinoma by serial passage *in vivo*. The original chick-embryo strain produced some oncolysis of lymphoma P388 ascites tumor, but the Ehrlich adapted strain had no such effect. Dr. Love concludes that adaptation of a virus to a

given tumor does not confer oncolytic power against other neoplasms.

PYRIDOXINE DEFICIENCY

Reports by others indicate that pyridoxine deficiency interferes with immune responses, and Drs. Couch and Smith conceived the idea of enhancing the oncolytic effect of adeno or Coxsackie B_3 viruses on epidermoid carcinoma of the cervix by clinically induced pyridoxine deficiency. This was accomplished by formulation of a semisynthetic diet which included the antimetabolite desoxypyridoxine. Reports of clinical pyridoxine deficiency induced at other research centers guided the local study and, in general, each of the four patients studied at the National Cancer Institute pursued courses consistent with the syndrome described by others. The mental symptoms were different in that mood swings with periods of stability and orientation were more frequent than expected. Only two patients exhibited marked lymphopenia. Marked impairment of renal function, elevated fasting blood sugar, and profound catabolic responses seen in each of the patients are new findings. Perhaps the hyperglycemia may be associated with the known toxicity of xanthurenic acid for the β cells of the islets of Langerhans.

The experience is too small to provide definitive information on enhancement of oncolysis by Coxsackie B_3 virus by pyridoxine deficiency. Although signs and symptoms of the clinical deficiency were reversed promptly by replacement of pyridoxine for the desoxypyridoxine in the diet, more information on some of the previously undescribed effects of pyridoxine deficiency should be sought from animal experimentation before further clinical studies are undertaken.

Drs. Dyer and Morris, having extensive experience with pyridoxine deficiency in rats, have studied biochemical aspects of the clinical syndrome. The urinary excretion of several tryptophan metabolites is almost identical in man and rat during pyridoxine deficiency. The urine contains large quantities of xanthurenic and kynurenic acids, as well as large amounts of 3-hydroxykynurenin and its acetyl-glucuronide and ortho-sulfate derivatives. These compounds, absent from the urine before induction of pyridoxine deficiency, disappear when dietary desoxypyridoxine is replaced by vitamin B_6.

Radiological Treatment of Cancers

Diversion of the urinary stream into a surgically created ileal pouch and X-irradiation of the pelvis in patients with cancers of the urinary bladder, class C or D, grade 3 or 4, provide marked symptomatic improvement in the limited experience of Drs. J. R. Andrews, Jack Levin, and H. D. Suit. Temporary control of the local tumors has been achieved, but 9 of the 11 patients studied have already died of distant metastases. The two patients still living have survived 25 and 34 months respectively.

Renal function among the patients with carcinomas of the urinary bladder whose ureters have been anastomosed to an ileal pouch has been improved, as shown by studies of blood-urea nitrogen, serum creatinine, and intravenous pyelograms. Endogenous creatinine clearance and fractional phenosulphonphthalein excretion have not improved to the same extent and, indeed, seem to become further depressed. Bacterial contamination of the urine is unaffected by the ureteral transplantation, even though the patients reveal no clear evidence of acute pyelonephritis.

Forty patients with cancers of the head and neck have been treated with protracted courses of 2-m.e.v. X-ray therapy. At least 1 year has elapsed since treatment was commenced in each case. The median survival is 10 months. Reactions of the skin and other normal tissues have been mild, probably as the result of 2-m.e.v. beams, rather than any increase in the time-dose relationship. The results are being analyzed further by Dr. J. R. Andrews.

A total of 184 courses of radiation therapy has been given during the year by Dr. K. C. Brace and associates in the service treatment of many types of cancer. One hundred sixty of the courses have utilized 2-m.e.v. therapy and five involved treatment with electron beam.

Mr. R. W. Swain is replacing the dosimeters used in conjunction with 2-m.e.v. Van de Graaff electrostatic generators with transmission ionization chambers connected to current integrators which can be preset to turn off the machine after

the prescribed dose has been given. Mr. Swain has also devised a means to permit semiautomatic plotting of isodose distribution in water using small ionization chambers.

Drs. J. R. Andrews, R. L. Swarm and associates have studied another patient with extensive chondrosarcoma of the thoracic wall whose neoplasm concentrated radioactive sulfur. A therapeutic dose of 388 mc of radioactive sulfate was followed after 2 months by another dose of 335 mc. Temporary retardation of tumor growth was followed later by increase in growth, requiring external beam-irradiation therapy. The isotope treatment produced only slight depression of leucocytes and platelets until 4 weeks after administration of the second dose, when thrombocytopenia became marked and leukopenia moderate. The blood picture returned to normal in 2 months, but 6 months after the second dose of isotope the patient developed a severe anemia and thrombocytopenia. Another patient treated in much the same way more than a year ago has developed no such syndrome which recalls experience with dogs reported by Dr. Brecher some years ago.

Dr. D. P. Tschudy has studied metabolic effects of X-irradiation in a patient with a lymphosarcoma of the lower extremity. Prior to treatment, the metabolic pool of nitrogen was slightly increased, but no change in the rate constant of metabolic pool turnover could be detected. A dose of X-rays amounting to 1,000 r was followed by marked enlargement of the pool with decrease in the same constant. Incorporation into body protein of amino acids labeled with N^{15}, however, was unchanged during this period.

In the experimental laboratory Dr. Suit has studied the influence of increased oxygen tension on the response of transplantable adenocarcinoma in C3H/BA to local X-irradiation. He reports no enhancement of therapeutic effect of the X-ray treatment when mice bearing this neoplasm are exposed to an environment of pure oxygen at 2 atmospheres during X-irradiation, as compared to an environment of air at 1 atmosphere. The result is the same with tumors measuring either 1 or 2 centimeters in diameter. A statistically significant difference in radiation effect was obtained with the smaller neoplasms.

The resignations during the year of Mrs. Hollcroft and Dr. Suit require complete rebuilding of the laboratory component of programs relating to therapeutic use of ionizing radiations. Difficulty in recruiting suitable patients for clinical radiological research has also retarded progress.

Chemotherapy of Cancers

Critical analysis of experience obtained throughout the world in the drug treatment of cancers reveals a small group of specific anatomical types of neoplastic diseases in which chemotherapeutic agents have produced definite beneficial effects, and a much larger group of cancers which remain relatively unaffected by systemic administration by chemotherapeutic agents. There are, therefore, at least two separable, broad, general problems with an infinite variety of specific subordinate problems. The substantial progress made in treatment of leukemias, lymphomas, choriocarcinoma, and adrenal cortical neoplasms emphasizes the desirability of quantitative comparisons of the efficacy of chemotherapeutic agents already available, and further exploration of chemical relatives of the effective agents for greatly enhanced therapeutic activity and research designed to elucidate the most effective means of administering the drugs now on hand. A search for new drugs with qualitatively different types of activity may also benefit patients with responsive types of cancer, but this becomes of paramount importance to patients suffering from the much larger number of neoplastic diseases which respond fleetingly or not at all to the currently available drugs. A variety of transplantable animal tumors is available for this research, and probably the variety must be increased if the necessarily empirical approach is to be fruitful. Increased emphasis on biochemical investigations with a view to categorizing individual or special types of cancers seems practical and desirable. Certainly a much greater use of patient material will be necessary before meaningful correlations between the therapeutic reaction of man and experimental animals can be established and, indeed, we probably do not know how to study therapeutic responses of cancers of the stomach, large intestine, or kidney at this time with any degree of precision. While the history of therapeutic research is sprinkled with episodes as dramatic as the penicillin story, most of the major advances

have required slow exploitation of small gains by systematic study.

The course of any research requiring creation of new knowledge is largely unpredictable. Having pointed out the possibilities of exploiting certain important developments obtained from the study of plasmacytomas, it seems appropriate to recount progress in correlated clinical and laboratory research in a different kind of cancer.

CHORIOCARCINOMA

The introduction of methotrexate therapy of choriocarcinoma stemmed from basic information on the need for folic acid in uterine and fetal metabolism, and, indeed, the initially effective therapeutic regimen designed by Drs. Hertz and M. C. Li deviated significantly from generally accepted doses and schedules of antifolic compounds. Fifty female patients with choriocarcinoma have now been admitted to the National Cancer Institute under Dr. Hertz's care. Twenty-one of the forty-four patients admitted prior to September 15, 1959, are free from clinical, radiological, or hormonal evidence of residual disease for periods ranging from 5 to 47 months. Eight have persistent choriocarcinoma, and 15 have died following an initial response to methotrexate therapy, with subsequent development of methotrexate resistance. Acquired drug resistance is an important obstacle to more effective management of this neoplasm. Methotrexate-resistant patients do not respond to subsequent treatment with nitrogen mustard, cytoxan, or actinomycin D.

Since May 1959, Dr. Hertz and associates have treated eight patients suffering from methotrexate-fast choriocarcinoma with Vincaleukoblastine, an alkaloid of *Vinca rosea*, initially described by Noble, Cutts, and Beer, and made available through courtesy of Eli Lilly and Company. Three of these women are now in complete remission, the longest period being 5 months. Two others had definite but rapidly reversible evidence of tumor regression; no response was obtained in the other three. Vincaleukoblastine depresses the bone marrow, causes central nervous system toxicity, alopecia, stomatitis, malaise, and local phlebitis at the site of injection; all of these effects are reversible. Nevertheless, it affects the course of methotrexate-fast choriocarcinoma, because it is the first alkaloid to retard cancerous growth.

To obtain a better understanding of problems associated with choriocarcinoma, Dr. Hertz adapted four different strains of human choriocarcinoma to grow in the cheek pouch of conditioned hamsters, but two of the strains no longer require pretreatment of the host with cortisone. Each of the tumor strains grows progressively for 2 to 3 weeks, when central necrosis occurs, followed by liquefaction and absorption of the graft in 1 to 2 weeks. Continued administration of cortisone does not influence this picture. Hamsters in which the neoplasm has been absorbed will not grow any of the strains of choriocarcinoma, and passive immunity can be conferred on other hamsters by injection of serum from the resistant animals. The resistant state is not induced by injection of several human tissues including normal placenta.

The problem of the immune relationship between choriocarcinoma and the host subject has been raised frequently because this neoplasm is derived from the zygote and contains genes from both parents. Dr. Hertz has undertaken a study with Dr. P. J. Schmidt (DBS) of the blood groups of choriocarcinoma patients and their husbands. No evidence of cross-immunization between the marital partners has been found, even among patients whose tissues were extensively invaded by tumor. The scientists infer that the embryonic neoplasm lacks significant antigenicity for the host, at least as far as circulating antibody formation is concerned.

The heterologous choriocarcinoma transplants produce large amounts of chorionic gonadotropin, which are readily detectable in the tumor, plasma, and urine but cannot be demonstrated in the host's normal organs or tissues. Choriocarcinoma also produces the gonadotropin in tissue culture. Dr. Hertz finds that neither slices nor homogenates of normal hamster organs inactivate the hormone. Unlike the normal placenta, choriocarcinoma displays no estrogenic, progestational, corticoid, thyrotropic, or adrenotropic activity.

Dr. Hertz is using the transplanted choriocarcinomas in screening for chemotherapeutic activity. Even though a particular tumor may have been taken originally from a patient whose lesion was completely resistant to methotrexate, the het-

erologous transplant still displays moderate sensitivity to the drug. The most potent tumor inhibitors found thus far are Vincaleukoblastine, noted above, and a related Vinca alkaloid, Leurosine. Other alkaloids of *Vinca rosea* have been inactive when given in toxic doses.

Thus a program originating in the laboratory has had important consequences in the clinic where new and additional problems have arisen requiring further laboratory research at an even more fundamental level. It is difficult to study choriocarcinoma in experimental animals and study of the physiology of chorionic gonadotropin presents problems. Serum and urine of the pregnant female monkey, *Macacus rhesus*, studied by Dr. Hertz, contain detectable amounts of chorionic gonadotropin only from day 20 to day 35 following timed mating. The hormone cannot be demonstrated in concentrates of these body fluids at any other time, nor is it found in any of the tissues including the chorion itself.

ENDOCRINE THERAPY

Administration of human pituitary growth hormone to 10 patients with metastatic carcinoma of the breast, 3 patients with cancers of the adrenal cortex, and 2 men with prostatic carcinoma has produced no exacerbation of disease nor other changes in the measurable parameters of tumor activity, according to Drs. Bergenstal and Lipsett. Excretion of urinary calcium is not influenced by injection of growth hormone during remission induced by hypophysectomy. Only those patients whose cancers continue to grow progressively after removal of the pituitary respond to growth hormone administration by some increase in urinary calcium excretion.

Dr. J. M. Van Buren (NINDB) has performed section of the pituitary stalk in 11 patients with advanced mammary cancers. The morbidity has been greater than in patients submitted to hypophysectomy, and the overall results are not as good. Drs. Bergenstal and Lipsett report decline in adrenal cortical activity among these persons to the levels reached in hypophysectomized individuals. Gonadotropin excretion is variable. Four patients demonstrated normal thyroid activity which could be suppressed with triiodothyronine. The investigators regard these findings as evidence for a variable degree of destruction of the anterior pituitary following stalk section. Unlike the dog, ACTH production seems to be the most sensitive index of this type of pituitary damage in man. Thyroidpituitary relationships in several patients have remained normal in the absence of vascular connections between the hypothalamus and pituitary.

o,p′–DDD

Drs. Hertz, Bergenstal, and Lipsett have used o,p'–DDD in the treatment of 14 patients with metastatic carcinoma originating in the adrenal cortex. All have shown some suppression of steroid output, and objective regressions of cancer have occurred in five. Ketosteroids reached low levels in two patients who responded to treatment, but a large proportion of the excretion was identified as dehydroepiandrosterone, which suggests persistently active carcinoma. Undesirable side reactions of o,p'–DDD encourage investigation of related compounds for better therapeutic agents.

SCREENING ACTIVITIES

The contract with Microbiological Associates, Inc., for quantitative screening of chemotherapeutic agents continues to be a highly satisfactory venture, under the direction of Dr. Zubrod, Dr. Abraham Goldin, and Mr. J. M. Venditti. Seventy-eight new compounds have been tested for therapeutic activity against leukemia L1210, using methotrexate as a reference standard, and 10 additional agents have been retested. A substantial amount of information has been obtained on the effect of dosage schedules. Some agents, especially cytotoxic antibiotics, are more effective when administered daily, while other agents are more efficacious when given at more widely separated intervals. Dosage schedules can influence therapeutic responses even among members of the same chemical class. Dichloroamethopterin, for example, exhibits about the same therapeutic effect when given twice daily or at intervals of 4 days while methotrexate is much less effective when the latter schedule is used.

Thirty-five compounds have been tested against sarcoma S–37 which Dr. Goldin has adapted to grow reproducibly for use in the screen. Treatment is usually started 3 days after tumor implantation and continues daily for 5 days. Seven compounds produce 40-percent inhibition of growth

on the 10th day after inoculation of the tumor when administered in doses causing death in not more than 20 percent of the animals. These compounds in optimal dose also increase the median survival time at least 40 percent over untreated control mice. Another five agents inhibit growth of S–37 without increasing survival time. S–37 has been particularly resistant to chemotherapeutic attack, and these results reported by Dr. Goldin and Mr. Venditti are encouraging.

Carcinoma 755 is the third transplantable neoplasm to be introduced into the quantitative screen. The reference standard against which other compounds are tested is 6-mercaptopurine, which produces 80-percent increase in survival time, and some tumor-free survivors are obtained when treatment is begun on the fourth day after inoculation and continued daily for 5 days. A large number of chemotherapeutic agents will retard the growth of this neoplasm and prolong the lives of the hosts when administered according to the same treatment schedule. Most of the more effective compounds are purine and pyrimidine antimetabolites.

Much of the research conducted by Drs. Dean Burk and M. W. Woods is directly concerned with the mechanism of drug action. The writer believes, however, that the total experience should also be viewed as a potential means of screening compounds for chemotherapeutic activity. The techniques seem capable of using clinical as well as experimental cancers, even though some technical difficulties may be encountered. Drs. Woods and Burk report that a high degree of malignancy is associated with greatly increased glycolytic capacity and lowered sensitivity to glycolytic inhibition of the anti-insulin type (Annual Reports, 1956–58). Generally speaking, those experimental neoplasms in which the hexokinase reaction is under strong insulin anti-insulin control respond to one or more chemotherapeutic agents better than do those cancers in which glycolysis is less readily inhibited by compounds of the anti-insulin type. One does not have to accept the theory advanced by Drs. Burk and Woods to recognize that these scientists have found a means of classifying experimental cancers with respect both to degree of malignancy and potential effectiveness of chemotherapeutic drugs.

Drs. Burk and Woods have studied the antiglycolytic effect of 5-fluorinated pyrimidines against Krebs-2 and Ehrlich ascites tumors, finding 5-fluorouridine the most active and 5-fluorocyosine inactive. The *in vitro* studies correlate closely with the capacity of each compound to inhibit tumor growth *in vivo*. On the other hand, a concentration of 400 P.P.M. of uridine is as active *in vitro* as is 20 P.P.M. of 5-fluorouridine. The antiglycolytic activity of the 5-fluorinated pyrimidines can be largely counteracted by increasing inorganic phosphate in the incubation medium, removal of oxygen, or by the use of agents that uncouple phosphorylation. The effect measured by Warburg manometry requires glucose; succinate, pyruvate, or glutamate cannot be substituted for glucose. Experiments conducted with Mr. J. C. Hunter describe the inhibition of aerobic glycolysis of rat-bone marrow by 5-fluorouracil, but not by 5-fluorodeoxyuridine and reflect the relative degree of toxicity of these compounds for mice.

Drs. Woods and Burk report that the *in vitro* mechanism of action of the 5-fluorinated pyrimidines appears to involve an inhibition of aerobic phosphorylation which lowers the availability of adenosinetriphosphate (ATP) for glycolysis. Insulin lowers the requirement for ATP and should counteract the metabolic effects of the 5-fluorinated pyrimidines. This it does in S91 melanoma and normal bone marrow, but not in insulin-insensitive ascites tumors Krebs-2 and Ehrlich.

Differences in metabolic characteristics of tumors respectively susceptible and resistant to action of a given chemotherapeutic agent are reported by Dr. Burk and Dr. K. M. Wight. 8-Azaguanine produces a prompt and marked increase in glycolysis *in vitro* of susceptible leukemic cells but not of resistant cells with concentrations of drug ranging from one-fourth to four times the pharmacological level commonly used to influence the growth of the sensitive leukemia *in vivo*. Respiratory inhibition occurs in both sensitive and resistant cells exposed to 8-azaguanine but is seen at lower doses with the susceptible line. The metabolic effects characteristic of the sensitive cells tend to disappear after repeated daily exposure to the drug *in vivo* and may be completely lost within a transplant generation.

The action of cytoxan on the metabolism of susceptible mouse tumors leukemia L1210, K–2

ascites, and Ehrlich ascites has also been studied by Drs. Burk and Wight. Treatment *in vivo* with removal of the tumor cells for study *in vitro* reveals pronounced inhibition of aerobic and anaerobic glycolysis as well as of respiration. Respiratory and glycolytic functions of a cytoxan-resistant strain of L1210, produced by Dr. Montague Lane, were usually not inhibited by treatment and are sometimes stimulated.

ANTI-LEUKEMIC COMPOUNDS

Cooperative Leukemia Group B in which Drs. Frei and Freireich represent the National Cancer Institute has completed its study of the relative effectiveness of methotrexate and 6-mercaptopurine given alone or in combination for the treatment of acute leukemia. The study includes experience with 328 patients, of whom 92 were hospitalized at Bethesda. The highest remission rate was attained when both drugs were given concurrently, but the rate was no higher than the sum of the remission rates produced with either compound alone. The remission rate was the same for either 6-mercaptopurine or methotrexate whether used as primary treatment or used following the administration of the other drug. Treatment with 6-mercaptopurine alone, however, produced a higher remission rate than was obtained with methotrexate. Overall rate of remission for all schedules was 50 percent of children, 15 percent in adults, but methotrexate was almost inactive in acute adult leukemias producing beneficial effects in less than 5 percent of those patients. Toxicity, comparable in all groups, required cessation of treatment after 9 to 25 days (average 14 days). Methotrexate produced more oral symptoms, 6-mercaptopurine, more gastrointestinal disorders.

In attempting to apply to the clinical problem knowledge of the biochemical mechanism involved in the resistance of experimental leukemias to 6-mercaptopurine, Dr. J. D. Davidson has encountered methodological problems. He has synthesized 6-mercaptopurine labeled with S35, but use of this material has necessitated development of new techniques for isolation of the drug's metabolites.

One problem confronting the clinical investigator relates to procurement of a sufficient quantity of a promising new drug to permit definitive clinical trial. The staff of the CCNSC has been most helpful in such matters, and the Lederle Division of the American Cyanamid Company cooperated to the fullest possible extent in making 3′,5′ dichloroamethopterin (DCM), but it took such a long time that no substantial progress can be reported in therapeutic trials against leukemias or other cancers in man. Studies are now underway, but the bulk of work with this new compound accomplished during 1959 relates to experimental cancer.

Dr. Loo and Dr. V. T. Oliverio can separate methotrexate (MTX) from DCM by column chromatography and can also extract it from body fluids. Only 10 percent is recovered unchanged from the urine after administration to man or mouse. Another 5 percent of the given dose is altered in some undetermined way.

DCM is the drug most effective against mouse leukemia L1210 and, indeed, some mice are actually cured of their advanced disease by use of this compound (Annual Report 1958). Dr. Goldin with Dr. M. A. Chirigos and Messrs. Venditti, S. R. Humphreys, and G. O. Chapman have conducted extensive experiments on DCM. Daily treatment must be continued 30 to 60 days to permit complete recovery from L1210. Ordinarily the tumor disappears in about two weeks but recurs promptly if treatment is stopped prematurely. Oral administration of both DCM and MTX is less effective in experimental leukemia than is subcutaneous injection.

Drs. Goldin, A. W. Schrecker, and J. A. R. Mead (Visiting Scientist) report that inhibition of formate incorporation into the acid soluble adenine of leukemic spleen is a reasonable measure of the effective doses of antifolic compounds given by parenteral injection. Maximum effect occurs 20 minutes after injection of the drug but one hour after oral dosage, and larger doses are needed to produce the same quantitative effect.

Some clue to a reason for the increased effectiveness of DCM over MTX in the management of experimental leukemia L1210 is afforded by other reports from the same investigators and Dr. R. A. Darrow. DCM has a smaller inhibitory effect on formate incorporation than does MTX. The dose ratio for equal response is about two, but the effect from MTX lasts longer. Extending

treatment intervals to 6 hours or more increases the dose ratio of DCM to MTX to 25. A single dose of DCM needed to inhibit formate incorporation for 24 hours must be of the order of 75 mg/kg, the same dose that produces maximum survival time of leukemic mice. A comparable dose of MTX cannot be tolerated.

Mice cured of L1210 will usually grow neither a second graft of this neoplasm nor cells from a subline of L1210, M46R, which resists treatment with antifolic compounds. If growth does occur, the new tumor remains localized to the site of implantation. BALB/C mice can be immunized against L1210. This type of immunity can be overcome, however, by treatment with MTX if the mice are inoculated with the antifolic-resistant M46R. If, on the other hand, immunity is induced by injections of spleen from strain DBA, the natural host of L1210, or by other experimental leukemias, antifolic compounds do not alter the resistant state.

These observations raise questions concerning the role that immunity plays in influencing the host-tumor relationship in experimental chemotherapy. Dr. Goldin and associates have performed an experiment in which the markedly resistant subline M46R has been affected by administration of an antifolic compound. Mice bearing both the sensitive form of L1210 as well as the resistant form M46R were treated with DCM. Their lives were prolonged for as much as 60 days, though treatment of M46R alone with either MTX or DCM extends the survival time to 20 days as compared with the 10-day life expectancy of untreated controls. Presumably the effective treatment of the sensitive tumor elicits an immune response in the host, which retards the growth of the resistant variant.

Drs. Goldin, Schrecker and Mead report further that large doses of MTX inhibit formate incorporation by both sensitive and resistant lines of leukemia L1210. Inhibition is less pronounced by treatment of the resistant cells with small doses of the drug. Such differences are quantitative rather than qualitative and are more pronounced in the solid tumor than in the leukemic spleen. Citrovorum factor only partially reverses formate inhibition induced by antifolic therapy. Perhaps the transport of citrovorum factor into the cells may be impaired by MTX administration, as was demonstrated for certain bacteria by Wood and Hitchings.

Experiments conducted by Mr. F. G. Dhyse suggest some relationship between other vitamins and folic acid. When biotin is added to cultures of *L. arabinosus* in excess of the quantity required for maximum growth, the cultures give rise to a fivefold increase in folic acid in the medium without any increase in growth rate. The folic-acid content of the bacterial cells is not affected, and the biotin must be added while the culture is growing actively. If excess quantities of pantothenate or riboflavin instead of excess biotin are added to the medium, excessive production of folic acid is not observed.

Dr. Michael Potter is also interested in folic acid and its antimetabolites and uses the tetraploid lymphocytic neoplasm P288 rather than L1210 as a tool. A folic-acid-deficient diet formulated by Dr. G. M. Briggs (NIAMD) produces better survival of mice bearing P288 resistant to antifolic drugs than of mice bearing the drug-sensitive counterpart.

In studying the acquisition of the resistant state, Dr. Potter inoculates 10 cells into genetically appropriate mice and 80 percent of the recipients develop leukemia regardless of whether the inoculum is derived from cells resistant or sensitive to the action of antifolic compounds. If the sensitive leukemia is treated five times within 10 days with small doses of MTX, transplantability falls to 5 percent. Cells isolated from P288-sensitive lines which have been treated with larger doses of MTX for longer periods of time grow in 30 to 60 percent of the mice into which they are transplanted. Furthermore, the appearance of a progressively growing mass at the site of inoculation is delayed for some time, suggesting some incomplete and hitherto unknown type of resistance.

Dr. Goldin, Mr. Venditti, and Dr. Frei describe a series of pyrazolopyrimidines that exhibits a wide range of antileukemic activity against L1210. The most effective of these chemicals, however, is far less potent than either 6-mercaptopurine or methotrexate.

Virus-induced leukemias have been introduced into the chemotherapy program by Dr. Moloney, Dr. Goldin, and Mr. Humphreys. No increase in survival time has yet been obtained with methotrexate, 6-mercaptopurine, or cytoxan, even though

definite reduction in the size of affected organs has been observed.

ALKYLATING AGENTS

A new alkylating agent, cyclophosphamide, developed in Germany, is commonly called cytoxan. Dr. Montague Lane reports an LD_{50} of 425 mg. for mice, 150 mg. for rats. The drug produces little gastrointestinal toxicity and causes hyperplasia of the megakaryocytes in the bone marrow and spleen. Cytoxan is more active than nitrogen mustard against leukemia L1210, including sublines resistant to antifolics, lymphoma L–2, carcinoma 241–6, and the Dunning rat leukemia and lymphosarcoma. The drug is most effective when administered once weekly. Dr. Goldin has confirmed Dr. Lane's finding with respect to the action of cytoxan on L1210 and reports it at least as effective as methotrexate. Many mice survive indefinitely when treatment is instituted early in the course of the transplanted leukemia. Cytoxan is also effective against adenocarcinoma 755 and S–37.

Studies of the toxicology of cytoxan in dogs and rats have been carried out under the direction of Dr. D. P. Rall at the Hazleton Laboratories. The advantage in terms of administration of large quantities of drug through the use of widely spaced dose schedules was clearly borne out for these species. Of particular interest is the demonstration in rats of a period following administration of large doses during which the animal appears to be refractory to toxic manifestations of cytoxan or methotrexate.

Cytoxan in man produces greater depression of leukocytes than of platelets, according to Dr. C. O. Brindley and Dr. Frei. Daily oral doses of 4 mg/kg are tolerated for weeks. Some therapeutic activity is displayed in patients with lymphosarcoma or ovarian carcinoma.

Leukemia group B is studying the effect of cytoxan therapy in acute leukemia. Drs. Freireich and Frei report no difference in toxicity when given daily or weekly, but somewhat more therapeutic benefit appears to be obtained from weekly doses. A total of 96 patients has been studied, of which NCI has contributed 30. Preliminary results indicate objective improvement in 30 percent of the patients.

Uracil mustard, another alkylating agent, has also been studied in the clinic. Drs. Brindley and Lane find it active against Hodgkin's disease, lymphomas, and chronic leukemias in oral weekly doses of 0.2 mg/kg. The drug depresses the bone marrow and occasionally causes severe gastrointestinal symptoms. The following oral doses are tolerated for 6 weeks with minimal toxicity:

Single dose	0.45 mg
Daily dose	0.015 mg/kg
Weekly dose	0.2 mg/kg

A comparative study of the effectiveness of thiotepa relative to nitrogen mustard in the treatment of Hodgkin's disease indicated superiority of the latter drug (Annual Report 1958). Reexamination of the data by the Eastern Solid Tumor Group disclosed some evidence that the dose of nitrogen mustard was closer to the maximum tolerated dose than was the dose of thiotepa used in the study. Therefore, the investigators undertook a study of dose-response relationships for both these alkylating agents. It soon became apparent that the dose-response curve is steep for nitrogen mustard, since therapeutic activity is lost when the usual dose is reduced 50 percent. Whether the therapeutic effects of thiotepa and nitrogen mustard on Hodgkin's disease are similar at maximally tolerated doses remains to be seen at the end of the study.

During the course of these several studies, Dr. Brindley has followed the activity of the enzymes lactic acid dehydrogenase and ribonuclease in the sera of many patients. When treatment is successful, the activity of these enzymes decreases. The activities are also likely to fall in preterminal stages of cancer. The use of serum enzyme activity for following the course of cancer is limited additionally because many patients never display any increases in lactic-acid dehydrogenase or ribonuclease.

Protection of bone marrow from deleterious effects of X-radiation by prior administration of 2 - aminoethylisothiuronium bromidehydrobromide (AET) encouraged Dr. Kelly to study its effectiveness in preventing bone-marrow damage associated with the use of nitrogen mustard. The Dunning-rat leukemia is highly sensitive to nitrogen-mustard therapy. No advantage was gained by pretreatment with AET when the cytotoxic

agent was given in small doses, but some protection was demonstrated against toxicity produced by larger doses of nitrogen mustard. A series of new compounds which generate free radicals is being screened for similar protective effects by Drs. Kelly and Loo.

TETRACYCLINES

Although tetracyclines do not appear to arrest growth of cancer, their important antibiotic properties and their ability to concentrate in certain tissues including some cancers makes desirable a study of the factors governing their cellular distribution. This is being accomplished by Dr. K. W. Kohn, working with Drs. Loo and Rall. Methods for extracting the drug from plasma, red blood cells, spinal fluid, urine, and bile were developed readily. Tissues presented a different problem because of their relatively high phosphate content. This was solved by precipitating the phosphates with lead ions at pH 5. The method is highly sensitive for certain tetracyclines, but other members of the family cannot be extracted by the procedure.

Study of plasma concentrations of tetracycline and dimethylchlortetracycline after single intravenous doses in dogs revealed first-order disappearance curves. Calculation of volumes of distribution indicated that dimethyl compound and, to a lesser extent, tetracycline are concentrated in some extravascular compartment of the body. The drugs entered the cerebrospinal fluid in concentrations approximating 20 percent of those found in plasma, though equilibrium was not attained. Concentrations in skeletal muscle 6 hours after administration were higher than in the plasma. Considerable amounts of both drugs were found to be associated with erythrocytes. While the disappearance curves from red blood cells were similar to those described for plasma, the presence of divalent metal ions had important effects which probably influence distribution of the tetracyclines *in vivo*.

Formation of complexes of tetracycline with calcium and other divalent metals is well-known. Dr. Kohn also describes complexes formed with barbiturates and with β-diketones and studies of the influence of divalent metal ions on complex formation. Tetracycline forms a unionized complex with barbital and either calcium or zinc ions. Magnesium and manganese fail to form extractable complexes. Pheno- and pentobarbitals are even more potent in causing extraction than barbital itself, and methyl substitution of a single nitrogen in the barbiturate nucleus does not change this property. If both nitrogens are methyl substituted, the molecule becomes inert. Such complexes do not change either the absorption spectra or optical density of the tetracyclines studied, suggesting that the barbiturate may not bind to the chromophoric part of the molecule.

In light of these findings, Dr. Kohn has performed equilibrium dialysis studies to determine whether tetracyclines bind to nucleic acids and proteins. The most striking results are obtained with deoxyribonucleic acids, which bind the drugs in the presence of calcium, zinc, or magnesium ions. Binding does not occur in the absence of divalent metal ions. Heat denaturation increases the binding ability of deoxyribonucleic acids. Ribonucleic acids bind tetracyclines less well and albumin binds to only a small extent. The degree of binding is increased in the latter cases by zinc ions but not by calcium or magnesium.

MISCELLANEOUS STUDIES

An interest in the chemotherapeutic effect of riboflavin antagonists has characterized Dr. Lane's activities for the last 5 years because some of these drugs retarded the growth of certain rat neoplasms but did not produce riboflavin deficiency in man (Annual Reports 1955, 1956). The Upjohn Company cooperated most generously in these studies, and some time ago Merck and Company made a different analog of riboflavin—galactoflavin—available for this research. Galactoflavin has produced typical riboflavin deficiency in two patients with disseminated cancer, and it may now be possible to determine the effect of this deficiency state on the growth of clinical cancers.

Limited experience in the treatment of cancer patients with either methyl-glyoxol-bis-guanylhydrozone or narcotine has not elicited any favorable responses.

A compound related to actidione, known as E37, has been studied by Dr. Rall in conjunction with the Hazleton laboratories. Female rats are more

susceptible to its toxic effect than are male rats, but such sex differences are not found in dogs. This drug which Dr. Goldin finds effective against leukemia L1210 produces a peculiar hemorrhagic and necrotic lesion in the lungs of dogs similar to the lesion described by investigators at the Sloan-Kettering Institute in the lungs of patients who had received this agent.

Service Functions

Requests for consultation by members of the Surgery Branch, NCI, have increased from 294 in 1957 to 437 in 1959. All of these requests are processed through Dr. Smith's office. The greatest change in demand has been for urological services which has increased 7 times over the 1957 figure. It is difficult to recruit and retain a staff of capable surgeons because of the obvious economic advantages of private or group practice.

The same considerations apply in an important degree to the generality of our senior clinical investigators. Our pay scale is just too low in relation to the opportunities that university medical schools can provide.

Mr. Joseph Albrecht and his group of excellent histopathology technicians have provided this staff with a total of 154,000 stained histological preparations of which 20,000 required special stains. The average cost was about $1.12 per slide, regardless of the stain used. This is an unusually low figure, especially when one considers that the cost of training unskilled recruits is included.

Members of the Pathologic Anatomy Branch performed 265 necropsies during 1959 of which 161 were performed for NCI. They examined 2,601 surgical specimens and accessioned 3,547 cytodiagnosis specimens. Many of their research contributions are included in the foregoing material.

Mr. R. J. Koegel's analytical group in the Laboratory of Biochemistry accepted 50 percent more specimens for microchemical analysis during 1959, despite a decrease of 15 percent in available man hours. His program on infrared spectrophotometric research has operated at a reduced level, but 20 percent of the samples submitted for empirical analysis are now studied by infrared techniques for purposes of structural interpretation and as an indication of chemical purity.

The Animal Production Section of the Laboratory Aids Branch, DRS, converted to a fee-for-service basis in July 1958. The staff of NCI was therefore required to predict the need for mice, rats, guinea pigs, hamsters, and rabbits for an entire year. The prediction proved correct within 10 percent of actual usage. This is no small feat, when one considers the number of persons involved, and emphasizes the ability and desire of the intramural research staff to accept responsibility, discharge it most capably, and cooperate with other organizational segments of NIH in solving difficult problems of mutual interest.

This long report is liberally sprinkled with problems requiring the attention of many people. Our colleagues are creative. The information issuing from the clinic and the laboratories is important to the cancer patient; yet some of it requires a period of further development to assay its utility. We would prefer to accomplish this within the framework of a direct or contractual operation of NCI and hope that we may be encouraged to do so.

NATIONAL HEART INSTITUTE

The Heart Institute is charged with responsibility for research aimed at improving methods for prevention and treatment of disorders of the cardiovascular system.

Organization of intramural research in the Heart Institute is based on the premise that progress toward any goal in science is best made by the creative efforts of individuals motivated by their own intellectual curiosity toward the solution of problems that interest them. It does not seem appropriate to attempt to divide research into the categories of "applied" or "basic," since there would be little agreement on how such a classification could be made or even on how the groups would be defined. One suspects that the scientist himself would accept the proposition that the man working on his own problems and following whatever leads may arise is doing basic research, while the man working on problems devised by someone else is doing applied research.

The problems which interest some individuals may have immediate practical significance; that which motivates others may not. Thus, within the Heart Institute there are men interested in improving the treatment of hypertension, in the improvement of diagnostic technics and surgical procedures for the correction of anatomical defects of the heart, while others are concerned with exploring the mechanism of chemical reactions or the relation between the structure of a protein and its biologic function.

Only through both types of activity can the long-term goals be achieved and the best assurance of maximum progress is a high level of general scientific productivity. The major responsibility of those charged with the leadership of intramural research is to assure maximum productivity by the selection of men (for the promise of their areas of interest as well as their capacity to contribute) and the provision of an environment and facilities most conducive to scientific accomplishment and interdisciplinary collaboration.

The pages that follow reflect the scientific progress within the individual research groups of the Heart Institute in the last year largely as seen by the leaders of those groups.

Laboratory of Cellular Physiology and Metabolism

SECTION ON CELLULAR PHYSIOLOGY

The work of the Laboratory of Cellular Physiology and Metabolism, Section on Cellular Physiology, continues to be aimed at elucidation of protein structure, the relationships between this structure and specific function, and with the synthesis of proteins with respect both to biochemical mechanism and genetic control.

During the past year the work of the Section dealt with these areas: (1) The development of methods for the study of protein structure and the application of these methods to ribonuclease, lysozyme, and several other proteins. These studies also interlock with investigations on the relationships between structure and function in biologically active proteins; (2) Investigations of the genetic control of the biosynthesis of proteins of bacteriophage with emphasis on the enzyme lysozyme, a catalyst employed by the phage particles for rupturing the cell wall of their host bacterial cell; (3) Investigations of the secondary and tertiary structure of certain fibrous proteins and fibrous protein models; (4) Biosynthesis of proteins in the hen's oviduct and a detailed study of certain lipid substances which appear to be intimately involved with the biosynthetic process; and (5) Studies on the metabolism of triglycerides by adipose tissue and liver.

(1) The complete structure of ribonuclease has now been worked out in detail, through the combined efforts of Dr. Werner Hirs and his colleagues at the Rockefeller Institute, and Dr. Anfinsen and his colleagues of the Section on Cellular Physiology. Certain inconsistencies between the results of the two groups have been investigated in some detail and resolved in the past few months. These inconsistencies were concerned mainly with two of the 124 amino acid residues of ribonuclease

whose positions in the polypeptide chain, as reported by Hirs et al., required inversion on the basis of the NHI data. This relatively minor point has been examined by a series of controlled proteolytic digestions and quantitative analyses. These detailed studies were of special interest since they involve the portion of the polypeptide chain which other studies suggest is involved in the enzyme's active center.

In a continuation of earlier research it has been shown that all four disulfide bridges in ribonuclease can be cleaved by reduction with mercaptoethanol and that the resulting inactive product can be converted to the orginal native molecule by simple exposure to atmospheric oxygen. Earlier uncertainties regarding the proper matching of half-cystine residues have now been resolved by the demonstration that the pairing of such sulfhydryl side chains is almost certainly identical with that found in the native protein and by the demonstration that regenerated protein is indistinguishable from native protein in immunochemical cross reactions. It has also been possible to show that approximately one-fifth of the polypeptide chain of the native molecule can be removed before the reduction-reoxidation procedure without destroying the "regeneratability" of the disulfide bonds in the remaining four-fifths of the protein. In terms of "genetic information," therefore, it seems possible to state that the information necessary for proper disulfide bridge formation is coded into only four-fifths of the molecule and that the rest of the chain must be present for other biological reasons. These studies are being continued with the aim of reducing the protein to a minimum size which will still permit reduction and reoxidation with the formation of an active regenerated substance. It is also planned to continue the work on stepwise reduction and stepwise reoxidation in an effort to prepare active intermediates which differ significantly in gross structure from the native enzyme. Preliminary studies have already indicated that several amino acids at the ends of the reduced, extended chain are superfluous from the standpoint of function and more drastic degradation is therefore indicated.

The species comparisons which showed differences in structure between sheep and beef ribonuclease—as reported in last year's annual report—now have been extended to porcine pancreatic ribonuclease. Although the latter is superficially identical with the bovine enzyme in covalent structure it is totally nonreactive with antiserum prepared against bovine ribonuclease (with first course serum, but reactive with second and third course serum). These immunological observations indicate the necessity for a more detailed study of comparative structure since they suggest that there may be some drastic, but not obvious, difference, perhaps in the nature of the pairing of disulfide bridges. Highly purified preparations of ribonuclease have also been made from spinach leaves and *B. subtilis*, although not in sufficient quantities or purity for attempting structural analyses. The species comparisons will be continued since they should lead to information on common denominators of structure which would inferentially suggest the location and nature of the active center of ribonucleases in general.

The variation of protein structure among various species is also being studied by preparing lysozymes from several bacteriophages and from the egg whites of a broad spectrum of birds. These studies, being carried out in part in collaboration with Professor Charles Sibley at Cornell, are at the moment mainly concerned with the development of simple, reproducible methods for the isolation of lysozyme from egg whites and such a method is now essentially free of difficulties. It involves adsorption of the very basic lysozyme protein onto the cation exchanger XE–64, followed by elution and purification on columns of the same resin. Comparisons of structure can then be made on the purified proteins by separation of peptides produced by tryptic and chymotryptic digests on paper sheets, using chromatographic and electrophoretic methods—the so-called "fingerprinting" technique. Preliminary results already indicate that the lysozymes from species to species will vary considerably less in structure than ovalbumins from the same species, supporting the hypothesis that enzymes can, in general, suffer less change during evolution than proteins whose functions are more concerned with storage or cellular architecture.

(2) A major effort is being made to determine whether or not there exists a direct correspondence between the arrangement of genetic subunits in specific genes and the structure of the protein controlled by this particular gene. Since lysozyme from bacteriophages is a relatively small

and easily isolated protein, and since mutant forms of bacteriophages should be relatively easy to isolate and subject to genetic mapping, this protein has been chosen for special study. A number of mutant forms of bacteriophage T2 and T4 have been isolated which, during growth, release lysozymes of varying heat stability into the surrounding medium. It is the present plan to make genetic crosses of these mutants for the purpose of gene mapping and to isolate the lysozymes in pure form for direct comparison of structures. A major aspect of the work at the moment involves the study of the sequential structure of both bacteriophage and egg-white lysozymes in order to provide baselines for future studies on the relationships between structure and function. It is also planned to investigate whether or not nonlethal mutations will, as might be predicted, only occur in those areas of the lysozyme molecule that are not essential for activity.

(3) The gross molecular structure of myosin, the protein unit of the muscle contractile mechanism, has been under active study by Drs. Harrington and Mihalyi. It was observed that the molecular weight of the myosin particle was decreased from 619,000 to 206,000 by concentrated guanidine solutions which tend to rupture hydrogen bonds and break protein polymers into smaller units. The conclusions reached from ultra-centrifugation studies were supported by sedimentation, diffusion and viscosity measurements. These results, when considered together with observed length and width and with the optical rotatory and X-ray diffraction properties of the myosin molecule, led to the conclusion that myosin is made up of three identical polypeptide chains, each wound into an α-helix and with the three strands twisted together to form a rope-like structure. Studies of the primary sequence of the myosin unit chain are now in progress. These should reveal whether all three are oriented in the same direction. The details of primary structure may then be used to explain the secondary and tertiary coiling and folding. Efforts are also being directed at determining the mechanism by which myosin subunits polymerize to form the aggregates characteristic of myofibrils. A second group of fibrous protein or protein-like molecules that have been thoroughly investigated are the collagens and collagen-like proline-glycine copolymers. Various proteolytic enzymes have been employed as specific probes of the second structure (that is, the internal coiling) of these long polypeptide chains and it appears that here, as in the case of myosin, these long molecules are made up of alternating amorphous and semicrystalline regions with differential sensitivity to proteolysis. The kinetics of proteolytic digestion suggests that neighboring charged groups strongly influence the susceptibility of sensitive bonds to hydrolysis.

(4) In the biosynthesis of proteins in the hen's oviduct, certain lipid components appear to be associated with an extremely active pool of amino acids. Dr. Hendler has separated these on alumina-silica columns in quantities for direct chemical study. When oviduct tissue or the bacterium *E. coli* is incubated with radioactive amino acids, the first metabolic pool to become labeled is a class of organic soluble substances which carry amino acids and peptide-like compounds. Whether or not the bond between the amino acids and peptides and the lipid moieties is covalent has not been established. The combined information on these interesting compounds suggests that they may be involved in the biosynthetic processes taking place in the so-called endoplasmic reticulum, which, it has been suggested, may be associated with protein synthesis. A study on the dissociation of this endoplasmic reticulum into its lipid components and ribosomal granules and the subsequent separation of these various components on ion exchange columns has been undertaken in collaboration with Drs. Peterson and Kuff of the National Cancer Institute. It is believed that information for making particular proteins is contained in the configuration of the nucleic acids of the ribosomal granules. On this basis one should expect that these granules will be heterogeneous and that it may be possible to fractionate them into classes, each responsible for a particular protein or group of proteins.

(5) Work on triglyceride metabolism and on the nature of the heparin-induced lipoprotein lipase—under investigation in this laboratory for a number of years—has been continued by Dr. Korn. Lipoprotein lipase has been subjected to further purification with the purpose of determining whether heparin is an integral part of the enzyme.

Another group of long-chain polysaccharides associated with the yeast cell wall has also been

investigated. These experiments, carried out by Dr. Korn in collaboration with Dr. D. H. Northcote at Cambridge University, have led to the isolation of fractions of yeast cell walls much more highly purified and better characterized than has previously been obtained. The studies on cell wall chemistry serve as models for the study and understanding of other conjugated proteins. The techniques for handling large conjugated proteins are relatively similar, whether the conjugated material is lipid or carbohydrate, and these studies should serve, therefore, as excellent background for the projected investigations of lipoproteins.

Dr. Rodbell has continued his investigation of the metabolic processes involved in the removal of chylomicrons of plasma. Rat epididymal adipose tissue does not distinguish between rat chylomicrons and synthetic fat emulsions with respect to uptake and metabolism, suggesting that chylomicron *proteins* are not essential for fat uptake or metabolism. Inhibition of lipoprotein lipase did not substantially reduce fat uptake, suggesting that this enzyme is perhaps necessary for chylomicron metabolism but not for transport into cells. C^{14}-labeled triglycerides were taken up from blood by the parenchymal cells of rat liver and these triglycerides were found to be associated with the microsomes and nuclei of the liver cells. The triglycerides are then converted to phospholipids and triglycerides characteristic of normal liver fats. These studies, in general, have implicated the microsomes in the absorption of exogenous fat by liver cells and it would appear that the endoplasmic reticulum may serve both as a channel for the entry of exogenous triglycerides as well as the site for metabolism and transformation.

SECTION ON METABOLISM

Studies on the basic physiology of fat absorption and fat transport

(a) Considerable progress has been made in studies of the metabolism of adipose tissue and the nature of its responses to hormonal factors. Last year it was reported that epinephrine added *in vitro* would stimulate the release of free fatty acids from adipose tissue. It has now been shown that glucagon and ACTH added *in vitro* also stimulate release of free fatty acids. Further studies revealed that all three of these hormones lead to an increase in the levels of active phosphorylase in adipose tissue and stimulate the uptake of glucose. It is of interest that this activity in the adipose tissue is quite analogous to the activities of these hormones on other peripheral tissues. Epinephrine and glucagon increase phosphorylase activity in the liver (but not in the adrenal) and ACTH increases phosphorylase activity in the adrenal (but not in the liver). Preliminary results suggested that these hormones might effect the observed increase in rate of release of fatty acids by inhibiting the synthesis of triglycerides. For this reason studies on the mechanism of triglyceride synthesis in adipose tissue were initiated. A cell-free system which will incorporate fatty acids into triglyceride has been derived from rat epididymal fat pads. This system carries out the first reported triglyceride synthesis in adipose tissue homogenates. The system requires α-glycerophosphate as a precursor and glycerol will not substitute for this requirement. ATP and Coenzyme A are required, presumably for the activation of the free fatty acids. Diglycerides of very high specific radioactivity have been isolated and are probably intermediates. Unlike the system in liver the adipose tissue homogenate does not accumulate phosphatidic acid but the requirement for α-glycerophosphate suggests that this is nevertheless an intermediate in the synthetic pathway. These studies are being pursued in the hope that with a better understanding of adipose tissue metabolism it may be possible to demonstrate the site at which the several hormones discussed above interact with the enzymatic mechanisms controlling fat deposition and release.

Heparin is known to lead to a marked increase in the levels of lipoprotein lipase in the serum. Studies completed this year show that addition of heparin to adipose tissue *in vitro* leads to a striking outpouring of lipoprotein lipase from the tissue into the medium. Also of interest is the finding that the levels of lipoprotein lipase in the adipose tissue of fasting rats is considerably lower than the level found in the tissues of carbohydrate-fed rats. Thus, the levels of lipoprotein lipase, rather than paralleling the rate of release of fatty acids, vary inversely with the rate of release of fatty acids. These results suggest that

the role of lipoprotein lipase may be in the uptake of fat rather than in its release.

(b) A study has been made of the fatty-acid composition of the chylomicron fat in patients fed large meals of different types of fat. It was found that the pattern of fatty acids in the chylomicron resembles very closely the pattern of the dietary fat used. These results are clearcut and disagree with results reported by Dole of the Rockefeller Institute, who claimed that there were large differences between the composition of fats fed and the fat in the chylomicrons during absorption. The disagreement may stem from the failure of the latter investigator to completely remove low density lipoproteins from chylomicrons prior to analysis.

Administration of carbohydrate by mouth or intravenously reduces considerably the rate of absorption of fat, as shown by studies carried out in rats with cannulated thoracic ducts.

(c) Kinetic studies on the utilization of injected C^{14}-fatty acids have been continued and analysis of these results shows that at least 50 percent of the fat utilized during fasting is transported through the serum as free fatty acid. Injection of epinephrine raises the net turnover of free fatty acids. During exercise there is a marked increase in net fatty acid utilization but the fraction accounted for by transport through the FFA fraction falls considerably.

Studies of dietary and hormonal factors determining serum lipoprotein levels

(a) Adrenal Control of Lipoprotein Levels. It has been shown that injection of epinephrine in oil not only elevates the plasma levels of free fatty acids (FFA) but also leads to an elevation of lipoprotein levels. The FFA response occurs early and is transient; the rise in lipoproteins does not occur until 12 to 24 hours after epinephrine injection. Studies completed this year showed that adrenalectomy or hypophysectomy abolished both the FFA and the lipoprotein responses to epinephrine. Pretreatment of the operated animals with cortisone or with ACTH, respectively, restored their ability to respond to epinephrine with both a rise in FFA and in lipoproteins. Administration of cortisone to normal dogs exaggerated the lipoprotein response to epinephrine injection. When the animals received extra cortisone as much as an 80 percent rise in serum cholesterol was obtained with three daily injections of epinephrine.

These results suggest a physiologic basis for the hypercholesterolemia of stress. It is well known that animals and patients under stress demonstrate hyperactivity of both the adrenal medulla and the adrenal cortex. This pattern of hormone production would appear to be adequate to explain elevations of both FFA and cholesterol (lipoproteins). Studies are currently in progress to evaluate the response of patients to exogenous epinephrine and cortisone.

(b) Studies of the effects of dietary fat on cholesterol excretion have been completed. Of major interest was the observation that a surprisingly large fraction of the cholesterol excreted in feces in man appears there in the form of cholesterol itself (35 to 80%). This is in contrast to the pattern in rats and other laboratory animals in which practically all of the cholesterol excreted appears in the feces in the form of bile acids. A study of eight patients fails to reveal any consistent effect of unsaturated fats on the rate of excretion of intravenously administered C^{14}-cholesterol in the feces. The mechanism by which dietary fats modify cholesterol levels has not been established. The effect may be on a redistribution of cholesterol within the body but this has not been established in man.

Parallel with the studies of cholesterol excretion, bile acid turnover studies have been done under various dietary conditions and in various clinical conditions. These studies were carried out in collaboration with Dr. Sven Lindstedt from Sweden. Results of the study are not yet complete. These collaborative studies are continuing in order to determine whether there are systematic differences in bile acid turnover in various forms of hypercholesterolemia.

(c) Studies of the production of lipoproteins by rat liver slices *in vitro* were continued and definitive identification of alpha-1-lipoprotein was obtained. This was done by preparing lipoproteins *in vitro* from a complete mixture of C^{14}-amino acids, purifying them, digesting with trypsin and chymotrypsin, and chromatographing the mixture of peptides in two dimensions. It was found that all of the peptides derived from

the alpha-1-lipoprotein coincided with peptides derived from alpha-1-lipoprotein prepared from normal rat serum. The identification of the beta-lipoproteins with serum beta-lipoproteins was inconclusive.

It was shown that the rate of cholesterol synthesis is not apparently a rate-limiting reaction in lipoprotein synthesis. Liver slices taken from cholesterol-fed rats (in which the rate of cholesterol synthesis is markedly suppressed) incorporated labeled amino acids into the protein moiety of lipoproteins at a normal rate. Conversely, accelerating the rate of cholesterol synthesis by injection of Triton did not increase the rate of synthesis of lipoprotein protein.

(d) In collaboration with investigators at the University of Maryland a study of modified milk fat was carried out. Dr. Shaw and his coworkers in the dairy department at Maryland University were able to alter the iodine number of milk fat by appropriate changes in feed. However, the changes were relatively small (increase in iodine number from 30 to 48) and no significant difference in the effects of fat of these two types was demonstrable in patients. Thus, it appears that unless a more radical change can be effected this approach to the problem of dietary fat will not be suitable.

(e) The technique previously described for incorporating cholesterol into lipoproteins has proved valuable for the incorporation of other nonpolar molecules. In particular, the technique serves to incorporate carcinogenic hydrocarbons so that these can be administered intravenously in known quantities and their metabolism studied. Studies of these hydrocarbons have been hampered because of their insolubility and the resultant uncertainty in evaluating absorption and distribution.

Studies on the metabolism of cholesterol and therapeutic agents useful in lowering serum cholesterol levels

(a) A new inhibitor of cholesterol biosynthesis produced by the Wm. S. Merrell Co. (MER–29) has been studied in animals and in man. This compound—1 - [p - (β - diethylaminoethoxy) - phenyl]-1-(p-tolyl)-2-(p-chlorophenyl) ethanol—was shown by Dr. Blohm to suppress markedly the incorporation of radioactive acetate into cholesterol and to lower the serum and tissue levels of cholesterol in rats. Studies in this laboratory with the collaboration of Dr. Erich Mosettig and Mr. Thompson of the Arthritis Institute have now established the probable site of action of the drug. It has been shown that 24-dehydrocholesterol accumulates in the liver of rats fed MER–29. It may account for as much as one-half of the total sterol in these livers. 24-dehydrocholesterol (desmosterol) has previously been shown to be a precursor of cholesterol in the rat. It differs from cholesterol only in having an additional double bond at the 24, 25 position in the side chain. Presumably it is converted to cholesterol by a simple reduction step. It will be of interest to explore the mechanism by which this new drug blocks this last step in cholesterol synthesis.

Clinical studies confirmed the work of others in that there was some lowering of serum cholesterol levels, although this was not marked. Some patients were studied on a diet free of cholesterol but this did not appear to magnify the response of the drug. It was possible to show that 24-dehydrocholesterol appears in the serum of treated patients in significant amounts. Because this sterol gives a lower color yield in the Lieberman-Burchard reaction the *apparent* drop in serum cholesterol obtained using the usual methods is misleading. While there is a slight decrease in total sterol it is smaller than would appear from the usual analyses. It will be important to evaluate the atherogenic potential as well as other metabolic effects of 24-dehydrocholesterol before extending clinical trials.

(b) A kinetic study of the distribution of C^{14}-cholesterol among the various tissues of the animal organism (rat and rabbit) has shown that every tissue, including brain tissue, takes up radioactive cholesterol from the serum. By extending the studies over a long time period it was shown for the first time that the specific radioactivity of the slowly metabolized cholesterol pools (brain, muscle, kidney) contained cholesterol of a higher specific radioactivity than that in the serum in the latter stages of the experiments. A simple mathematical model satisfactorily accounts for the observed results on the basis of isotopic exchange.

Studies on hypoalbuminemia and the mechanisms responsible for it

(a) A new clinical syndrome, *exudative enteropathy*, or *protein-losing gastro-enteropathy*,

has been described. This is a condition characterized by loss of plasma proteins into the intestine with a resultant lowering primarily of the level of serum albumin, but also that of other serum proteins as well. The patients in this category have previously been described as having "idiopathic hypercatabolic hypoproteinemia." By the use of a non-metabolizable polymer of molecular size comparable to that of albumin it has been shown that these patients lose into the G.I. tract much larger amounts of injected macromolecules than do normals. The fate of the polymer, polyvinylpyrrolidone, which is of molecular size comparable to that of serum albumin, probably reflects quite well the fate of circulating albumin molecules.

The diagnostic test using I^{131} labeled PVP is technically simple. Many medical centers have received samples prepared at NIH and a large number of cases have already been uncovered. A commercial firm is planning to produce labeled polymer for routine clinical use.

(b) Biopsies of intestinal mucosa were obtained in 6 cases of protein-losing gastroenteropathy. In 5 of these a common lesion consisting of markedly dilated lymphatics within the villi was demonstrable. This, combined with the fact that many cases have had chylous effusions, suggests that there may be a common etiology somehow associated with the lymphatic system.

(c) Dr. Gordon carried out similar studies in patients with Asiatic cholera in Bangkok. There was not evidence of excessive loss of protein into the intestinal tract. This negative finding is not consistent with the generally accepted concept that there is serious desquamation of the intestinal mucosa in cholera.

Studies on the mechanisms of protein synthesis and degradation

Conclusive evidence of the incorporation of amino acid analogues into crystalline proteins was obtained and published. A comprehensive review of "The Specificity of Protein Biosynthesis" was prepared and published in *Advances in Protein Chemistry*. Preliminary studies that have revealed the presence in mammalian tissue of peptides apparently conjugated to nucleotides were completed and published. This phase of the laboratory program has now been, temporarily at least, discontinued.

Basic studies on the structure of proteins and the nature of the clotting process

Investigations of the fundamental mechanism of fibrin formation were continued with particular reference to the proposed role of tyrosine residues. By the application of highly sophisticated spectrophotometric methods it was shown that the tyrosine residues not titrated in fibrinogen are probably not involved in hydrogen bonds but rather masked by some sort of hydrophobic bonding. These studies are being continued, using careful kinetic analysis of pH changes in order to clarify the mechanisms of the fibrinogen-fibrin transformation.

Studies on the disturbed metabolism of lipids in nephrosis and on the immunochemical mechanisms involved

(a) A comprehensive study of the serum lipid pattern in patients with nephrosis has forced a revision of the usual concept that only the very low density lipoproteins are elevated. Many patients were found to have the most marked elevation in the β_1-lipoprotein fraction. It was observed that during improvement due to steroid therapy the lipoprotein pattern undergoes shifts toward the higher density β-lipoproteins. These findings refute the theory of Gitlin that the defect in nephrosis is a deficiency in the conversion of very low density lipoproteins to higher density lipoproteins.

(b) It has been repeatedly shown that infusion of albumin lowers the lipid levels in nephrosis. Surprisingly it now appears from studies on nephrotic rats done in this laboratory that infusion of inert macromolecules such as dextran and polyvinylpyrrolidone also causes a decrease in lipid levels.

(c) Intravenous infusions of glucose generally cause some decrease in serum cholesterol level and little change in serum triglyceride levels. In three cases of nephrosis, however, glucose infusion caused a marked rise in triglyceride levels and in low density lipoprotein levels. The significance of these results is not yet determined but will be investigated further.

Development of techniques for radioassay in the liquid scintillation spectrometer

The new approach described in last year's report has now been in use for over a year and has proved to be a very valuable adjunct in radioassay. It has been shown that the method has a wide range of applicability. It has been effectively used for assay of tritium, C^{14}, Ca^{45}, and it has been shown in pilot studies that it is applicable for counting P^{32} and I^{131}. The method has received wide acceptance, particularly for the assay of weak beta emitters. Studies are being continued to determine whether the method can be used for pure gamma emitters and weak X-ray emitters.

SECTION ON ENZYMES

The activities of the Section on Enzymes have been directed toward elucidation of the following diverse fundamental biochemical processes: (1) the metabolism of heterocyclic compounds, (2) the metabolism of three carbon compounds, (3) cellular differentiation and protein synthesis, (4) anaerobic oxidative phosphorylation and electron transport, (5) nucleotide decomposition, (6) the metabolism of onium compounds, (7) the metabolism of isoprene derivatives, and (8) the metabolism of amino acids.

The Metabolism of Heterocyclic Compounds

(a) Riboflavin Degradation (Drs. E. R. Stadtman, P. Z. Smyrniotis, and L. Tsai). Previous studies in this laboratory have shown that the oxidative dissimilation of riboflavin to ammonia and CO_2 by an aerobic bacterium involves the intermediary formation of 1-ribityl-2,3-diketo-1, 2,3,4-tetrahydro-6,7-dimethylquinoxaline (compound I) and 3,4-dimethyl-6-carboxy-α-pyrone (compound III). Evidence has now been obtained showing that 3,4-dimethyl-2,3-quinoxalinediol (compound II) and oxamide are intermediates in the conversion of compound I to compound III. The conversion of riboflavin to compound I involves a cleavage of the pyrimidine ring (ring C) with a stoichiometric formation of urea and CO_2. This transformation is of special interest since from the overall chemical point of view it can be represented as a simple hydrolytic process; however, it occurs only in the presence of molecular oxygen. The conversion of compound I to compound II involves a cleavage of the N-ribityl linkage. This cleavage is also of unique interest since it too requires molecular oxygen. Although the exact fate of the ribityl moiety is still unknown, the oxygen requirement is not restricted to oxidative degradation of the side chain since oxygen is required also for the cleavage of the N-hydroxyethyl, N-methyl, and N-acetaldehyde analogues. The further degradation of compound II to a mixture of oxamide and the α-pyrone derivative is obviously a complicated process. This conversion is inhibited by arsenite, iodoacetate and hydroxylamine and is activated by various oxidizable substrates such as ethanol, lactate, pyruvate and glucose. Further studies on the mechanism of the individual reactions in these various transformations are in progress.

(b) Phenazine-1-Carboxylic Acid Biosynthesis (Dr. M. Levitch). The bacterium *Pseudomonas aureofaciens* Kluyver offers a unique opportunity to investigate the biosynthesis of the heterocyclic phenazine ring system since this organism produces unusually large quantities (1.0 gm/liter) of phenazine-1-carboxylic acid during growth. Further insight into the mechanism of this biosynthetic process has been sought by measuring the incorporation of isotope carbon into phenazine-1-carboxylic acid when bacterium is grown in a medium supplemented with various C^{14}-labeled compounds. Of numerous compounds tested, the most effective precursors are acetate, bicarbonate, alanine, serine and methionine. Methods for the stepwise degradation of the phenazine derivative to permit a determination of the distribution of labeled carbon from the various precursor compounds are now being investigated. It is hoped that the results of these studies will suggest an intelligent approach to the problem of phenazine biosynthesis at the enzyme level.

(c) Alkaloid Biosynthesis (Drs. E. Kravitz and P. R. Vagelos). Studies of the biosynthesis of opium alkaloids by tissue preparations of the poppy plant, *Papaver somniferum*, have been undertaken as an additional effort to obtain basic information on the biochemistry of heterocyclic compounds. The poppy plants were supplied by the USDA Plant Industry Station in Beltsville, Md. Progress to date has been restricted to the

development of optimal experimental conditions for the *in vitro* synthesis of alkaloids by tissue slices and the development of procedures for the isolation and separation of the various alkaloids produced. Alkaloid synthesis has been followed by measuring the incorporation of isotopic carbon into the alkaloid fraction after incubating the plant preparations with methyl-C^{14}-methionine or β-C^{14} serine, which were introduced by the vacuum infiltration technique. It has been found that with 4 to 5 week old plants the isotope is incorporated predominantly into narcotine and papaverine, whereas in older plants labeled morphine and codeine were also produced (identification of the various alkaloids is still tentative). Studies with tissue slices derived from various parts of the plant have revealed that the roots are by far the most active sites of alkaloid synthesis. In future studies efforts will be made to develop cell-free preparations of roots that are capable of catalyzing alkaloid synthesis.

An ion exchange procedure using Dowex-1-OH^- and Dowex-50-H^+ has been devised for the separation of the major opium alkaloids.

In addition to the above investigation, studies have been initiated to investigate the biosynthesis of the ergot alkaloids by the fungus *Claviceps purpura.*

The Metabolism of Three-carbon Compounds

(a) Propionic Acid Oxidation (Drs. P. R. Vagelos and W. Sly). In previous studies, Dr. Vagelos has shown that cell-free enzyme preparations of the bacterium, *Clostridium kluyveri*, catalyze the oxidation of propionate by a pathway involving the intermediary sequential formation of propionyl CoA, acrylyl CoA, β-hydroxypropionyl CoA, malonylsemialdehyde CoA, and malonyl CoA. Further studies of this metabolism have led to the discovery of a curious exchange reaction between the carboxyl group of malonyl CoA and added $C^{14}O_2$. This exchange is absolutely dependent upon the presence of catalytic amounts of an acyl CoA derivative of a saturated fatty acid having 4 to 16 carbon atoms. In view of the fact that malonyl CoA and CO_2 have been shown recently to be involved in the biosynthesis of fatty acids, it appears probable that the observed exchange reaction represents one step in fatty acid synthesis. The exact mechanism of this reaction is therefore of immediate interest and is under further investigation.

The enzyme catalyzing the TPN-coupled oxidation of malonyl-semialdehyde CoA to malonyl CoA has been partially purified and is under further study.

Incidental to these investigations has been the development of a good general method for the chemical synthesis of β-ketothiolesters.

(b) The Role of Biotin and Vitamin B_{12}-Coenzyme in Propionate Metabolism (Dr. E. R. Stadtman in collaboration with Mr. P. Overath and Prof. F. Lynen in the Max Planck Institute fur Zellchemie, Munchen, Germany). Previous studies by Flavin et al., with animal enzymes, and studies by Whitely, Carson, Wood and Delwiche, with enzymes derived from propionic acid fermenting bacteria, have established an intermediary role for succinyl CoA and methylmalonyl CoA in the metabolism of propionic acid. A consideration of the fact that propionic acid formation represents the major metabolic process catalyzed by bacteria belonging to the genus *Propionibacteria* and that these organisms possess unusually high concentrations of vitamin B_{12} coenzyme and biotin, have prompted an investigation to determine if these vitamins are involved in propionic acid metabolism. Propionic acid metabolism in cell-free extracts of *Propionibacterium shermanii* was measured by the overall incorporation of 1-C^{14}-proprionate into succinate. After treatment with protamine and charcoal and then dialysis, cell-free extracts lose their ability to catalyze the incorporation of labelled propionate into succinate. This ability is restored by the addition of catalytic levels of acetyl CoA and a light-labile factor present in boiled extracts. The latter factor is completely replaced with low concentrations (10^{-8}M) of pure dimethylbenzyimidazole-B_{12}-coenzyme (supplied by H. A. Barker). A role of biotin in the propionate exchange system is indicated by the fact that the reactivated enzyme is completely inhibited by avidin but not by avidin which has been pretreated with biotin. In light of the recent report that the succinyl-CoA isomerase activity of rat liver is lowered in B_{12} deficiency, the above findings form the basis of a working hypothesis that the propionate-succinate exchange is the net result of two

556044—60——5

vitamin coenzyme-linked reactions: (1) the B_{12}-coenzyme dependent isomerization of succinyl CoA to form methylmalonyl CoA, and (2) the reaction of methylmalonyl CoA with biotin-enzyme to form a biotin-enzyme-CO_2 complex and propionyl CoA. The reversible exchange of propionyl CoA with free labeled propionate and reversibility of the other postulated reactions could account for the observed results. This hypothesis is under investigation.

(c) Propionic Acid Fermentation by Clostridium Propionicum (Dr. H. Goldfine). In continuing studies on the anaerobic fermentation of three carbon compounds it was found that cell-free extracts of *C. propionicum* convert pyruvate, lactate and serine predominantly to acetate and CO_2, whereas α-alanine and β-alanine are converted mainly to propionate. Evidence was obtained supporting the conclusion that propionate formation from β-alanine proceeds by the following pathway: β - alanine$\rightarrow\beta$ - hydroxypropionate $\rightarrow\beta$-hydroxypropionyl CoA$\rightarrow$acrylyl CoA$\rightarrow$propionyl CoA$\rightarrow$propionate. The first step, i.e., the conversion of β-alanine to β-hydroxypropionate involves the release of ammonia and is obligately dependent upon the presence of catalytic amounts of pyruvate and α-ketoglutarate. The latter observation and the demonstration that β-alanine serves as an amino group donor to form α-alanine and glutamate from the corresponding α-keto-acids together with the further discovery that the DPN-linked oxidative deamination of α-alanine occurs only in the presence of α-ketoglutarate, supports the conclusion that the formation of β-hydroxypropionate from β-alanine occurs by a transamination of the amino group of β-alanine to pyruvate, thence to α-ketoglutarate, and then the release of the amino group as free ammonia by the action of glutamic dehydrogenase. As yet no evidence has been obtained for the formation of malonyl semialdehyde as the expected intermediary in β-alanine transamination.

The Biochemistry of Cellular Differentiation and Protein Synthesis.

(a) Amino Acid and Protein Metabolism in the Slime Mold (Dr. B. K. Wright, Mr. G. McNeil and Miss Minnie Anderson). Studies on the turnover of amino acids and protein during cellular differentiation of the slime mold *D. discoideum* have been continued. It has been found that the differentiation process is associated with a net decrease in protein content, but that active protein synthesis, as measured by the incorporation of S^{35} methionine into the protein fraction, occurs throughout all stages of development. At preculmination the methionine in protein is replaced by the endogenous pool S^{35}-methionine at a rate of about 7 percent per hour. A unique feature of this metabolic system is the discovery that the size of the "free" endogenous methionine pool is not influenced by changes in the exogenous methionine concentration. Although fixed in size at any given stage of development, the endogenous methionine pool can nevertheless undergo exchange with exogenous S^{35}-methionine, and the extent of this exchange (i.e., the specific isotope content of the pool methionine at equilibrium) is a linear function of the exogenous S^{35}-methionine concentration. This curious phenomenon remains as yet unexplained. The results suggest the possible existence of a heterogeneous endogenous methionine pool, in which the exchangeability of various parts is differentially influenced by the external methionine concentration.

Following momentary exposure of the organism to S^{35} methionine, the separation of cellular proteins into various arbitrary classes by means of solubility in ethanol and by DEAE column chromatography has revealed marked differences in the rates of isotope incorporation into the various protein classes. From such studies evidence has accumulated which indicates that methionine molecules in various parts of the amino acid pool are "fixed" with respect to the proteins into which they are incorporated. It appears that, on the average, pool methionine molecules which exchange readily with exogenous S^{35}-methionine are most readily incorporated into certain protein fractions which attain a relatively high specific radioactivity, whereas pool methionine molecules exchanging poorly with exogenous S^{35}-methionine are preferentially incorporated into protein fractions attaining a relatively low specific radioactivity. It is evident from the results obtained that the slime mold is particularly well suited for further studies on the biochemistry of protein metabolism.

(b) The Chemotactic Hormone, Acrasin (Dr. B. K. Wright and Mr. G. Liddel, in collaboration with Dr. E. Heftmann of NIAMD). Acrasin is the chemotactic hormone involved in initiation of aggregation at the onset of differentiation. A sterol with acrasin activity has been isolated from *D. discoideum* as a pure crystalline compound and has been identified as Δ^{22}-stigmasten-3B-ol. As judged by the lack of hormone activity in other fractions during purification and by the fact that the recovered acrasin accounts for most of the hormone activity of the crude cellular extract, it is concluded that this sterol is the major active compound present after acid hydrolysis. Since it is not as active as crude acrasin, attempts to isolate a conjugated form of this sterol are in progress.

Anaerobic Oxidative Phosphorylation and Electron Transport (Drs. E. B. Brown and E. R. Stadtman).

The reduction of crotonyl CoA to butyryl CoA by reduced pyridine nucleotide is associated with a standard free energy change of −14,000 calories and it has been postulated that this oxido-reduction system may be coupled with phosphorylation. In preliminary reports from another laboratory evidence has been presented to support the conclusion that ATP is produced during the reduction of crotonyl CoA to butyryl CoA by extracts of *Clostridium kluyveri.* Results of the present studies on this enzyme system suggest that the observed phosphorylation may not be associated with the reduction of crotonyl CoA *per se* but that it is derived indirectly by a dismutation of crotonyl CoA to butyryl CoA and acetyl CoA, followed by the formation of ATP from the latter compound via acetyl phosphate.

Nucleotide Decomposition (Drs. E. B. Brown and E .R. Stadtman).

Four separate ferrous iron-dependent nucleotidases were identified and partially purified from cell-free extracts of *C. propionicum.* Two of these enzymes are mononucleotidases sharing a remarkable degree of resistance to heat but differing in their sensitivity to versene inhibition; the other pair of enzymes are heat sensitive dinucleotidases separable on the basis of versene sensitivity. Successive action of the di- and mono-nucleotidases catalyzes the irreversible decomposition of diphosphopyridine nucleotide to adenosine, nicotinamide mononucleotide and two equivalents of orthophosphate.

The Metabolism of Onium Compounds

(a) The Anaerobic Fermentation of Choline (Drs. H. Hayward and T. C. Stadtman). An organism capable of deriving its carbon, nitrogen and energy for growth from the anaerobic dissimilation of choline was previously isolated from the soil and was shown to catalyze the conversion of choline to one mole of trimethylamine and one-half mole each of acetate and ethanol. This organism has now been identified as a new species belonging to the genus *Vibrio* and has been given the name *Vibrio cholinicus.* Studies with cell-free extracts of the organism have shown that choline degradation involves the intermediary formation of acetaldehyde which then undergoes a dismutation to form ethanol and acetate. By means of sedimentation in an ultracentrifuge, crude sonic extracts have been separated into a particulate fraction and a soluble fraction, both of which are needed to catalyze the decomposition of choline. The enzymes catalyzing the dismutation of acetaldehyde are present in the soluble fraction. This dismutation is catalyzed by the joint action of a TPN-specific ethanol dehydrogenase and an acetaldehyde dehydrogenase. Although the detailed mechanism of acetaldehyde oxidation has not been elaborated, it is significant that no dismutation occurs in the absence of TPN, ADP or a sulfhydryl compound; moreover, the reaction is markedly stimulated by the addition of ferrous iron or other divalent cations and by coenzyme A.

It has been further established that the dissimilation of choline by crude extracts is associated with the esterification of orthophospate to form ATP. The possibility that this phosphorylation is associated with electron transport is suggested by the fact that phosphorylation is inhibited by 2,4-dinitrophenol in concentrations known to uncouple oxidative phosphorylation. Preliminary, as yet inconclusive, evidence has been obtained that betainaldehyde is an intermediary in choline degradation.

The discovery that cell-free extracts of the *Vibrio* organism contain large amounts of a cytochrome pigment spectrally similar to animal cytochrome c was previously reported. A functional

role of this cytochrome in choline metabolism is suggested by the observation that cell-free extracts catalyze its reduction in the presence of choline.

(b) The Metabolism of Sulfonium Compounds (Dr. C. Wagner). The hydrolysis of sulfonium compounds at neutral pH is associated with a standard free energy change of about 21,000 calories. In an effort to investigate the possibility that cleavage of the sulfonium bond can be energetically coupled with cellular metabolism (as for example by the synthesis of ATP), an organism has been isolated from the soil that is capable of growing anaerobically with dimethyl-β-propiothetin as the major source of energy and carbon. Preliminary studies have been made to determine the nature of the fermentation process. It has been found that the decomposition of propiothetin is accompanied by the formation of propionic and acetic acids.

The Metabolism of Isoprene Derivatives

(a) Cholesterol Degradation. (Dr. M. G. Horning in collaboration with Prof. S. Bergstrom and Dr. H. Danielsson at the Karolinska Institute, Stockholm). Incubation of human red blood cells with cholesterol-4-C^{14} results in the formation of several degradation products which have been separated into three major classes, acids, diols, and triols, by means of reverse phase chromatography. Although neither cholic acid nor chenodeoxycholic acid could be detected in the acid fraction, one of the acids produced appears to be identical with a compound formed when cholesterol is incubated with liver mitochondria. This compound may be a di- or tri-hydroxy coprostanic acid. 3β,5α,6β-trihydroxycholestane was identified as a component of the triol fraction and one of the diols was identified as 7β-hydroxycholesterol. These results indicate that blood may have an important role in the metabolism of cholesterol. Future studies will be directed toward a more detailed analysis of this metabolic process at the enzyme level.

(b) Isoprenoid Degradation (Dr. W. Seubert). In order to facilitate studies of the biochemistry of polyisoprene metabolism, an aerobic bacterium has been isolated from the soil that can utilize a simple diisoprene derivative, citronellol, as its sole carbon and energy source for growth. This organism has been identified as a new species belonging to the genus *Pseudomonas* and has been designated *Pseudomonas citronellolis*. In addition to citronellol, higher analogues, such as farnesol and farnesoic acid, and the cyclic isoprenoid, β-ionone, are also utilized for growth. Results of various experiments to ascertain the mechanism of isoprenoid dissimilation have shown: (1) Citronellic acid accumulates in the culture medium as a transient intermediate during growth of the organism on citronellol; (2) In the presence of various isoprenoids, arsenite-inhibited resting cell suspensions catalyze the incorporation of $C^{14}O_2$ into acetate. This incorporation does not occur in the absence of isoprenoids or when the isoprenoids are replaced by straight chain saturated fatty acids; (3) Incubation of arsenite-inhibited cell suspensions with $C^{14}O_2$ and citronellic or farsenoic acids leads to the accumulation of β-keto-acids; (4) Cell-free extracts catalyze the incorporation of $C^{14}O_2$ into acetate in the presence of either dimethylacrylyl CoA, geranionyl CoA or farnesyl CoA; (5) Extracts catalyze the incorporation of $C^{14}O_2$ into acetoacetate in the presence of dimethylacrylyl CoA. These observations provide indirect support for the working hypothesis that the oxidation of polyisoprenoids involves a stepwise degradation of the terminal isoprene moiety by reactions analogous to those involved in the oxidation of isovaleric acid. Thus the oxidation of farnesol would be visualized to occur by the following reaction sequence:

I II III
Farnesol→farnesoic acid→farnesyl CoA→gera-
IV V
nylacetoacetate→geranionyl CoA→dimethylal-
VI VII
lylactoacetate→dimethylacrylyl CoA→acetoace-
VIII
tate→2 acetyl CoA.

According to this mechanism a fixation would occur at steps III, V, and VII.

The Metabolism of Amino Acids

(a) Threonine Biosynthesis (Dr. M. Flavin and Mr. C. Slaughter). The enzyme, threonine synthetase, catalyzes the conversion of 0-phosphohomoserine to threonine and orthophosphate. This novel reaction involves the elimination of an 0-phosphoryl group from the alpha position of

homoserine and the simultaneous introduction of an hydroxyl group into the beta position. In a continuation of studies on the mechanism of this reaction, the enzyme has been purified 500-fold from Neurospora extracts and some of its properties have been determined. Activity of the purified enzyme requires the presence of added pyridoxal phosphate. A further insight into the reaction mechanism has been obtained from studies with H_2O^{18} and D_2O carried out in collaboration with Dr. Tetsuro Kono of the McCollum-Pratt Institute. When the reaction is carried out in the presence of H_2O^{18}, O^{18} is incorporated into threonine but not into phosphate. O-phosphothreonine is not decomposed. From these observations it must be concluded that the phosphate group of phosphohomoserine is removed through cleavage of the C-O bond by an elimination reaction rather than by hydrolysis. When the reaction is carried out in the presence of 100 percent D_2O, two atoms of deuterium are incorporated into threonine, one in the α-position. On the basis of these results it is tentatively proposed that threonine biosynthesis involves the intermediary formation of a Schiff base of vinylglycine and pyridoxal phosphate. The further observation that only 0.1 atom of solvent hydrogen per mole is incorporated into threonine when the reaction is carried out in $H^3{}_2O$, indicates a high degree of discrimination against tritium. Tritium ions add to the postulated vinylglycine intermediate at only 2 to 3 percent of the rate of proton addition.

(b) The Reductive Deamination of Glycine. (Dr. T. C. Stadtman). Further studies have been made with the soluble enzyme system form *Clostridium sticklandii* that catalyzes the reduction of glycine to acetate and ammonia by 1,3-dimercaptopropanol, with the simultaneous esterification of orthophosphate to form ATP. Attempts to purify the enzymes involved have revealed that a minimum of four proteins are essential for catalysis of the overall reaction; hence the mechanism is more complicated than was anticipated. The conversion of glycine to acetate and the coupled phosphorylation are inhibited by antimycine A. This and other indirect evidence supports the belief that a quinone derivative is involved. Analysis of the lipid fraction of *C. sticklandii* failed to detect the presence of tocopherols or quinones of the vitamin K or Coenzyme Q types. During the course of these experiments, it was found that the characteristic reddish orange color of extracts of *C. sticklandii* is due to the presence of remarkably high concentrations of the adenine vitamin B_{12} coenzyme. Nutritional studies revealed that the concentration of the B_{12} coenzyme is a function of the rate of one-carbon metabolism by the cell. For example, supplementation of the culture medium with formate (which is fermentated largely to acetate) results in a much higher synthesis of the B_{12} coenzyme. The potential significance of the B_{12} coenzyme in one-carbon metabolism was further indicated by the discovery that two species of methane producing bacteria are exceptionally rich sources of the coenzyme. No evidence could be obtained to indicate that the B_{12} coenzyme is involved in the reduction of glycine to acetate. Evidence to the contrary was obtained by showing that two other strains of *Clostridia* capable of catalyzing the reduction of glycine to acetate, do not contain appreciable amounts of the B_{12} coenzyme.

(c) The Fermentation of γ-Aminobutyric Acid (Mr. J. Hardman and Dr. T. C. Stadtman). Previous studies in this laboratory have shown that the fermentation of γ-aminobutyrate by *Clostridium aminobutyricum* involves the intermediary formation of succinic semialdehyde and γ-hydroxybutyrate. The overall conversion of γ-aminobutyrate to form γ-hydroxybutyrate by cell-free extracts of this bacterium was found to be an oxidation-reduction process in which α-ketoglutarate and glutamate have catalytic roles. The reaction involves a transamination of the amino group of γ-aminobutyrate to α-ketoglutarate with the formation of succinic semialdehyde and glutamate; the latter compound then undergoes oxidative deamination by a DPN-specific dehydrogenase to form α-ketoglutarate and DPNH. The oxidation of glutamate is finally coupled with the reduction of succinic semialdehyde to form γ-hydroxybutyrate. The enzyme catalyzing the latter reaction has been purified about 20-fold from crude extracts. It is a DPN-specific dehydrogenase showing marked substrate specificity. It appears to be a zinc-sulfhydryl enzyme. A requirement for two proximal sulfhydryl groups is suggested by the observation that the enzyme is inhibited by concentrations of arsenite that selec-

tively react with disulfhydryl compounds. Aging of the purified enzyme results in a drastic loss of enzyme activity which is curiously restored by the addition of AMP.

(d) Anaerobic Metabolism of the Dibasic Amino Acids, Ornithine and Lysine (Dr. V. Tarantola). Although ornithine was originally reported by Stickland and Woods, in their classic studies on the Stickland reaction, to undergo reductive deamination, it would appear that the mechanism does not involve a direct reductive deamination. The final reduced and deaminated product, δ-amino valerate, produced by extracts of *Clostridium lentoputrescens*, appears rather to be formed via a pathway involving a preliminary formation of a keto acid derivative of ornithine by a transamination followed by ring closure and reduction to proline. A further reductive ring cleavage of proline by proline reductase and 1,3-dimercapto propanol yields δ-amino valerate.

A new clostridium, as yet unidentified, has been isolated from swamp mud that is capable of growing on lysine as a single amino acid substrate. The products—ammonia, acetate, and butyrate—indicate that the organism probably catalyzes the same coupled oxido-reduction reaction originally discovered in *C. sticklandii*. The new organism is unique in that it can grow as a result of this fermentation and thus may be much more suitable as experimental material for study of this interesting cleavage of the lysine molecule. In *C. sticklandii* the formation of acetate and butyrate from lysine requires lipoic acid but the enzyme system proved to be very unstable.

Laboratory of Chemistry of Natural Products

The work of the Laboratory during the past year may be summarized in four categories: (1) studies in the structural chemistry of naturally occurring substances, particularly the *Amaryllis* alkaloid group, (2) the development of new methodology for lipid studies, and the application of new methods to lipid problems, (3) studies of the callicrein-callidinogen-callidin system, with particular regard to isolation procedures and the development of assay methods, and (4) work on reactions related to biochemical transformations involving amine oxides and hemiacetals.

(1) The work in structural chemistry continued and extended past studies. A new ring system, not previously known to exist for either a synthetic or a natural substance, was found for the alkaloids montanine, coccinine, and manthine. The structural relationships between these compounds and other members of the *Amaryllis* alkaloid group was established through a transformation linking the new series with another of the known groups. Since stereochemical relationships are of profound importance in determining the direction of biogenetic reactions, and usually lead to major variations in degree of physiological action, the previous structural studies were extended through the determination of the configuration and stereochemical relationships of the ethanophenanthridine compounds. The C:D ring fusion is *cis*. Of interest is the fact that two groups of compounds of opposite stereochemical configurations occur in this field. These are based on the (+)- and (−)-crinane series. A number of additional structural determinations were made; structures were established for 1-acetyl-lycorine, epicrinine, nerbowdine, 6-hydroxycrinamine, criwelline, and haemanthamine.

This work provides a considerable amount of new information about alkaloid structures, and about naturally occurring ring systems. The isolation work was mostly concerned with the new compounds discovered during the year, but attention was also given to the problem of galanthamine isolation. This substance was introduced into Russian clinical medicine last year as a new and superior drug for the treatment of myasthenia gravis. Earlier chemical work in Russia led to the proposal of an incorrect structure for this compound; the correct structure has since been established through work in Japan and in this laboratory. Current studies have resulted in the development of a compound of still greater activity. Pharmacological studies of these substances are being carried out by Dr. R. L. Irwin (NINDB) and Dr. B. Holmstedt (Karolinska Institute, Stockholm).

Other alkaloid studies included those of the *Lunasia* and *Cassia* compounds. Degradative studies of cassine were combined with instrumental data to lead to a proposed structure; the substance is a reduced pyridine compound with a long alkyl side chain. The *Lunasia* work was

terminated with several structural determinations.

Present work in this category is directed toward completion of the *Amaryllis* structural studies, with particular regard to stereochemical problems, and to the development of biosynthetic investigations.

(2) Until very recently most work in the lipid field was characterized by the use, out of necessity, of poor and inexact methods. This circumstance, more than any other, was responsible for the very slow rate of development in lipid chemistry and lipid metabolism over the last 30 years. The apparent relationship of lipid metabolism to atherosclerosis, and the modification of human serum cholesterol levels by dietary fatty acids, has stimulated efforts to improve the methodology of lipid chemistry. In general, two broad areas of work were involved. The problem of identifying and measuring amounts of individual fatty acids was the more difficult of the two. Preliminary experiments showing that this could be done by gas-liquid chromatography were carried out in England in 1952, but 5 years later gas chromatographic methods were still ineffective for biological applications. Preliminary studies indicated that the existing detection methods were not satisfactory and a continuing exchange of information with several U.S. laboratories was started in an effort to develop instruments and separation methods that would provide both the sensitivity and resolution required for work with lipid. In this laboratory the characteristics of the argon detector were studied, and conditions for linearity defined. A study of liquid phases and of coating procedures was also carried out. Since the success of the method rests on the proper functioning of the column as well as the detection system, a new method for coating liquid phases was developed, and new procedures were worked out for preparing polyester liquid phases with desirable separation factors and high stability. Perhaps the most important factor involved in the use of polyester phases lies in the removal of exchange catalysts. From studies made in various ways it is believed that the polyester procedures developed here have solved this problem.

The development of column chromatographic procedures for the separation of lipid classes has also been pursued. Existing methods were evaluated and suitable modifications developed so that, by using silicic acid columns, the following classes can now be separated effectively: hydrocarbons, cholesterol esters, triglycerides, cholesterol, cephalins, lecithins, sphingomyelins, and lysolecithins (work in another laboratory has shown that monoglycerides may also be separated, when present).

Several laboratory studies using these and related methods are in progress. A detailed study of the lipids of severely atherosclerotic patients has been started. This work is in collaboration with Dr. Michael DeBakey (Baylor University) and Dr. B. G. Creech (Methodist Hospital, Houston). The characterization extends to serum lipids, the lipids of the arterial block tissue, and adipose tissue.

A method for the qualitative and quantitative estimation of the long chain base fraction of sphingolipids has been devised. This work disclosed the presence of a new long chain base in human sphingomyelin.

A comparison of the lipids (serum and aortic) of rabbits fed cholesterol with those of normal rabbits is in progress; this is in collaboration with Dr. D. B. Zilversmit, who carried out the feeding experiments.

Current work involves the continuation of methodological studies and the extension of new methods to laboratory problems. The chief new area of methodology work is capillary chromatography. The highest degree of resolution obtained so far at 180–200° for fatty acid work is about 50,000 theoretical plates. While this is far beyond that obtainable by any other method, it seems likely that a separating capacity of 300–500,000 theoretical plates can be reached. Further, with this resolving power it should be possible to separate steroidal substances, if suitable liquid phases and modified techniques can be developed. This problem is under study in several laboratories. The extension to steroids is needed to study cholesterol formation and degradation more effectively. A high degree of resolution is also needed to separate positional and stereoisomers for unsaturated fatty acids.

(3) The callicrein-callidinogen-callidin system has been studied with particular regard to isolation and assay procedures. This work was carried out in collaboration with Dr. S. J. Sarnoff and Dr. M. E. Webster. Methods were developed for the preparation of purified fractions of calli-

crein (plasma, pancreatic and urinary) and for callidinogen. The objective is the characterization of these substances which may be of great importance in the functioning of the circulatory system. The problem is made difficult by the complexity of the protein and polypeptide mixtures which must be dealt with. A new assay method (Dr. Webster) is expected to be of considerable help.

Current work is concerned with the perfecting of additional chromatographic purification methods.

(4) Amine oxide studies were resumed through an investigation of the catalyst requirements for the reaction. This work was started by Dr. J. C. Craig at the University of Sydney, Australia, and is now being continued at the same laboratory. A variety of iron complexes were used. It was found that both α-hydroxy and α-amino acids were excellent complexing agents, and that the nature of the iron complex was important in determining the effectiveness of the rearrangement reaction. The data suggested that the mechanism involved a sequence of one electron shifts, with the transient formation of iron IV. The overall reaction leads to the removal of a methyl group from nitrogen, and it provides a model for the reaction of biological demethylation.

A study of hemiacetal formation for long chain compounds was carried out. This work arose from observations on plasmalogens and related lipid substances in which an aldehyde reaction is clearly involved. It was found that long-chain aldehydes (palmitaldehyde) were highly reactive substances, and that both trimer formation and hemiacetal formation proceeded readily. The formation of esters from hemiacetals was also studied. These are models for studying lipid aldehyde reactions. Dr. Craig will continue this work at the University of Sydney.

Laboratory of Chemical Pharmacology

DEVELOPMENT OF NEW DRUGS

Drugs for Arthritis, Gout, and Muscular Spasm—a Résumé

Largely because of studies in this laboratory with phenylbutazone and zoxazolamine, three derivative drugs for treatment of arthritis and gout and one for muscle spasm are now available. Two of these were introduced in the past year. The four drugs are: (1) Oxyphenbutazone (in arthritis), (2) Sulfinpyrazone (Anturan) (uricosuric agent), (3) Zoxazolamine (Flexin) (uricosuric agent), (4) Chlorzoxazone (Paraflex) (for treatment of muscle spasm). Two other potent phenylbutazone derivatives with prolonged uricosuric action, a para-methylsulfone derivative of phenylbutazone and a keto derivative of oxyphenybutazone, may be of value as *long acting* uricosuric agents.

Additional studies of phenylbutazone analogues confirm the view that these compounds act in the ionic forms and that an alkyl side chain is necessary for antirheumatic activity. Our continuing search for a nontoxic antirheumatic agent will be guided accordingly.

Reserpine Analogues

Last year, studies with reserpine, in collaboration with Ciba Pharmaceuticals, indicated that the trimethoxybenzene ester linkage might be unnecessary for activity. A nonester reserpine has been found to release peripheral amines selectively without releasing brain amines. It is now in clinical trial for treatment of hypertension.

BIOGENIC AMINES

Drugs Acting Through Release of Brain Amines

Whether the tranquilizing action of reserpine is due to loss of brain norepinephrine (NE) or is associated with inability of brain to bind serotonin (HT) is a crucial question. Dr. Brodie and his associates have shown that small doses of a reserpine analogue, dimethylaminobenzoyl methylreserpate (Su 5171), deplete stores of brain NE considerably, without releasing stores of brain HT appreciably and without eliciting sedation. Larger doses of Su 5171 elicit sedation only if they affect 50 percent or more of brain serotonin binding sites.

Animals subjected to cold (or other stress), and then given reserpine, are not sedated; brain NE but not brain HT is depleted. In contrast to effect on reserpine, stress had no effect on chlorpromazine action. Hypophysectomized rats, subjected to cold and then given reserpine, are sedated and brain HT stores are released, indicating that a

pituitary substance is needed for "stress" to prevent reserpine action.

These results support the view, for which further pharmacologic evidence has been obtained, that "automatic" functions of brain are integrated by antagonistic neuronal systems: an adrenergic system (blocked by chlorpromazine); and a serotonergic system (stimulated by reserpine). If reserpine, as postulated, acts centrally by stimulating a neuronal system (trophotropic) which integrates parasympathetic with somatomotor and psychic functions, then the drug should increase parasympathetic output from the CNS. Reserpine elicits profuse salivation from the cannulated salivary gland, by a central parasympathetic action. Previous studies have shown that reserpine-induced miosis and enhanced light reflex are a reflection of central parasympathetic stimulation. Since chlorpromazine does not increase central parasympathetic tone but instead decreases sympathetic tone, the two drugs must act centrally on opposing autonomic systems.

Drugs Acting Through Release of Peripheral NE

Studies with syrosingopine (Singoserp) show that this compound can release peripheral NE, without releasing brain amines. This confirms the view that reserpine lowers sympathetic tone not by a central action, but by depleting peripheral NE and suggests that this drug may be useful in hypertension since it is likely to be effective in doses that do not produce depression.

Studies with Guanethidine (Su 5864), a Ciba product, show that the drug probably lowers blood pressure by depleting peripheral NE but not brain NE. It presumably acts by a different mechanism than does reserpine since it does not release serotonin, and releases NE more slowly than does reserpine.

Drugs Acting Through Blocking the Metabolism of Amines (Monoamine Oxidase (MAO) Inhibitors)

Toxicity—Certain MAO inhibitors, especially hydrazine derivatives of phenylethylamine, produce, in dogs, degenerative lesions in the inferior olivary nucleus and in the pyriform lobe accompanied by neurological symptoms. JB 516 (Catron) produces such effects in dosage as low as 0.5 mg per kg daily. Preliminary data suggest similar results in cats, but not in rabbits and monkeys.

Effect on Brain Amines.—Previous studies using MAO inhibitors have established a rapid turnover for brain HT but a slow turnover for NE; however, excitation elicited by MAO inhibitors is temporally related to rise in brain NE. Turnover of dopamine has also been found to be extremely rapid (50% in 15 minutes), indicating that MAO is important in the metabolism of this catecholamine in brain.

Further evidence favors the view that the chief role of MAO is not to metabolize physiologically released NE, but to regulate amounts of NE and HT stored in neurons. Thus, the two enzymes, O-methyltransferase and MAO, may have quite different roles, the former modifying released catecholamines, the latter regulating the amount in storage.

Mechanism of Action of the Anti-depressant Drug, Imipramine (Tofranil)

This drug, structurally related to chlorpromazine, is an antidepressant but is not a MAO inhibitor. It does not elicit excitation in normal man or in animals, but acts only in the depressed individual. Studies in this laboratory have shown that Tofranil blocks a number of the central actions of reserpine (but not of chlorpromazine). It blocks the ability of reserpine to potentiate alcohol and barbiturates in mice and rats and to produce sedation, ptosis, and miosis in rats. It does not, however, interfere with release of brain amines by reserpine. Preliminary evidence indicates that chlorpromazine, despite its depressant action, also has a delayed action in blocking reserpine. These findings are tentatively explained by the working hypothesis that phenothiazines and related compounds may have two actions: central adrenergic blocking and central serotonin blocking. Chlorpromazine exerts both effects, with antiadrenergic predominating. Tofranil exerts both actions with antiserotonin predominating.

Mechanism of Uptake of Catecholamines by Brain Tissue

In previous studies it has been shown that platelets take up HT and catecholamines through active

transport, by a mechanism blocked by reserpine. Similar studies are being undertaken to ascertain whether the uptake of amines by brain also involves active transport. On incubation *in vitro* with plasma, brain slices including hypothalamus, thalamus, rhinencephalon, and pituitary, but not cerebellum, take up epinephrine. In contrast, brain slices from animals pretreated with reserpine do not concentrate epinephrine.

Studies on Distribution and Role of NE, HT, and Their Synthetic Enzymes in Nervous Tissue

(a) Micromethods for the estimation of 0.04 γ of NE and 0.20 γ of HT were developed.

(b) The constancy of the ratio of 5HTP to DOPA decarboxylase activities throughout the cat brain indicates the same decarboxylase acts on both substrates. The concentration of amines and enzymes is low not only in cerebellum and cortex but also in those sensory nuclei which are situated in the brain stem. In contrast, they are high only in those parts of brain associated with "automatic behavior," e.g., reticular formation, hypothalamus, and rhinencephalon.

(c) Levels of brain amines are being related to gross behavior and drug action at various ages. At birth the level of NE in rats is only about 20 percent of adult level, while the HT level is about 40 percent adult level. The levels increase with age and the results suggest an association of brain amine levels and development of behavioral patterns. Studies of guinea pigs are underway, since these animals are born more fully developed than rats.

(d) Reserpine decreases by 90 percent the NE content of the superior cervical ganglion in cats. The post-ganglionic response to a preganglionic electrical stimulation is markedly enhanced. This suggests that normally NE may have a role in the ganglionic response to acetylcholine.

Histamine Studies

The development of a specific and simple fluorometric procedure for histamine assay in tissues should help in studies of this substance whose physiological role and biosynthesis are still unknown. Application of the method to brain has shown that contrary to many reports, there is little histamine present and this may be associated with non-nervous vascular tissue. By use of compounds that inhibit MAO but not diamine oxidase, it has been shown that MAO has no role in metabolizing histamine *in vitro* and presumably *in vivo*. It has also been found that the reserpine releases histamine from rabbit platelets but from no other tissue. Evidence indicates that this release may be mediated by free HT.

PASSAGE OF DRUGS ACROSS MEMBRANES

Blood-Brain Barrier

Kinetic data from a study of the penetration into CSF of 20 drugs with diverse structures and physical properties now provide considerable confidence in the assumption that the blood-brain barrier acts as an inert lipoid boundary to drugs. Only the undissociated forms of the drugs appreciably penetrate the CSF and at rates determined by their heptane/water partition ratios. However, certain parts of brain, including both lobes of the pituitary, the intercolumnar tubercle and the area postrema have no blood-brain barrier to N-acetyl-4-aminoantipyrine, sulfaguanidine, radioactive sodium, and labelled epinephrine. In contrast to their slow passage into CSF, water-soluble substances such as sucrose and phenol red readily leave the CSF. They may leave via the arachnoid villi and the rate may be related to the turnover of CSF.

Penetration of Drugs into Cells

The penetration of a number of drugs from plasma into red cells has been studied. Substances generally cross this boundary much more rapidly than blood-intestinal or blood-brain barrier perhaps because of large surface/volume relationship. The relative rates of entry of organic bases seem to be determined by lipid solubility, but the passage of organic acids does not fit the lipoid barrier pattern. Instead, organic electrolytes follow a pattern not unlike that shown by the usual mineral ions. Thus, sulfonic acids penetrate many times faster than quaternary ammonium ions, and acids at the steady state may occupy only a fraction of total volume of cell water in contrast to bases which occupy the total volume of cell water.

Passage of Purines and Pyrimidines Across the Intestinal Tract

A transport mechanism for the absorption of uracil and thymine is present in the intestinal mucosa. Active transport has been shown *in vitro* using everted intestinal sacs. The mechanism requires oxygen and is saturated at low concentrations of pyrimidine. Thymine transport is blocked by uracil, hypoxanthine and other pyrimidines and purines.

DRUG METABOLISM

Substances (Antimetabolites) Metabolized by Relatively Specific Enzymes of Intermediary Metabolism

Studies of the metabolism and mechanism of action of the anti-tumor agent, 6-chloropurine, have been continued. Last year the isolation of a new substance, 6-chlorouric acid, was reported. Studies this year show that the purine skeleton of 6-chloropurine is incorporated into the adenine and guanine of both RNA and DNA. Furthermore, 6-chloropurine inhibits the turnover of both RNA and DNA, as followed by p^{32} *in vivo*, in liver slices, and in isolated liver nuclei.

Substances Acted on by Extremely Nonspecific Enzymes Not Involved in Intermediary Metabolism

Further studies have been made on the oxidative enzymes in liver microsomes.

(a) Microsomal Sulfoxidase. The enzyme system that oxidizes 4,4′-diaminodiphenyl sulfide and chlorpromazine to the corresponding sulfoxides has been definitely characterized as another microsomal enzyme (a sulfoxidase) requiring TPNH and O_2.

(b) Nicotine. The key step in oxidation of nicotine is not cotinine, as suggested in last report, but hydroxylation of the carbon atom next to nitrogen in side chain to yield a cyclic aldehyde (hydroxynicotine). This supports the view that the first step in demethylation is hydroxylation of the methyl group. Elucidation of this reaction may indicate how ring systems are split in the body.

(c) Mechanism of Microsomal Drug Oxidation. This mechanism involves direct utilization of oxygen and may share the same donor of "active" oxygen as cholesterol. A similar mechanism may be involved in the hydroxylation of steroid rings to form corticoids. In previous work it was shown that TPNH is oxidized in the absence of drug to yield H_2O_2. Two drugs, 4,4′-diaminodiphenyl sulfide and p-ethoxyacetanilide, do not affect the rate of TPNH oxidation, but cause a decrease in formation of H_2O_2 equivalent to the formation of their metabolites. This supports the view that TPNH and O_2 react in microsomes to form a "hydroxyl donor." In the absence of drug substrate, a part of the donor participates in normal hydroxylation reactions (e.g., cholesterol formation), and the rest breaks down to H_2O_2. In the presence of drug, a part of the "hydroxyl donor" is used by a number of non-specific "drug enzymes" to hydroxylate foreign compounds.

(d) Induced Enzyme Formation. Administration of certain drugs, e.g., phenylbutazone, aminopyrine, 3-4,benzpyrene, phenobarbital, increases the ability of rats to metabolize the same or closely related drugs. This increased activity is also shown *in vitro* by liver microsomes. This effect may explain, in part, the tolerance to barbiturates. A particularly interesting observation is that barbiturates lower coumarin anticoagulant levels in man. The tolerance of hyperthyroid subjects to drugs prompted a study of effects of thyroxin on drug metabolism. Pretreatment of rats with thyroxin decreases the duration of zoxazolamine paralysis and increases the activity of liver microsomal enzyme that metabolizes the drug.

(e) Biochemical Evolution. It was shown in previous studies that the appearance of drug metabolizing enzymes is associated with adaption to living on dry land. In continuing studies of the ontogenetic development of drug enzymes, we find that liver microsomes in chicken embryos, unlike those in the mammalian embryo and the tadpole, contain enzymes that oxidize drugs and which require TPNH and O_2. The possession of these enzymes by the chicken embryo may be related to its nonaqueous milieu.

STUDIES IN BIOCHEMICAL BEHAVIOR

Preliminary experiments in rats on the temporal relation between pituitary stimulation and various responses were undertaken. Typical

stimuli were cold, intradermal formaldehyde, ethionine, reserpine, ethanol, dibenamine, carbon tetrachloride, 3-methylcholanthrene and Tofranil. (No responses are obtained in hypophysectomized animals.) (1) Adrenal ascorbic acid is rapidly reduced, returning to normal in about 5 hours. With persistent stimuli (cold and methylcholanthrene), adrenal ascorbic level returns to higher than normal value. This probably reflects the induced, increased synthesis of ascorbic acid in the rat, since in guinea pigs which do not make ascorbic acid, the adrenal levels remain low in persistent stress. (2) Plasma corticosteroid levels are maximal in 1 to 4 hours and then return to normal. With persistent stress, the high levels are maintained. (3) Plasma-free fatty acids (FFA) usually are maximal in 1 to 4 hours and then return to normal, except in persistent stress. Anomalous results are obtained with electroshock which lowers FFA and with Tofranil which produces a delayed response. (4) Although many stimuli cause a rise in FFA only some of them result in deposition of liver triglyceride. This suggests that something must also happen in liver to cause fatty deposition. (5) The activity of tryptophane peroxidase (TPO) in liver is increased by all stimuli. (6) A chemical picture typical of "stress" is elicited by the "antistress" compounds, reserpine and chlorpromazine.

Specific Studies

(a) Reserpine. It has been reported that reserpine pretreatment inhibits the responsiveness of the pituitary-adrenal axis to stressful stimuli. Surprisingly, reserpine depletes adrenal ascorbic acid in the rat, increases the level of circulating corticoids and increases the level of liver tryptophane peroxidase. No effects are observed in hypophysectomized rats. Preliminary results indicate that the effect on pituitary may be related to change in brain amines.

(b) 3-Methylcholanthrene. This compound in rats causes prolonged pituitary stimulation lasting up to 48 hours. The effects are depletion of adrenal ascorbic acid, increase in plasma corticosteroids, increase in plasma FFA, increase in liver TPO and antagonism of reserpine sedation. Other carcinogenic agents do not elicit this marked response. 3-Methylcholanthrene affected the pituitary-adrenal axis in doses as low as 0.1 μg/kg. The mechanism of this effect is under study.

(c) Effect of Various Pituitary Stimuli on Various Body Enzymes. The most sensitive index of pituitary stimulation found thus far is the increase in liver TPO. Not only is TPO increased by stimuli previously mentioned but by certain barbiturates. None of the stimuli is effective in hypophysectomized animals. Of interest is the finding that tryptophane in doses said to act as specific inducer of TPO, also decreases adrenal ascorbic acid, and increases plasma corticoids and FFA.

The effect of many drugs in stimulating the biosynthesis of L-ascorbic acid in rats, and which has been shown in this laboratory to result from a stimulation of glucose metabolism via glucuronic acid, l-gulonic, etc., is also a pituitary response, which occurs in adrenalectomized rats, but not in hypophysectomized animals. Drugs that increase ascorbic acid synthesis also increase the activity of liver microsomal enzymes. Whether this effect on liver microsomes is an adaptive response to reduce toxicity to foreign compounds or is a more generalized response remains to be seen. Other enzymes that are stimulated by drugs are those in liver that convert galactose to glucuronic acid.

(d) Effect of Drugs on Triglyceride Mobilization and Deposition. Triglyceride deposition in rat liver is produced by CCl_4, ethanol, and ethionine. The fatty acids appearing in the neutral fat of liver appear to be mobilized from fat depots (collaboration with Dr. Marjorie Horning), but ethanol may also stimulate their synthesis in liver. Triglyceride deposition may be blocked by large doses of adrenergc blocking agents.

Isolation of Cardiotonic Substances from Mammalian Tissues

In addition to lysolecithin, previously reported, other active factors, all acidic lipids, have been obtained from mammalian tissues. These include: (1) Beef blood factor—probably cis vaccenic acid. (2) Beef heart factor—an acidic factor which, from infrared spectra, may be a lactonized hydroxy fatty acid. (3) Rabbit serum factor; infrared also suggests that this is an unsaturated lactonized hydroxy acid.

It is believed that these substances may influence the passage of ions through membranes, as a result of which the muscle contractility may be increased.

STUDIES WITH ASCORBIC ACID

Ascorbic acid is synthesized in rats from glucose through D-glucuronic acid and L-gulonic acid. During the past year evidence has been obtained for an enzyme system in rat liver which synthesizes D-glucuronic acid through uridine nucleotide precursors. The occurrence of such reactions in the intact animal was confirmed by experiments showing that D-galactose is a considerably better precursor of L-ascorbic acid than is D-glucose.

Information has also been obtained on the metabolism of L-ascorbic acid. An enzyme system in guinea pig and rat tissues which decarboxylates L-ascorbic acid has been purified and two sugar acids, L-lyxonic and L-xylonic acids, have been identified as products of the reaction. A strain of yeast has been adapted to grow on L-ascorbic acid as its sole carbon source; this observation may furnish a powerful tool for studying the mechanisms involved in ascorbic acid metabolism.

Laboratory of Technical Development

GAS CHROMATOGRAPHY

Considerable effort has been devoted to the development of methods of increasing the sensitivity and versatility of gas-chromatography techniques. The radiofrequency (RF) discharge detector system has been thoroughly evaluated and shown to be applicable to both standard columns and capillary columns and to provide high sensitivity. Comparisons of the RF detector with argon ionization detectors showed an approximately 10-fold greater sensitivity for the RF discharge detector. Variations in conditions produce changes in the baseline with the RF detector, whereas similar variations yield, with the argon detector, changes in sensitivity with consequent errors in the size of the peaks but with a stable baseline. This makes the records produced with the argon detector superficially more attractive since the errors are less obvious. At present it is simpler to use the argon detector but improvements in the radiofrequency excitation source may change this. Studies of the radiofrequency discharge as a source of ionization which can be measured downstream from the gas discharge and to improve the radiofrequency excitation systems are continuing.

The need for a satisfactory system for the radioassay of C–14-labeled compounds was pointed out by the Committee on Lipid Analysis and a program was undertaken to solve this problem. The method developed consists of capturing the effluent in short sections of column using anthracene crystals coated with one of several liquid phases shown not to interfere with the counting. These short columns permitted direct radioassay by the usual automatic scintillation counting techniques. The efficiency of this system is very close to theoretical limits. In addition to the above noted gas chromatographic techniques, Dr. Karmen participated in several studies in cooperation with other laboratories, notably in the studies on chylomicron composition after fat ingestion and a study of binding of unesterified fatty acids to various serum proteins with Drs. Bragdon and Shafir, respectively. An exploration of the application of zeolite molecular sieves to analysis of respiratory gases was undertaken as a project for one of the high school teachers on summer training. Although the attempt to produce a zeolite sieve to separate all of the respiratory gases in one step was not successful, the study suggested several other potential uses of these sieves.

Since it would be particularly advantageous to have a detector for gas chromatography columns that would not only be sensitive but would also give information as to the molecular weight of the gas, a gas chromatographic detector, based on the measurement of sound velocity, was developed. From a consideration of the parameters contributing to the change in velocity, it would appear that if the quantity were known an estimate of the molecular weight could be obtained from the sonic velocity curve. The sensitivity of ordinary methods of sound velocity measurement suggested that the method would be of no use unless some high sensitivity system for sound velocity measurement were developed. An ingenious electronic-circuit

system, far exceeding expectations as to sensitivity, was devised by Mr. Noble. The method of frequency multiplication and phase comparison available with this apparatus makes possible sensitivities which can be utilized only if suitably stable sample chambers can be developed. Test runs using this sound velocity system showed it to possess sensitivity exceeding that of the thermal conductivity system. The relative complexity of the electronic equipment and the requirement for a split stream differential system can be matched by an expectation of very high sensitivity and an extremely small cell volume. The system in current operation has a cell volume of 0.08 ml and this can be reduced several fold without sacrifice of capability. This equipment in its present form, with a Linde sieve type 13X, can yield an analysis of 5 μl of air for oxygen and nitrogen content in 0.4 minutes.

MICROANALYSIS BY EXCITATION IN LOW PRESSURE HELIUM DISCHARGE

Several aspects of the helium discharge and its ability to excite spectral emission were investigated. It was found that the discharge would excite molecular and atomic emission from volatile organic compounds. This suggested the possibility of spectral analysis of the glow as the fractions appeared from the chromatograph column. The emission spectra were taken and studied for several types of compounds. The complexity of the spectra produced by mixtures of gases does not encourage specific analysis by this means. Since spectral analysis showed a relatively clear zone above 500 millimicrons, it was concluded that organic materials in blood and urine specimens would not produce interfering bands that would overlap the region used for determination of the alkali metals. The work on emission spectroscopy by means of helium discharge for the determination of alkali metals is now continuing after a period during which the apparatus was modified and these spectra explored. The apparatus is now improved with stabilized methods of measurement, stable excitation sources and a new chamber allowing volatilzation of the sample on a heated platinum wire. In addition, preparation of measuring pipettes has been facilitated by the use of a commercially available quartz tubing 33 μ I.D. available under the name of Santo tube from Monsanto.

ERRORS IN CATHETER-RECORDING SYSTEMS AND THEIR CORRECTION

The completion of Mr. Noble's work on a precise variable frequency hydraulic pressure generator has made possible accurate analysis of the performance of various intracardiac pressure-measuring systems. It has been shown that pressure curves taken by cardiac catheterization, as normally performed, are distorted in shape and time. As these errors may be wrongly interpreted as due to the vascular system rather than the instruments, several efforts have been made to develop a catheter-tip transducer. A solution developed by Mr. Noble makes it possible to compensate electrically for the errors introduced by the catheter. The simple electronic circuit can be adjusted for the specific catheter so that all the errors due to aging of the catheter and the particular pressure transducer used are compensated at once. Tests of the efficacy of the system are being run in collaboration with Dr. Guy Barnett of the Section on Cardiodynamics. The current method utilizes the recently developed hydraulic pressure generator to make and confirm the compensation, but a relatively simply method of setting the compensator can be developed using a simple, transient pressure pulse applied to the catheter after it is in place.

COMPUTER FOR ANALYSIS OF OVERLAPPING DISTRIBUTIONS

The analog computer for analysis of overlapping distribution functions was completed this year and tested for ability to resolve overlapping peaks. The instrument's utility will be more completely evaluated by application to resolution of absorption spectra by Dr. Hayes, who has prepared and purified for this purpose some specific compounds with known absorption spectra. In addition, the application of the instrument to the detailed analysis of infrared spectra is anticipated.

EVALUATION AND QUANTIFICATION OF VALVULAR REGURGITATION

An investigation of methods of quantitating mitral valve regurgitation has been undertaken

by Dr. Peter Frommer in collaboration with the Cardiology Section of the Surgery Branch. Methods have been reviewed and the possibilities of the dye dilution technique utilizing radio-opaque dye and serial X-ray films are under consideration. The exploration has been facilitated by the use of a simple analog computer which provided easily changed parameters with idealized curves produced to indicate the effects of backflow and dilution. From the examination of the properties of the analog system, it would appear that the radio-opaque dye dilution technique has promise but puts some stringent requirements on the instrumentation and clinical procedures. The analog system proved so convenient and informative in the evaluation of partial mixing and regurgitation that a paper on the method is in preparation.

FLOW METER DEVELOPMENT

Dr. Frommer has guided the investigation of the possibility of utilizing for flow measurement the Coriolis force produced when a fluid is made to gain and lose angular momentum as it passes through a rotating loop of pipe. This type of flow meter would measure actual mass flow, be independent of viscosity and provide a convenient form of differential flow meter. A rather large model system was constructed and was sufficiently promising to encourage further work on more compact designs. This was a summer project almost entirely performed by a summer student employee.

Investigation of nuclear magnetic resonance phenomena in the measurement of blood flow and their potentialities in analytical instrumentation is continuing. Mr. Kudravcev has constructed several types of nuclear resonance apparatus and circuits have been developed which yield a considerable reduction in the size and in the instability noted in previous equipment. It still appears feasible to evolve a flow meter system sensitive to volume flow using nuclear resonance principles. The possibility that such a device can be made without requiring intimate electrical contact may compensate for the complexity of the apparatus. Application to pulsating flows has not yet been minutely examined. If the volume flow measurement should prove impractical, the velocity technique may have value. Considerable information has been gained with regard to construction of simplified nuclear magnetic resonance systems and this will presumably be useful in the development of analytical devices should development of a practical flow meter prove impossible. The analytical characteristic of the system may also be applied to flow measurement as a method for determining the concentration of a nuclear "dye" as it goes past the detector. Fluorine, for example, is easily detected and could be used with present apparatus in a dye dilution system. Highly fluorinated materials such as freon could probably be utilized.

THEORETICAL ANALYSIS OF TRANSPORT

Dr. Stephenson has evolved several contributions to the theoretical analysis of transport in biological systems, and this work has resulted in two papers on the subject in the *Bulletin of Mathematical Biophysics*. This theoretical material has been helpful in the analysis of Dr. Frederickson's experiments on fatty acid metabolism. The data have been analyzed and programmed for computation on the IBM. The investigation of the physics of the ultrarapid freezing of water in biological materials is continuing. A fuller understanding of the process may lead to information on the structure of biological material and aid in the design of methods for preservation of material by freezing and drying, both for banking of biological materials and the preparation of material for electron microscopy.

PHOSPHORESCENCE ANALYSIS

An investigation of the application of phosphorescence to the analysis and characterization of biological materials is being pursued by Dr. Hayes with the loan of the phosphorimeter from the American Instrument Company. One of the first problems was to find a solvent that would be suitable for investigation of water soluble materials. A survey of materials that would form a satisfactory glass at a temperature at which phosphorescence observations could be made was undertaken, and it was found that propylene glycol at minus 80° formed a satisfactory glass. At the temperature of liquid nitrogen, however, the

solvent would crystallize and spoil the optical properties of the system. Accordingly, liquid gases for the cooling of the sample were surveyed to find one which would provide temperatures between those avilable from carbon dioxide and liquid nitrogen. Nitric oxide was found satisfactory. Utilizing the new system, a survey of biological compounds of biological interest is currently in progress. Evaluation of phosphorimetry as a method for analysis and characterization of materials will be pursued.

ULTRASONICS

Dr. Weissler's investigation of the effects of ultrasonic irradiation of hemoglobin has shown that the products of ultrasonic irradiation of water participate in the destruction or conversion of the hemoglobin molecule, and that additives in the form of dissolved gases or chemical agents modify the form of degradation. This work suggests the possibility of modifying the destructive effects of ultrasonic irradiaion when utilized for liberation of biological materials from cells and tissues by high powered ultrasonic disintegration. In addition, the selection of the environment may provide for selective isolation of particular enzymes by protecting one at the expense of the other.

Laboratory of Cardiovascular Physiology

STUDIES ON THE HEART

The Diastolic Pressure—Myocardial Segment Length Relation in the Ventricle. Observations on the Contribution of Atrial Systole

The relation between left ventricular diastolic pressure and the simultaneously recorded changes in the length of a segment of left ventricular myocardium was intensively studied. The shape of this curve laid the basis for a clearer understanding of those circumstances under which atrial systole will produce its greatest contribution to the elongation of the fibers of the ventricular myocardium. Further, when considered together with the curve relating pressure to stroke work it gave added support to the position that, in any given metabolic state, the force of contraction of the ventricle is a function of the fiber length from which the contraction begins. These investigations show the importance of atrial systole for ventricular filling.

The Influence of Cardiac Sympathetic and Vagal Nerve Stimulation on the Relation Between Left Ventricular Diastolic Pressure and Myocardial Segment Length

The full curve relating ventricular pressure to myocardial segment length showed clearly the lack of any change in this relation with sympathetic or vagal stimulation. These observations made it clear that the family of curves relating filling pressure to stroke work is accompanied by a family of curves relating initial fiber length to stroke work. A corollary of these data is that, under sympathetic stimulation, the ventricle will produce more external work from any given initial fiber length as well as from any given end-diastolic pressure. This constitutes a substantial advance.

In the course of these studies it was found that tachycardia, without concomitant sympathetic stimulation, so impinges on diastole (especially if the stroke volume is high) that the ventricle does not have sufficient time to acquire its "normal" diastolic pressure-length relation. The addition of sympathetic stimulation at the same heart rate so condenses systole that it permits the same ample diastole that would have occurred at a lower heart rate. The technical and conceptual advances in these studies have helped to bring into clearer focus the importance of the simultaneous positive inotropic effect on the ventricle when tachycardia is induced by the sympathetic outflow.

The Regulation of the Ventricle's Contraction: The Influence of Cardiac Sympathetic and Vagal Nerve Stimulation on Atrial and Ventricular Dynamics

With the advances described above it was possible to systematize understanding of the means by which the central nervous system can produce acute changes in the performance of the heart other than by the well known effects on rate.

The pertinent observations may be stated as follows:

1. At constant heart rates efferent stimulation of the vagus nerve exerts a profound depressant effect on the strength of the atrial contraction and can thereby influence ventricular filling and ventricular stroke work; mean atrial and thus venous pressure are elevated at any given level of cardiac work or cardiac output during vagal stimulation despite the fact that the vagal stimulation used does not alter the performance characteristics of the ventricle. The effects of vagal stimulation are blocked by atropine.

2. Stellate ganglion stimulation or norepinephrine infusion augments the strength of atrial contraction and thus the atrial contribution to ventricular filling. The augmented atrial contraction takes place in a shorter period of time.

3. Stellate ganglion stimulation or norepinephrine infusion increases the external work and power produced by the ventricle from any given filling pressure and fiber length.

4. There is a family of curves representing the relation between end diastolic fiber length and stroke work as well as a family of curves representing the relation between filling pressure and stroke work.

5. When taken together with the well known sympathetic and parasympathetic effects on heart rate, the above data are believed to comprise a reasonably comprehensive description of the means available to the central nervous system for directly inducing acute changes in the activity of the heart.

On the basis of these observations, Dr. Sarnoff and his colleagues propose what they refer to as "the law of the innervated heart" as follows:

1. If the effective catechol amine stimulus remains constant, the contraction of the ventricle varies with its end diastolic pressure and fiber length. If the end diastolic pressure and fiber length remain constant, the contraction of the ventricle varies with the effective catechol amine stimulus.

2. The central nervous system has available efferent neuronal pathways to the heart by means of which it can vary ventricular end diastolic pressure and fiber length while keeping the effective catechol amine stimulus constant, means by which it can increase the effective catechol amine stimulus, or both.

The Regulation of Ventricular Contraction by the Carotid Sinus: Its Effect on Atrial and Ventricular Dynamics

The role of carotid sinus baroreceptors in circulatory regulation was reevaluated and showed that a dominant aspect of the carotid sinus regulatory activity is to augment or diminish the contraction of the ventricle. The basis for this conclusion is as follows:

1. Carotid hypotension diminishes venous distensibility. The net effect of such a change, if it alone occurs is an increased ventricular end diastolic pressure and fiber length and thus an augmented ventricular contraction. Splenic contraction would have the same effect.

2. Carotid hypotension augments and shortens the atrial contraction. The net effect of such an atrial augmentation, if it alone occurs, is an increased ventricular end diastolic pressure and fiber length and thus an augmented ventricular contraction.

3. Carotid hypotension directly augments the work produced by the ventricle from any given end diastolic pressure or fiber length and produces more complete systolic emptying.

4. Carotid hypotension augments ventricular power as well as contractility, since it shortens the systolic time for any given amount of work produced, provides more rapid relaxation, and thus diminishes filling impedance. If this alone occurs, it provides for a longer interval of diastolic filling than would otherwise occur and thus produces an augmented ventricular contraction, a factor which becomes especially important at high heart rates.

5. The catechol amines secreted by the adrenal medulla in response to a lowering of carotid sinus pressure would be expected to reenforce the effects enumerated under 1 through 4 above.

In each experiment, over the range of aortic pressures and flows observed, the increase of ventricular stroke work was several times the simultaneously observed increase in total peripheral resistance when carotid pressure was lowered. Thus rather than acting primarily to safeguard blood flow to the vital organs such as the brain and heart, the baroceptor acts not unlike a voltage regulating element which causes an increased input into an electronic system so as to maintain a constant voltage when the current requirements of the system it is supplying are increased. Thus

the carotid sinus helps to regulate the blood flow to all the tissues.

A Comparison of the Hemodynamic Effects of Pacing the Atrium and Ventricle at the Same Rate

By causing the atrium to contract while the atrioventricular valve is closed, the importance of the atrial contribution to ventricular filling was further shown.

By closing the door, so to speak, on the atrium during atrial systole and thus depriving the ventricle of the filling pressure and fiber length it would otherwise have achieved, the ventricular end diastolic pressure was significantly lowered and the external work produced was thereby lessened. It was further observed that the amount of work produced by the ventricle from any given end diastolic pressure was lower during ventricular pacing than during atrial pacing. Analysis of high speed ventricular pulse contours showed that the total ventricular effort is appreciably less concerted and synchronous. Thus, when the first fibers are contracting, the flaccidity of those which are not as yet activated tends to impose the same hydraulic limitations as a ventricular aneurysm, e.g. diminish the effectiveness of the contraction. Similar considerations apply to the last contracting fibers.

The Analysis of Coronary Sinus Blood for Catechol Amines Before, During and After Sympathetic Stimulation of the Heart

The concentration of norepinephrine in coronary sinus blood rises sharply during cardiac sympathetic nerve stimulation. An interesting lead, however, is the massive outpouring, after the cessation of sympathetic stimulation, of a substance which assays chemically as norepinephrine by the Weil-Malherbe and Bone technique. This is probably not norepinephrine since the heart's action does not conform to the presence of large amounts of the active substance at that time.

The Influence of the Vigor of Atrial Systole on Closure of the Mitral Valve

In the dog with heart block, between two and five atrial A-waves, and their reflections on ventricular diastolic pressure, can be studied in the absence of the disturbances ordinarily produced by ventricular activity. As evidenced by the staircase pattern or its absence in the ventricular diastolic tracing, it can be determined whether the mitral valve has closed after any given atrial systole. The "nonclosing" atrial systoles are transformed by sympathetic stimulation into "closing" atrial systoles. The greater speed of the decline in atrial pressure is seen after the strong atrial A-wave causes the leaflets of the mitral valve to close, since the increased rate of change of atrial pressure after a strong atrial systole produces an initially higher velocity of refluxing blood and thus is likely to close the valve.

Auto-Regulation of the Performance Characteristics of the Ventricle

The ventricle of the isolated heart, beating at a constant rate and free of reflex or hormonal regulatory influences, requires no longer a time to put out any given stroke volume against a high aortic pressure than against a low one. If the work of the ventricle is increased solely by increasing aortic pressure, the ventricular function curve is much steeper than if the ventricular work is increased solely by increasing stroke volume. When oxygen consumption is increased by increasing aortic pressure while holding stroke volume constant, there is a greater relative increase in coronary blood flow and a narrowing of the arteriovenous O_2 difference. Conversely, when O_2 consumption is increased by increasing stroke volume while holding mean aortic pressure constant, the increase in coronary blood flow is less marked and there is a widened arteriovenous O_2 difference.

Reexamination of these phenomena showed that the effects of raising aortic pressure by increasing the resistance to left ventricular ejection produced alterations in the pulse contours which were in every respect similar to those observed after the administration of norepinephrine.

There is a body of evidence suggesting that the responsiveness to catechol amine stimulation is a function of the biochemical environment of the stimulated effector organ. It is proposed that increased aortic pressure, by increasing coronary flow relative to O_2 utilization, so alters the biochemical environment of the myocardium as to render it more responsive to catechol amines and thus increases the effective catechol amine stimulus. This hypothesis is to be investigated further.

STUDIES OF CIRCULATORY REGULATION—EXERCISE

The total flow to both lower extremities was metered, before and during exercise, while the arteriovenous O_2 difference, arterial and venous pO_2 and pH were recorded. Prior to exercise, three types of sympathetic stimulus were applied: (a) the reflex increase in sympathetic tone consequent to lowering carotid sinus pressure, (b) the emphatic sympathetic stimulation resulting from stimulating the central cut end of the vagus nerve, and (c) the injection of constricting doses of norepinephrine into the arterial line supplying the lower extremities. These were then repeated during simulated exercise induced by electrical stimulation of the muscles of both lower extremities. In a second type of experiment the blood flow to each lower extremity was separately metered so that one extremity, the resting extremity, could act as the control while the opposite extremity was exercised.

The results of both types of experiments make it clear that with augmented muscular activity, the vascular bed of the active area can disregard a sympathetic stimulus to which it would ordinarily be responsive. This might be termed the functional sympathectomy of activity. The basis of this phenomenon is not yet known but it does not appear to be pO_2.

All of the studies in this laboratory have been greatly facilitated by the improvement of instrumental and recording techniques, developments which have been largely carried out in this laboratory with some advice and assistance from the Laboratory of Technical Development.

ABDOMINAL PRESSORECEPTORS

It was earlier observed that splanchnic or pancreatic vascular hypotension will produce tachycardia and a pressor response in the cat. In current experiments the lower abdominal aorta, near the bifurcation, is perfused at constant flow and the femoral arterial pressure recorded. The increased femoral artery pressure in the area perfused at a constant flow, which takes place when the coeliac and superior mesenteric artery are occluded, suggests that a reflex increase in peripheral vascular resistance takes place. It remains, however, to exclude the possibility that the observed elevations of pressure in this vascular bed are not due to the influence of collateral channels.

STUDIES OF THE CALLICREIN SYSTEM

The objective of these investigations was to obtain an increased understanding of the physiological significance of this system for circulatory regulation. Callicrein, a hypotensive proteinase, acts on callidinogen, an alpha-2 globulin in plasma, to release a polypeptide called callidin which produces the observed vasodilatory effect. Since various callicreins are found in the urine, pancreas, plasma, saliva, etc., and these callicreins may be differentiated by differences in their susceptibility to proteolytic inhibitors, it is proposed that the callicreins are limited to local vasomotor regulation, the magnitude of the effect being determined by the activity of the tissue or organ involved.

During the past year, semiquantitative methods have been developed for the biological determination of some of the various components, e.g. callicrein, callidinogen, callidin, and two plasma callicrein inhibitors. The callidin inhibitor can be estimated by measuring the rate of destruction of callidin following its liberation from callidinogen. However, a quantitative method for the measurement of this inhibitor remains to be developed. With the techniques currently available, plasma and urine from patients with orthostatic hypotension show a greater deviation from their normal controls than do plasma and urine from patients with essential hypertension.

Plasmin, the proteolytic activity of serum found in the euglobulin fraction, has definitely been distinguished from plasma callicrein. Other factors in plasma which have not yet been distinguished from plasma callicrein are the glass activated proteinase of Anderson and coworkers, Hageman's factor, and the permeability factor of Miles and Wilhelm. Studies of comparison of these factors have been initiated and no differences have yet been found.

Because plasma callicrein was inhibited *in vivo* with diisopropylfluorophosphate, a known inhibitor of esterolytic activity, it was thought possible that the callicreins might be capable of digesting synthetic esters. Tosyl L-arginine methyl ester

(TAME) has been found to be a substrate for the various callicreins. Crude urinary callicrein attacks this substrate at the same ratio of activity as does highly purified hog pancreatic callicrein (obtained from Dr. Moriya, Japan), and it is possible that urinary callicrein may be the main esterase excreted in the urine. The addition of acetone to human plasma, a procedure which causes the activation of plasma callicrein, activates at least two esterases; one inhibitable, the other not, with soy-bean trypsin inhibitor (SBI). To date, correlation between SBI inhibitable proteinase and plasma callicrein has been consistent. It is possible that the SBI resistant proteinase may be the enzyme (callicreinase) responsible for the liberation of callicrein from its inactive precursor (callicreinogen). Plasma callicrein, when measured as a proteinase inhibitable by SBI, appears to be eight times more active as a TAME esterase than is either urinary or pancreatic callicrein. Further studies will be required to determine whether acetone causes the activation of more than one proteinase inhibitable with SBI.

REFLEXLY INDUCED CHANGES IN RENAL FUNCTION

During stimulation of the isolated stellate ganglion of the anesthetized dog there is a diuresis which is not related to the changes in arterial blood pressure. The diuresis is characterized by a rapid onset, little or no change in urine solute or chloride concentration, an increase in glomerular filtration rate and a rapid cessation with the "stoppage" of stimulation. Also, the diuresis occurs in the presence of a lowered left atrial pressure. It was further found that cervical vagotomy diminishes the extent of the diuresis whereas vagotomy at the level of the diaphragm does not appear to modify it. From the results of these experiments it was suggested that the increased urine flow during stimulation of the stellate ganglion could be attributed to a peripheral vasodilatation elicited via the baroreceptors in response to the changes in dynamic pressure effected by stellate stimulation. Vagotomy effectively deprived the organism of a portion of the pressure-stabilizing system, thus lessening the extent of the reflex changes induced by stellate stimulation and thus lessening the extent of the changes in renal vasomotor tone, glomerular filtration rate, and urine flow.

Laboratory of Kidney and Electrolyte Metabolism

Five major areas of research are being pursued in the Laboratory of Kidney and Electrolyte Metabolism. These include: (1) studies on the mechanism of electrolyte excretion and acidification of the urine in intact animals; (2) studies on the mechanism of urinary dilution and concentration; (3) studies of electrolyte and water transport in isolated systems, both living and artificial; (4) studies of the control of aldosterone secretion in dogs; and (5) studies of a mammalian cardiotonic protein system.

Drs. Orloff and Burg have completed their examination of the effect of the cardiac aglycone, strophanthidin, on electrolyte excretion in the chicken. As noted in the previous annual report they were able to elicit unilateral natruresis in the chicken by injecting strophanthidin into the renal portal venous system. Associated with the diminution in Na^+ reabsorption they also observed interference with both K^+ and H^+ secretion. These effects have since been shown to be inhibited to a considerable degree by the simultaneous administration of K salts. The effects of strophanthidin are unlike those observed following the injection of other pharmacological inhibitors of Na reabsorption. In the latter instance a fall in Na reabsorption is generally attended by reciprocal, rather than parallel changes in the secretion of K and H ions. On the basis of these data, as well as the results of studies of the kinetics of K^+ transport in renal cortical slices of rabbits, to be summarized below, it has been concluded that strophanthidin exerts its renal effect by inhibiting a contraluminal Na-K exchange pump. The latter pump is thought to be similar to that present in most living cells and presumably involved in the maintenance of the intracellular concentrations of Na and K. By interfering with the normal processes of Na ejection and K uptake on the contraluminal border, transluminal transport of these ions as well as of H^+ is depressed.

The thesis that strophanthidin affects a hypothetical contraluminal exchange system derives further support from other studies of Drs. Orloff

and Burg. These workers reexamined the effect of various inhibitors, including strophanthidin, on K transport in rabbit renal cortical slices. The methods used were described in detail in the previous report. They confirmed their earlier findings that strophanthidin reduces intracellular K and raises that of Na; and that the effect is due in part at least to specific interference with K influx. These changes are consistent with an effect on linked Na-K exchange, analogous to that observed in red cells, muscle, etc. by other workers.

Drs. Orloff and Burg were unable to demonstrate an antagonistic effect of adrenal steroids and strophanthidin on electrolyte transport as had been described by others. The effects of the aglycone on electrolyte excretion are not altered by potent adrenal steroids (including aldosterone); the strophanthidin effects on K^+ and Na^+ accumulation in cortical slices of both normal and adrenalectomized animals are not altered by salt-active steroids; nor is the inhibition of PAH accumulation by strophanthidin altered by aldosterone.

Drs. Kahn, Brenes, Earley, and Orloff are investigating the effects of the infusion of ammonium salts on electrolyte excretion in the chicken. They have confirmed an earlier finding of this laboratory that the infusion of a series of ammonium salts into the leg veins elicits a profound natruresis. The mechanism of this effect is being actively studied.

Drs. Jaenike and Berliner have concluded studies utilizing a modification of the stop-flow technique described originally by Malvin and Wilde. The technique involves occlusion of the ureteral outflow in the anesthetized dog for a variable period of time, release of the occlusion and collection of serial urine samples. The composition of these samples is thought to reflect to a considerable extent the composition of the "stopped" intraluminal fluid at various levels in the nephron. The modifications introduced were designed to permit critical examination of distal tubular functions by effectively eliminating renal pelvic dead space (filling it with mineral oil) and isotopically labeling distal convolution fluid (subcapsular injection of K^{42} during stop flow). The data confirm that vasopressin markedly affects the permeability to H_2O of the distal convolution and further that electrolyte is also abstracted in this area. These results are consistent with a view of the mechanism of urinary dilution and concentration previously described by Dr. Berliner and his associates. Utilizing this technique Drs. Jaenike and Berliner have also been able to reaffirm the view that K^+ and Na^+ are exchanged in the distal system and have clarified a number of factors influencing this process.

These investigators are at present studying the effect of vasopressin on the permeability of the collecting system to urea in the dog. The data support the interpretation that as vasopressin enhances bulk flow of water along its osmotic gradient out of the collecting system into the interstitium, urea movement is accelerated in the same direction. This concept is of considerable significance with respect to the role of urea in the concentrating mechanism. The medullary urea/urine urea concentration ratio approaches unity in the presence of vasopressin at a time when flow of H_2O out of the collecting system into the medulla is high, and is considerably lower when no vasopressin is present and there is limited movement of water (and of urea).

Dr. Bray has investigated the osmotic pressure of rat kidney slices by a modification of a technique which depends on the direct observation of the relative thawing time of previously frozen slices. The presence of a progressive increase in osmotic pressure from cortex to inner medulla in kidney slices of either dehydrated or non-dehydrated rats has been confirmed. The cortex, which, for the most part, is isosmotic, contains some tubules which are definitely hypotonic. The hypotonic tubules are not seen in the outer medulla. In contrast, the inner medulla which is considerably more concentrated, contains no detectably hypotonic structures. The collecting ducts in the inner medulla generally seem less concentrated than surrounding structures. In animals undergoing a water diuresis, the concentration gradient is less marked; the cortex resembles that of dehydrated animals as does the outer medulla. The inner medulla on the other hand is considerably less concentrated than in dehydrated rats but the smaller structures (loops, capillaries) are definitely hypertonic and, as expected, the collecting ducts are distinctly hypotonic.

A clinical situation pertinent to the problem of urine concentration and dilution is being studied

by Drs. Earley and Orloff. Nephrogenic diabetes insipidus, a disease characterized by inability to elaborate a hypertonic urine due to insensitivity to vasopressin has been reported to respond to a limited degree to chlorothiazide, a diuretic agent. This observation has been confirmed and the mechanism of the drug's action is under study.

Dr. Hoffman is continuing his investigation of the characteristics of electrolyte transport in human red cell ghosts (hemolyzed red cells). He has established that the ghost system transports Na^+ in exchange for K^+ essentially as does the intact red blood cell. Thus electrolyte transport in ghosts has three components. (1) active transport; (2) passive transport, and (3) exchange diffusion. Active transport of Na out of cells requires the presence of K in the extracellular phase and is blocked by strophanthidin. Although this is similar to the situation in intact human cells, the kinetics of the process differs, and indicates, in contrast to the red cell findings, that a single Na compartment exists.

In view of the precise definition of the ghost transport system, it has been possible to utilize it as an assay system in an attempt to define the metabolic intermediates involved in active transport. Dr. Hoffman has been able to show that electrolyte is immediately and completely dependent upon the availability of adenosine triphosphate; further, any reaction capable of generating ATP stimulates the transport system as does ATP alone. It appears that the pump itself is, or has an an intimate component in its structure, ATPase. These observations together with related findings in other laboratories constitute a major advance toward an understanding of the mechanism of active electrolyte transport.

In association with Dr. Hoffman, Dr. Sidel and Dr. Ryan are exploring the possibility of measuring the rate of flow of H_2O across the red cell membrane under an osmotic gradient utilizing a rapid flow system developed by Dr. Tosteson, a former member of this laboratory. The method allows for rapid and continuous separation of extracellular fluid from a flowing system of suspended cells. They are also examining the effect of various liquid membranes (organic solvents) on the movement of Na and K from aqueous phases separated by the solvent membrane. They have established that the addition of cephalin to the membrane confers a slight degree of specificity to the movement of Na and K from one aqueous phase to the other. Other parameters are being studied.

Dr. Cotlove, in association with Dr. Hogben (now at George Washington University), has examined the kinetics of chloride transport across isolated frog gastric epithelium. They have established that chloride movement is more rapid across the luminal than the nutrient membrane of the epithelial cell. Using tracers and an inhibitor which affects only active transport and exchange diffusion (DNP), they have concluded that the drug interferes with luminal transport only, presumably indicating this to be the site of linked, carrier-mediated, transport. Dr. Cotlove has also continued his examination of the so-called "true" chloride method, described in detail in the last report.

Dr. Davis and his associates have extended their studies of the mechanism of aldosterone secretion in dogs and have restudied the pituitary role in this process. Although administration of a low salt diet or constriction of the thoracic inferior vena cava stimulates hormone secretion in normal animals, no such effect was observed following hypophysectomy. Administration of ACTH restores the capacity to respond to vena cava constriction with an increase in the aldosterone output in adrenal vein blood. That this effect of ACTH is permissive is indicated by studies in unanesthetized dogs. The stress of anesthesia and operation induces ACTH secretion and a high corticosterone output in the usual anesthetized animal. In the unanesthetized trained dog corticosterone output is low, indicating that ACTH secretion is not increased. Nevertheless caval constriction stimulates aldosterone output just as in anesthetized animals, without an increase in corticosterone secretion. It is probable that ACTH secretion is normal in the secondary hyperaldosteronism of caval constricted animals, and that ACTH merely serves to support aldosterone production at a high level rather than initiate its secretion.

Attempts to characterize the precise mode of stimulation of aldosterone secretion are continuing. It is now apparent on the basis of work reported in the past by this group that an unknown trophic hormone is involved in the increased se-

cretion of aldosterone in animals with constrictions of the inverior vena cava. Efforts to isolate and identify this hormone are being carried out in collaboration with Dr. Titus.

Chronic denervation of the cervical common carotid, the carotid sinus, and the cervical portions of the external and internal carotid had no significant effect on either electrolyte balance or aldosterone excretion in normal dogs or dogs with constrictions of the vena cava. However, in one dog aortic denervation subsequent to caval constriction and cervical carotid denervation diminished aldosterone excretion without affecting sodium balance. Furthermore, in one other dog, constriction of the abdominal arteries (coeliac, superior, and inferior mesenterics) increased arterial pressure and aldosterone secretion markedly. Although as yet inconclusive, these data are concordant with the possibility of neural receptors somewhere in the vascular tree. Studies of this nature are being pursued. Neither midbrain transection nor pinealectomy has been shown to appreciably affect aldosterone secretion and animals with such lesions appear to respond normally to stimuli known to enhance aldosterone output in unoperated controls.

Drs. Hajdu and Leonard are continuing their study of a mammalian cardiotonic protein system, discovered and characterized by them in the past. Recent efforts have been devoted to the assessment of the physiological significance of this system in man and animals. The concentration of the cardiotonic system is distinctly elevated in a small group of patients with uncomplicated hypertension and in patients with aortic stenosis. It is markedly diminished in a considerable proportion of patients suffering from congestive heart failure of unknown origin (that is not due to the usual causes, such as hypertension, rheumatic heart disease, etc.). The activity virtually disappeared from the plasma of patients subjected to extracorporeal circulation and returned to normal in all but one who developed irreversible vascular collapse. These preliminary data warrant the tentative hypothesis that the system has specific cardiotonic function in man, the activity increasing in a compensatory fashion in disease states associated with increased isometric tension of the left ventricle, and being diminished in severe myocardial insufficiency. Animal studies are in progress in an attempt to delineate the function of the system in a more precise manner.

Laboratory of Clinical Biochemistry

The problem of amine formation and metabolism has been reinvestigated to determine the nature of the decarboxylation of the various amino acids and the routes of metabolism of the resulting amines. By the use of column and paper chromatography it was possible to demonstrate that human urine from normal individuals (particularly following administration of monoamine oxidase inhibitors) contains at least 25–30 amines. In addition to those already known, it was possible to identify ortho-tyramine, meta-tyramine, and phenylethylamine. The presence of so many amines suggested either that there must be many individual amino acid decarboxylases or that the available enzymes were not very specific. The enzyme known as 5-hydroxytryptophan (5HTP) decarboxylase, when purified, was found to decarboxylate DOPA, 5HTP, tryptophan, phenylalanine, tyrosine and histidine, at rates in the order listed. Further work is proceeding on the assumption that a single enzyme, analogous to L-amino acid oxidase in its lack of specificity, is responsible for catalyzing all these reactions. This finding is of great significance from a theoretical as well as a practical standpoint. First, it means that a large number of dietary and metabolically formed amino acids continually give rise to a spectrum of amines. The amount of a given amine which appears in the tissues is then dependent upon the affinity of the decarboxylase for the precursor amino acid, the concentration of the amino acid in the tissues, the presence of competing amino acids, and finally on its rate of destruction by metabolizing enzymes. The latter two factors may vary significantly in many pathologic conditions. As for amine metabolism, it is now apparent that a variety of mechanisms are available. Although all of the aromatic and cyclic amines are metabolized, to some extent, by monoamine oxidase (MAO), there are more specific enzymes available for the metabolism of some of the amines. Thus histamine may be metabolized by diamine oxidase as well as an N-methyl pherase. In the case of the epinephrines, it appears that once they are introduced into the circulation, methylation is the major route of metabolism.

However, it would appear from studies of Dr. Udenfriend and his associates that metabolism of norepinephrine in brain, heart and other organs is largely due to MAO. The complexity of the effects produced by inhibitors of amine metabolism is no longer surprising when account is taken of the fact that serotonin and norepinephrine are but two of the many amines affected. The marked central effects of tryptophan and tryptamine on patients receiving MAO inhibitors comprise but one example of the significance of the totality of amines.

In view of all these factors an investigation of the biochemical effects of inhibitors of amino acid decarboxylation in patients was undertaken in collaboration with the Section of Experimental Therapeutics. α Methyl DOPA was found most effective in this respect and following its administration tyramine formation was found markedly diminished; the excretion of tryptamine and serotonin was also decreased and it appears that there may have been some detectable effects on norepinephrine formation. Simultaneous studies with purified mammalian decarboxylase indicate that α-methyl DOPA inhibits formation of all aromatic and cyclic amines including histamine. The finding that α-methyl DOPA is a potent antihypertensive agent (see report from Section of Experimental Therapeutics) is most interesting and gratifying. However, the biochemical findings indicate that the mechanism of its action is not yet clear and it does not appear to be attributable entirely to diminished formation of norepinephrine.

In view of these interesting and important findings, studies of a number of additional aspects of amine metabolism have been undertaken. Conversion of tryptamine to 6-hydroxytryptamine was shown to be catalyzed by liver microsomes and TPNH. It may be that ortho-tyramine may be formed from phenylethylamine in a similar manner. The mechanism of formation of meta-tyramine is not yet clear but may involve intermediate formation of meta-tyrosine through the action of phenylalanine hydroxylase. Methods have been developed for the determination of tryptamine and tyramine in tissues and in urine. Studies with kynuramine, the amine derived from kynurenine, have led to a simple and direct spectrophotometric procedure for assaying MAO. The aldehyde formed from kynuramine cyclizes so readily to 4-hydroxyquinoline that in the crudest preparations spectrophotometry can be used as a rapid and direct assay for oxidative deamination. This method may be expected to facilitate markedly steps leading to purification and characterization of MAO.

In studies of catecholamine metabolism the enzyme dopamine β-oxidase was shown to be present in as high concentration in hypothalamus and caudate nucleus as in adrenal medulla. However, little if any was found in the higher centers of the brain. The mechanism of the dopamine β-oxidase catalyzed reaction has been under investigation in collaboration with Dr. Witkop's laboratory. One of the results of these studies was the demonstration that dopamine gives rise to 2,4,5-trihydroxyphenylethylamine upon chemical oxidation and that this product appears in the urine when dopamine-C^{14} is administered to animals and patients with pheochromocytoma.

Studies of the mechanism of serotonin uptake by platelets have continued. Using saline media it has been possible to obtain additional evidence that this process is one of active transport. Requirements for K^+ and $PO_4^{\equiv}$ were demonstrated, the demonstration of inhibition by digitoxin being further evidence of a K^+ requirement. A relationship between serotonin uptake and glycolysis was also shown, including marked inhibition by fluoride.

Mechanisms whereby amino acids penetrate into various mammalian cells are also under study. It has been possible to show that *L*-tyrosine is taken up from blood into brain *in vivo* by a process of facilitated transport. The evidence is that the *L*-isomer penetrates rapidly and several times faster than the *D*-isomer and more rapidly than non-amino acid congeners. The uptake of *L*-tyrosine is markedly inhibited by other aromatic amino acids including tryptophan and fluorophenylalanine but is not inhibited by alanine, histidine or lysine. Although tyrosine is rapidly taken up into muscle too, this process is not inhibited by other amino acids. Using rat diaphragm muscle it has thus far not been possible to detect any evidence of active or facilitated transport of tyrosine, the amino acid entering by diffusion only. These findings are contrary to conclusions put for-

ward by Christiansen and others. The studies will be extended to other tissues and to other amino acids.

There have been some interesting findings relating to γ-aminobutyric acid. Transamidination has been shown to occur in brain and to yield γ-guanidinobutyric acid. A peptide containing γ-aminobutyric acid and histidine and possibly another amino acid has been found in brain. In beef brain it is present in amounts as high as several milligrams per cent. Histidyl-γ-aminobutyric acid has already been synthesized by Dr. L. Cohen of Dr. Witkop's laboratory to help in the investigation of structure.

Studies on the metabolism of amino acids unique to collagen have been continued. It was shown that ascorbic acid which influences collagen formation does not do so by influencing hydroxyproline formation. Ketoproline increases hydroxyproline levels in tissues. Investigation of this phenomenon showed that ketoproline inhibits hydroxyproline metabolism by liver and by bacteria, and is itself converted to hydroxyproline. A mammalian enzyme system which catalyzes this conversion in the presence of DPN was studied and found to be distinct from other dehydrogenases. Studies in this laboratory have corroborated the reported presence of ketoproline in actinomycin and have shown that ketoproline is of the *L* configuration. The metabolism of another amino acid found only in collagen was also investigated. Hydroxylysine was found to be metabolized by achromobacter and liver microsomes to 5-hydroxypipecolic acid and 1-amino-5-hydroxyadipic acid. A sensitive and specific method for measuring hydroxyproline in tissues was developed. It has been applied to studies on urinary hydroxyproline in man and animals. Using proline-C^{14} it was shown that in rats urinary hydroxyproline becomes labelled. The rate of disappearance of C^{14} from urinary hydroxyproline in adult rats indicates three components. The first may represent a rapid conversion of proline to free hydroxyproline; the second represents a hitherto unknown peptide material with a fairly rapid turnover (half life $\overline{ca}$ 15 days); the third is typical of the slow turnover of collagen hydroxyproline which is known to obtain in adult animals.

SECTION ON EXPERIMENTAL THERAPEUTICS

The principal areas of investigation in this section may be grouped for descriptive purposes as (1) studies on vasoactive substances, (2) metabolism of amino acids in man, and (3) action and metabolism of drugs.

Vasoactive Substances

Accumulated experience in this laboratory on the differential assay of urinary catecholamines in pheochromocytoma (18 cases) plus a review of such assays reported in the literature (60 cases) indicates the procedure has usefulness in localization as well as diagnosis of the tumor. With exception of two tumors of the Organs of Zuckerkandl, excessive excretion of epinephrine represented a tumor in the adrenal area. In collaboration with Dr. J. Pisano (LCB) methods were developed for measuring the m-O-methyl metabolites of epinephrine and norepinephrine. In studies on the urine of 15 patients with pheochromocytoma, the free catecholamines accounted for 0.4–6.7 percent, the methoxy-catecholamines for 17–42 percent and 3-methoxy-4-hydroxymandelic acid for 57–78 percent of the total excretion of catecholamines plus metabolites. It is felt that catecholamine assays may be supplanted by measurements of metabolites for the initial chemical detection of pheochromocytoma.

A method for estimating catechol-O-methylpherase activity in man has been developed. It consists of administering the d-isomer of isoproterenol and determining the percentage of the dose excreted in the urine as the O-methyl metabolite. Use of this technique along with methods for measuring monoamine oxidase (MAO) activity *in vivo* have revealed no differences between normal subjects and those with primary hypertension. This suggests that if an abnormality in the metabolism of norepinephrine in hypertension exists, the defect lies in biogenesis of the amine or in some other mechanism for inactivation such as protein-binding.

The further study of amines excreted in the urine of patients receiving MAO inhibitors has led to conclusive identification of m-tyramine and

phenylethylamine. In two cases of phenylketonuria the rise in urinary phenylethylamine upon the administration of MAO inhibitors was considerably greater than normal while the rise in m-tyramine excretion was subnormal. The first finding represents the only demonstration of excess amounts of a centrally-active compound in phenylketonuria and the latter suggests that the defective hydroxylation of phenylalanine in this condition may involve the meta as well as the para position.

The cardiovascular actions of various amines are being investigated systematically in dogs and in patients. Many different amines increase contractile force and potentiation of the effect of these amines has been observed during MAO inhibition in the case of phenylalkylamines which lack a B-hydroxyl group and/or alkyl substitution on the amino group. These factors also determine susceptibility as substracts for MAO. Most attention has been given to norepinephrine, serotonin, dopamine, tryptamine and tyramine. Curiously, the administration of Ritalin produced an effect opposite to that of MAO inhibitors, with potentiation of the cardiac effects of norepinephrine and serotonin but not of dopamine, tryptamine and tyramine. In hypertensive subjects, potentiation of pressor responses to dopamine by MAO inhibitors was closely related to the degree of enzyme inhibition (as measured by urinary tryptamine) whereas potentiation of the pressor effects of norepinephrine and methoxamine occurred only when there was also sympathetic blockade as manifested by orthostatic hypotension.

In cooperation with the Clinic of Surgery, extensive studies of cardiac contractile force responses in man have been done using the strain gauge arch technique. The findings were similar to those in the dog and thus resolve such old arguments between pharmacologists and clinicians as to whether norepinephrine is a cardiac stimulant in man and whether cardiac glycosides increase the contractile force of the "normal" human heart. The answer to each question is yes.

Metabolism of Amino Acids in Man

The method of assay for hydroxyproline (OPR) in tissues and urine has been simplified considerably so that it is now possible to measure OPR routinely. A more extensive survey of connective tissue disorders has begun and arrangements have been made to perform a broader study of Marfan's Syndrome in 48 affected families under the care of Dr. V. McKusick of Johns Hopkins Hospital. Study of the specific activity of urinary OPR in rats after injection of C–14 proline has given a measure of the turnover rate of OPR and presumably also of body collagen. Similar studies in patients appear feasible.

Recently, the compound α-methyl-dihydroxyphenylalanine (αM-DOPA) has become available for clinical studies. This substance has been found in LCB and elsewhere to be an effective inhibitor of various amino acid decarboxylases *in vitro* and *in vivo* in laboratory animals. Administration of the compound (2.0 gm./day) to human hypertensives has been shown also to produce decarboxylase inhibition as indicated by a decrease in the excretion of tryptamine and tyramine following standard loading doses of tryptophan and tyrosine respectively. In the course of this work, a hypotensive response was also observed (see next section). αM-DOPA is only the first of several decarboxylase inhibitors to be studied and thus attempts are being made to develop procedures for use as indices of decarboxylation. Patients with pheochromocytoma, carcinoid syndrome, phenylketonuria and urticaria pigmentosa may prove helpful in these studies because of the exaggerated formation of amines in these conditions. By the same token, specific decarboxylase inhibitors may be useful therapeutic agents in these disorders.

Action and Metabolism of Drugs

Five different MAO inhibitors have been evaluated for biochemical and pharmacologic activities in human hypertensives: iproniazid (Marsilid), 1-phenyl-2-hydrazinopropane (JB–516, Catron), dl-phenylcyclopropylamine (SKF–385), nialamide (Niamid) and phenylethylhydrazine (Nardil). Although administration of each of these agents produced postural hypotension, a precise correlation could not be made between the degree of enzyme inhibition and hypotension. However, the dose of each drug required to produce an increase of urinary tryptamine to levels of 500–700 μgm./day was of a magnitude similar to that reported to give optimal psychiatric effects. JB–516 is still the most potent and con-

sistently effective hypotensive agent to be found among this group of drugs. Studies at George Washington University Hospital on 30 patients over a period of 6 months confirmed its effectiveness particularly when used in combination with chlorothiazide. However, the development of visual toxicity (diminished acuity as well as color perception) which has been only slowly reversible in three cases seriously limits the use of this agent in the management of hypertension. The daily dose which may be administered safely is less than 12 mg./day in our opinion and this amount is insufficient for control of the blood pressure in many hypertensives. The d and l isomers of JB–516 have been found to be equally effective for reducing the blood pressure but it is not yet known whether one of them will be devoid of visual toxicity. Complete absorption of JB–516 has been shown through the unusual experiment of demonstrating equivalent effects on urinary tryptamine excretion by single oral and intravenous doses of the drug.

Studies of the actions of MAO inhibitors on the autonomic nervous system in dogs have shown sympathetic (but not parasympathetic) ganglionic blockade with harmine and iproniazid but not with several other inhibitors.

The finding that αM-DOPA produces lowering of the blood pressure in patients with hypertension is under active investigation. A uniform and significant decrease in both supine and standing blood pressure has been observed in several cases during short-term studies.

Since June 1959 studies of anti-fibrinolytic agents have been conducted in collaboration with Professor J. Waldenstrom and associates of Malmo, Sweden. Several aliphatic amino acids were found to be inhibitors of plasminogen activation *in vitro*, the most potent being Δ-aminovaleric acid, Δ-aminolevulinic acid and E-aminocaproic acid (E-ACA). Administration of the latter compound to two patients with pathologic fibrinolysis (secondary to leukemia and cirrhosis) has shown it to be effective in man. A method for chemical assay of E-ACA in urine was developed and about 60 percent of a single dose (6 gm.) given intravenously or orally to patients was found to be excreted within 12 hours. The hypothesis that the early stages of atherosclerosis are related to deficient fibrinolysis is an attractive one and for this reason a continued interest in synthetic and naturally occurring inhibitory substances is contemplated.

SECTION ON CARDIODYNAMICS

It is the ultimate objective of this section to study the physiological behavior of the cardiopulmonary system of human subjects as they go about their usual daily activities and as they are subjected to various physiologic, psychic, pharmacologic, and other stressful interventions, both in health and disease. The measurement of a large number of physiologic variables under conditions most nearly simulating normal activity has made the development of new instrumentation methods of prime importance. Further advances of this broad approach will depend largely upon development of (1) new highly specialized instrumentation and (2) some new sophisticated biophysical and physiologic approaches. The activities of the Section have been determined largely by these needs.

The accurate measurement of pressures has remained a problem of high priority in the Section since new approaches developed in this laboratory both in the vascular and the pulmonary field have placed ever increasing demands on the accuracy of the measurements. A paper reviewing progress in this field will appear shortly. This section has worked with the Laboratory of Technical Development to develop an electrical pressure correction device which will instantaneously and continuously correct dynamic response errors in various pressure manometer systems.

A major advance in the field of blood-flow measurement was achieved when the catheter-computed pressure gradient method for the instantaneous and continuous estimation of aortic blood velocity was developed for use in the intact man. This advance has opened the way to measuring the power output of the heart from moment to moment, the kinematics of cardiac ejection, the power loss at diseased valves, and the distributed impedances and junctional admittances in the vascular tree. Measurements of this kind will be necessary to evaluate the abnormal physical properties and energetics of the vascular system in myocardial disease, coronary attenuation, arteriosclerosis, hypertension and related conditions. To

date, 19 studies, using the catheter-computer method for instantaneous blood velocity, have been performed without major complication and the data are undergoing analysis. The resources of the mathematical and computer section of NIH have been called upon for processing some of these data.

Various electrical analogs of the vascular system are being devised and tested with the use of a Donner Analog Computer. The relationship between the vascular visco-elastic properties and the transmitted pressure and flow wave are being studied in animals. Preliminary results indicate among other things that under certain circumstances it should be possible to infer the character of the central pulse from measurement of peripheral pressure pulses. The significance of this is two-fold: (1) Pressure pulse data may be useful in indirect determination of the visco-elastic properties of intact human vascular systems by the use of a relatively simple computer unit. (2) It may be possible to compute the instantaneous blood flow from the heart using peripheral pulse information.

The implication of this latter possibility is important in that it may make it possible to measure cardio-circulatory function under circumstances close to those of normal activity. The development of miniaturized transducers, amplifiers, telemetering systems and tape recording will be necessary for the ultimate realization of this goal. To this end pilot studies using a tape recorder have been started to investigate the feasibility of multichannel tape recording in the physiologic application. Progress to date has suggested that many improvements must be made; however, the approach appears entirely practical and the acquisition of an improved system of this type will be necessary for the continuation of this work.

Pilot studies in animals, using the blood velocity catheter, are in progress to determine the effect of impairment of the coronary circulation. Acute attenuation of the coronary circulation either by ligature or embolization of the coronary tree produces dramatic and reproducible changes in the blood velocity curve. Methods are being developed for producing chronic coronary insufficiency in dogs. Pilot studies in dogs are in progress to determine the pressure-diameter relationships in various parts of the systemic and pulmonary vascular bed so that inferences regarding flow can be made from the blood velocity curve. When these studies are completed, the problem of systemic and pulmonary vascular impedance can be better evaluated. Preliminary data indicate that although there is a marked divergence between the shape of the pressure and velocity curves in the aorta, their shapes become almost identical as either the systemic or pulmonary capillary bed is approached. This indicates that the arteriolar-capillary-venular bed probably behaves as a pure resistance without significant reactance.

Vascular resistance in the pulmonary bed is being studied both by the catheter blood velocity technique and by conventional dye dilution curve methods to establish comparison of the methods and to establish the effect of intrathoracic pressure and various pharmacologic agents on resistance. These studies are being carried out both in man and in animals. A new method was developed for simplifying and making more reproducible the calibration of dye dilution curves.

Since the direct measurement of the intrathoracic pressure in man is hazardous and in itself alters the normal function of the lung, studies are in progress to establish the relationship of intrathoracic pressure to intraesophageal pressure. The determination of intrathoracic pressure is necessary for determination of the transmural stress on the intrathoracic structures in most of the foregoing studies.

New methods of studying the mechanical behavior of the lung were developed. Detailed studies of the unified pressure-flow-volume-time relationship of the living human lung were carried out in normal, cardiac and emphysematous subjects. A relationship between the maximum achievable expiratory flow and degree of lung inflation was discovered which theoretically has far-reaching physical and physiological implications.

Studies are being undertaken jointly with the Section on Experimental Therapeutics to assess the changes in flow and resistance occurring with the administration of inotropic and vaso-active agents in normal controls and patients with various disease states. The new approach to indicator dilution curves makes it possible to do a sizeable number of these determinations with markedly reduced amount of time and effort.

Other studies are being undertaken to determine the incidence and character of arrhythmias in patients after closure of atrial septal defects. These are done as a joint project with the Clinic of Surgery, NHI. Study of the characteristics and possible mechanisms of formation of ectopic beats during right and left heart catheterization is being pursued by members of the Section in association with Dr. Albert Kistin of the Beckley Memorial Hospital, Beckley, W. Va. This includes development of better intraesophageal and intracardiac electrodes.

Studies of coronary flow dynamics in human patients are to be undertaken in an attempt to develop methods that are valid and clinically more useful. The analysis of nitrous oxide gas by newer techniques including gas chromatography has been explored and has promise. It also appears worthwhile to try to assess the possible use of isotope tracer substances with external counting as a means for finding some index of myocardial blood flow.

SECTION ON CLINICAL ENDOCRINOLOGY

The activities of the Section on Clinical Endocrinology may be grouped into four general areas, as follows: (1) studies on the function and metabolism of steroids and their role in disease states; (2) studies of the abnormalities in water metabolism found in various disease states; (3) studies of calcium metabolism, with special reference to the effects of parathyroid hormone, the effects of vitamin D, and the effects of calcium on renal function; and (4) studies of the permeability of arteries to large molecules.

Steroid Metabolism

Studies in the area of steroid metabolism include further exploration of the control of aldosterone secretion, clinical studies in hyperadrenal corticism, and measurement of relation of steroid function to structure.

Afferent pathways mediating control of aldosterone secretion were explored. The decreased aldosterone secretion which occurs upon release of construction of the inferior vena cava had been found to require the presence of the vagus nerves. It is likely that they arise in the area of the auricles and the great vessels. Studies were carried out to define the pathways required for the increased secretion of aldosterone which occurs upon application of caval construction. It was found that constriction of the carotid arteries was also an effective stimulus to increased secretion of aldosterone. Exploration of the carotid arteries in the dog revealed the presence of an area with slight baroceptor function in the region of the thyrocarotid arterial junction. This baroceptor function could be abolished by denervation of this area. It was found that denervation in this area also abolished the rise of aldosterone secretion following constriction of the carotid arteries as well as that which follows constriction of the inferior vena cava. Analysis of the results suggests that an important stimulus to increase of aldosterone secretion depends upon arterial pulse pressure, and that the intracarotid pulse pressure is the most important variable.

In patients with aldosteronism, direct measurements of blood volume, pulse pressure, and potassium balance have been carried out. Extracellular fluid volume was changed by loading subjects with sodium or depleting them of sodium. Changes in intravascular volume were produced by the infusion of albumin. Finally, the action of aldosterone on the renal tubules was blocked with the use of aldosterone antagonists. With these measurements, it was hoped that primary aldosteronism, with autonomous secretion from a tumor, or unexplained "primary" hypersecretion from hyperplastic glands, could be distinguished from secondary aldosteronism. (Numerous studies, previously reported, support the view that changes in intravascular volume have a major role in the control of aldosterone secretion in man, as in the dog with experimentally produced aldosteronism.) It was found that measures which changed intravascular volume would induce changes in aldosterone secretion in patients with secondary aldosteronism, but not in patients with primary aldosteronism. The effect of aldosterone-blocking agents was more complex. Whereas in most cases of primary aldosteronism, secretion of aldosterone did not rise when the effect of the hormone was blocked, exceptions were seen when large increases in serum and total body potassium followed the use of the blocking agents. Under these circumstances, aldosterone secretion might rise in pri-

mary aldosteronism, as it regularly did in secondary aldosteronism.

It was found that potassium deprivation would lower aldosterone secretion, and restoration of the deficit would elevate aldosterone secretion, as previously reported. In carefully controlled balance studies, it was shown that this phenomenon could be produced without changes in the blood volume, as measured directly with double isotope dilution methods.

Two patients with potassium-losing renal disease were studied in an attempt to elucidate the relationship between aldosterone secretion and potassium loss. It was found that aldosterone secretion would change markedly in response to changes in sodium intake and, thus, could not be considered "primary" or "autonomous." The degree of potassium loss appeared to depend, in turn, upon the secretion of aldosterone. One subject was explored and found to have hyperplastic adrenal cortices. The potassium loss was greatly improved following subtotal resection.

Studies of aldosterone secretion in patients with postural hypotension have been continued. In view of the evidence that arterial pressure has an important role in the control of secretion, it was considered worthwhile to measure the efficiency of the control of secretion in as many subjects with this disorder as possible. Subjects were classified according to the location of lesion, using a series of tests, including mental arithmetic (which may reveal a normal efferent system and locate the essential lesion in afferent pathways) and peripheral nerve block or vasoconstrictor agents (which may reveal an inactive, hypersensitive efferent system and locate the lesion in efferent pathways). It was found that, whereas patients with afferent lesions may show an inability to secrete aldosterone with sodium depletion, patients with efferent lesions usually retain this property.

Patients with Cushing's syndrome have been studied for relative dependence of hydrocortisone secretion upon blood levels of hydrocortisone or hydrocortisone analogs. These studies were done to clarify the locus of the essential lesion in Cushing's syndrome. All patients with hypersecretion of hydrocortisone were tested first with moderate doses of hydrocortisone analog. Under these conditions patients with Cushing's syndrome were found to excrete tetrahydrocortisone in unaltered quantities. They could be distinguished clearly from patients with ovarian disorder who may, at times, show hypersecretion of hydrocortisone, but a ready fall of secretion with the suppressive steroid. The patients with Cushing's syndrome were then subjected to suppressive doses four times as large. The response of the urinary steroids to this procedure allowed the separation of the patients into two groups: patients whose hydrocortisone secretion was not suppressed had adrenal cortical adenomas; with one exception, those who did show suppression had hyperplasia of the adrenal cortex. Subjects with Cushing's syndrome are being further studied by measuring the response to agents which block the 11-hydroxylation of steroids. It is hoped, in this way, to distinguish Cushing's syndrome of pituitary origin from that of hypothalamic origin.

Methodology has been developed for fractionation of 17-ketosteroids, and patterns are being determined in patients with Cushing's syndrome, patients with the adrenogenital syndrome and patients with ovarian disease. In this way, it is hoped that more can be learned about the specific enzymatic defect in the various disorders. In particular, patients with the adrenogenital syndrome may be classified in this way, as regards the presence or absence of 11-hydroxylase.

The factors influencing the protein-binding of steroids *in vivo* have been further studied. The amount of hydrocortisone bound could be greatly increased by administration of estrogen. As this does not alter the metabolic effects attributable to hydrocortisone, it appears probable that the metabolic activity of circulating hydrocortisone depends solely upon the "free" fraction. A number of steroids were tested for their ability to bind to serum proteins. Studies of the effect of fasting and of surgical trauma on the binding of steroids are now in progress.

Studies of the relation of steroid structure to steroid function have been continued, both in metabolic balance studies in man and in acute studies of renal sodium and potassium excretion in the adrenalectomized dog. It is hoped, with such studies, to achieve an understanding of the essential features in steroid structure responsible for the various metabolic effects, and also to allow prediction of structural changes which might enhance the activities of steroids.

Studies of Disturbed Water Metabolism

Studies have been carried out to define the abnormality in water metabolism in a number of diseases in which there is limitation of free water clearance, water retention, and hyponatremia. In patients with cirrhosis, it was found that the antidiuresis resulting from "physiologic" doses of pitressin were not more marked and did not last longer than the effects produced in normal subjects. Furthermore, all subjects could excrete free water after a water load, albeit in minimal amount. The defect in free water clearance in these subjects was attributable to excessive sodium reabsorption in the proximal tubules, and it was shown that they could excrete normal or even increased amounts of free water when proximal sodium was "delivered" to distal sites with the use of mannitol. A similar effect could be produced with infusion of sodium chloride and under these circumstances the increase of free water occurred even without increase of solute excretion. Preliminary results have been obtained in patients with cardiac failure and a similar mechanism appears to be responsible for the defect in free water clearance.

In Addison's disease there is a defect of free water clearance in the presence of large amounts of sodium in the urine. It was shown that a marked increase in free water clearance could be obtained by expansion of total extracellular fluid volume with sodium chloride or of intravascular volume with albumin. In the absence of any steroid therapy, these studies are being pursued to determine whether steroid therapy has an additional effect and whether hemodynamic changes alone would explain the defect in free water clearance or is antidiuretic hormone hypersecretion also involved.

Study of an additional patient with hyponatremia and bronchogenic carcinoma has shown that urinary sodium is, in part, dependent upon sodium intake but related to a much greater extent to water intake. The findings support the view that the syndrome results from sustained inappropriate secretion of antidiuretic hormone and does not result from renal or adrenal disease.

Studies of Calcium Metabolism

Studies of the essential metabolic abnormalities in primary hyperparathyroidism have been continued. With the use of a rigorously controlled test of the effect of phosphorus deprivation together with a low calcium intake, a number of new patients with hyperparathyroidism have been discovered. The adequacy of phosphorus deprivation has been estimated from the extent of the decline in urinary phosphorus. When the phosphorus deprivation is adequate, patients with hyperparathyroidism have shown in all cases a rise of urinary calcium and, in most cases, a fall of serum phosphorus. The addition of calcium loads distinguishes patients with hyperparathyroidism from those with hypercalciuria of other origin in that the latter, but not the former, show a corresponding increase in urine calcium. The addition of glycogenic steroids serves to differentiate patients with hyperparathyroidism and "idiopathic" hypercalciuria on the one hand from those with sarcoidosis on the other. In patients with sarcoidosis studied thus far, the urine calcium has fallen with glycogenic steroid.

Calcium metabolism in known sarcoidosis cases has been studied with the use of balance techniques. These patients have further been subjected to all the tests currently in use in this laboratory for hyperparathyroidism. This includes the determination of the Tm of phosphorus and the response of serum and urine phosphorus to a standard calcium infusion during a period when a constant diet is given. The balance studies have been so designed that the effects of vitamin D (to which these patients are said to be hypersensitive) and the effects of glycogenic steroid (which are said to block the effects of vitamin D in this syndrome) could be assessed separately and together. In this way, we are exploring the hypothesis that the defect in calcium metabolism in sarcoidosis is essentially one of hyperabsorption of calcium, that this results from abnormal sensitivity to vitamin D, and that the effects are reversible with steroids.

An attempt to prepare radioactively labeled vitamin D is in progress. This is to be used to determine the fate and distribution of vitamin D. The effects of vitamin D will be assessed in a preparation of rabbit intestine and also in dogs and rats that are parathyroidectomized and maintained on calcium alone.

The defect in the renal concentrating mechanism in all types of hypercalciuria has been studied with renal function techniques. We have confirmed that this defect is pitressin resistant.

It has been found that it may occur in the presence of hypercalcemia or hypercalciuria without hypercalcemia. It has been shown to be independent of solute load. A quantitative measure of the extent of the defect may be obtained by measuring TcH_2O in the manner of Zak, Brun and Smith. With this technique it has been shown that the defect may be produced in patients with "essential" hypercalciuria within 2 weeks by high calcium feeding, and that it may be eliminated by a similar period of calcium deprivation. In patients with hyperparathyroidism it was possible to return urinary calcium to normal with versene and sodium phosphate. With this treatment the concentrating defect did not improve. These studies will be extended to determine whether hypercalcemia alone is sufficient to produce the defect and to determine the points of similarity of this defect with that resulting from potassium depletion.

Studies of Aortic Permeability to Large Molecules

Studies of the movement of protein and lipid through arterial walls have been continued in rabbits and dogs. Labeled albumin and cholesterol have been administered and the rate of accumulation and loss from the aortic wall has been measured as a function of depth of penetration and of longitudinal sites in the aorta. Comparable studies on animals with the hypercholesteremia of hypothyroidism have been instituted. The localization of atherosclerosis, as judged from these studies on cholesterol deposition, appears to depend more upon a decreased rate of cholesterol removal from more distal areas than upon increased rate of deposition. The deposition rate of cholesterol and protein in the aorta may best be explained, from available data, as a function of the circumferential tension in the aortic wall.

Surgery Branch

There has been a continuing interest in methods for the precise detection and localization of circulatory shunts. The usefulness for this purpose of various inert gases, as well as the application of indicator-dilution curves, has been previously demonstrated. A study of the use of indicator-dilution techniques in determining drainage pathways of the pulmonary veins in the presence of atrial septal defect, has shown that, by appropriate injection and sampling, the presence or absence of anomalous pulmonary venous drainage can be precisely determined. The validity of the method was proved in 29 patients. The injection of a radioactive or colored indicator into a heart chamber, with sampling at a proximal site, makes possible detection of pulmonary or tricuspid valvular regurgitation and estimation of the magnitude of the regurgitant flow. Localization of the site of origin of a left-to-right shunt by means of indicator-dilution curves has, in the past, required injections into the left side of the heart. The difficulties of this technique were found to be obviated by a new method in which sampling was carried out within one of the right heart chambers following the intravenous injection of dye. This simplified technique indicated the correct site of entrance of the shunt in all patients in whom it was applied. A study was also made of the effect of injections of vasopressor agents on indicator-dilution curves in patients with shunts as well as valvular regurgitation. Whenever a left-to-right shunt was present, its magnitude, as measured by indicator-dilution curves, was increased when vasopressor drugs were given.

An important advance in the field of indicator-dilution methodology has been the adoption following the work of Clark of ascorbic acid as an oxidation-reduction indicator. By means of a platinum electrode incorporated within a needle the concentration of this reducing substance can be recorded continuously without sampling arterial blood. The principal advantages have been in the study of children and experience to date demonstrates that the contour of oxidation-reduction dilution curves is virtually identical to those obtained with blood sampling through a photoelectric densitometer. Similarly, the usefulness of solutions of cold saline for recording indicator-dilution curves has been investigated further. Difficulty with the method has been the establishment of a stable base line, but a new electronic circuit has, in pilot studies, apparently obviated this. Curves recorded after the injection of cold solutions with thermistor sensing in a peripheral artery have the same advantages as those described for oxidation-reduction curves.

It has been shown in several laboratories that when a gamma-emitting substance, such as radioactive diodrast, is injected intravenously the passage of the isotope through the heart can be detected with a scintillation counter placed over the patient's chest. Such curves, recorded in more than 100 patients admitted to the service, demonstrated that the contour of the precordial dilution curve indicates presence or absence of a left-to-right circulatory shunt. The method is valuable in screening patients and may obviate postoperative catheterization in patients subjected to operation for the correction of such shunts.

Finally, the principle of isotope dilution has been applied in the study of abnormal communications between the systemic and portal venous systems. In both patients and animals a solution of radioactive krypton ^{85} was injected into the spleen. When no abnormal communications existed, the appearance of the gas in expired air was greatly prolonged. When esophageal varices or a patent portacaval anastomosis was present, however, the gas appeared immediately and in high concentration. It is probable that this method is safer and more sensitive than portal venography for assessing patients with portal hypertension and varices both before and after operative treatment.

An important group of studies has centered around the clinical use of the artificial heart and lung machine. The instrument itself has been further refined to permit constant observation of the oxygen tension in blood returned to the patient, precise control of the volume of blood returned to the patient, and yet another electronic device has been developed which maintains constant the volume of blood contained within the patient and the extracorporeal circuit. All of these improvements have resulted in clinical provisions more closely approximating the normal physiologic state. A detailed investigation has also been made of the bacteriology of the heart-lung machine. These studies indicated that in virtually all instances bacterial contamination of the apparatus occurs, but that it could be minimized by special assembly techniques and the installation of bacterial filters at all points where room air has access to the circuit.

In the early experience with open heart operations, flaccid paralysis of the heart was often induced by injection of solutions of potassium citrate into the coronary bed. It was noted in several patients that effective ventricular contraction did not resume after cardiac arrest and two studies of this phenomenon were undertaken. In an experimental study it was shown that left ventricular function was severely impaired after administration of either potassium citrate or acetylcholine, but that intermittent occlusion of the ascending aorta without a chemical agent had no demonstrable effect on ventricular function. In 15 of 19 patients subjected to potassium citrate arrest a distinctive type of myocardial necrosis, most prominent in the left ventricle, was found. In the hearts of 19 other patients in whom this agent had not been employed no lesions of this type were discernible. These physiological and anatomic observations have led to the abandonment of the technique of elective asystole in the course of cardiovascular operations. Intermittent aortic occlusion can be employed in virtually all procedures in which aortotomy is not necessary. When the aortic valve must be exposed for long periods it has been found that effective myocardial contraction can be maintained for nearly 2 hours by direct perfusion of the left coronary artery. This technique is thus employed whenever aortic-valve operations are necessary.

When the heart is divorced from the peripheral circulation in the course of cardiopulmonary bypass, there is a unique opportunity for studying the effects of various drugs and procedures on myocardial contractility, and the central and peripheral effects of these agents can be separately assessed. In nearly 80 patients a myocardial strain gauge arch has been placed at the beginning of the thoracotomy and in the course of the operations injections of various pharmacologic agents have been made and their effects on myocardial contractility studied. It has been found that acute digitalization increases the contractility of the nonfailing heart. The effects of various vasopressor agents have been compared. Norepinephrine and epinephrine produced identical increases in cardiac contractile force but vasoxyl gave no such response. It has also been possible, with the strain gauge arch, to study the effects of various anesthetic agents such as fluothance, demerol and muscle relaxants, drugs given commonly in the course of cardiac operations. Studies of contractile force have further indicated

556044—60——7

the safety of aortic occlusion and coronary perfusion in the course of open procedures.

The development of methods for catheterization of the left side of the heart by the transbronchial, the percutaneous and the retrograde arterial routes has been described in previous reports. In the past year the transseptal method of left heart catheterization developed in this laboratory has been employed in more than 100 patients. In this technique the left atrium is entered by a needle passed from the right atrium in the course of right heart catheterization. The method provides opportunity for the prolonged measurement of pressures in the left side of the heart with the patient in a comfortable basal condition. No complications have been encountered in this limited experience. Preliminary experiences also indicate that selective angiocardiography, with injection into the left atrium through the transseptal needle, is a convenient and useful method in the study of patients with both congenital and acquired lesions.

Mitral commissurotomy, in the past, has usually been performed with dilatation of the valve with the finger inserted from the left atrium or with a knife passed from this approach. More recently, however, the valve has been opened by means of a dilator inserted from the apex of the left ventricle, the commissurotomy being controlled by a palpating finger passed from the left atrial appendage. A detailed study of the results of operation in patients operated upon by the latter method indicates that a superior hemodynamic result almost invariably can be obtained. Twelve patients have been operated upon for the second or third time for mitral stenosis. In no patient could restenosis of the valve be documented and it is felt that in most instances the obstruction was residual rather than recurrent. In 8 of the 12 patients a good hemodynamic and clinical result was obtained by an effective repeat operation. In the course of both open and closed operations for the correction of mitral stenosis and mitral regurgitation, valves have been encountered which are so badly damaged that a corrective procedure has been either unsatisfactory or impossible. A prosthetic mitral valve, suitable for entire replacement of the diseased valve, has been designed and preliminary studies of its usefulness have been carried out in animals. The present, and most promising model is constructed of urethane foam, reinforced with plastic mesh cloth. Studies of the effects of various plastics and plastic surfaces on the coagulation mechanisms of the blood have also been initiated. Such information will probably be of paramount importance in selecting the material for fabrication of prosthetic mitral, as well as aortic, valves.

In normal patients or animals, general body hypothermia at temperatures of 28–30° permits total arrest of the circulation for periods of only 10 or 12 minutes. If the metabolic demands of the body could be further reduced by abolition of the thyroid gland this safe period of circulatory interruption might be extended. In an investigation of this possibility dogs were rendered myxedematous by the injection of I^{131}; survival could be regularly obtained after 20 minutes of circulatory interruption. Another experimental study concerning hypothermia has been an evaluation of the effects of quinidine on myocardial function. This drug is commonly administered during general hypothermia to prevent arrhythmia. Preliminary results thus far indicate that quinidine itself has a depressant action on myocardial function.

The major problem of total replacement of the heart is immunologic. Even if means can be found to obviate these difficulties, however, many technical problems remain. In acute studies in animals the transplanted heart is completely denervated and this is considered, in most instances, the cause of death. An experimental study of the totally denervated heart *in situ* is underway and with refinement of the operative technique chronic survivors have been obtained. An attempt to determine the optimal method for storing an excised heart prior to reimplantation is also in progress. The comparative value of perfusion with blood and various physiologic solutions is being studied and attempts are being made to determine whether the beating or arrested heart is most suitable for long-term preservation.

No adequate operation is available for the treatment of patients with complete atresia of the pulmonary artery or true truncus arteriosus. An experimental study has been made of methods for total replacement of the pulmonary artery by the insertion of a plastic graft into the outflow tract of the right ventricle and suturing the other end of the graft to one or both pulmonary arteries. Death has occurred in some animals for technical reasons,

but in the majority of survivors the prosthesis has been proved patent at the time of sacrifice or when angiographic studies were carried out.

Eight patients have now been studied in whom obstruction to outflow from the left ventricle was caused not by discrete narrowing in the region of the valve or subvalvular region, but by massive left ventricular hypertrophy of unknown etiology. An attempt has been made to reproduce this lesion in the experimental animal. Constrictions of the ascending aorta were made either by excision of a portion of the wall of the aorta or by banding it with a tape of plastic material. Large pressure gradients between the left ventricle and aorta have been created and serial cardiac catheterizations are being carried out to determine the progression of the lesion. The studies have not been in progress long enough to determine if massive left ventricular hypertrophy can be induced by this technique.

Considerable effort (in the Section of Cardiology) has been directed toward elucidating the manner in which various hemodynamic factors modify the heart's performance. The relationship between left ventricular end-diastolic pressure and circumference has been systematically investigated in the dog. It was observed that with tachycardia, ventricular end-diastolic pressure rose at a constant end-diastolic circumference. At a constant heart rate, acutely induced hypothermia had a similar effect, and it is suggested that abbreviation of the phase of ventricular filling by both of these interventions is responsible. On the other hand, deterioration of the heart's performance, i.e. acute heart failure, resulted in an augmentation of end-diastolic circumference at any end-diastolic pressure. This appears to be a true alteration in ventricular distensibility. However, alterations in aortic pressure and cardiac output did not modify ventricular distensibility. The latter observations indicate that myocardial oxygen consumption is not primarily dependent on the end-diastolic size of the heart.

In studies of the circulatory responses to acutely induced hypervolemia in man, it was observed that a striking augmentation of cardiac output occurred only when the activity of the autonomic nervous system had been reduced by ganglionic blockade. This investigation shows that in studying the validity of Starling's law of the heart in man, circulatory changes occurring secondary to activity of the autonomic nervous system, rather than of the heart itself, must be excluded. Indeed, in several subjects a consistent relationship between ventricular end-diastolic pressure and stroke work was observed in the presence of ganglionic blockage and, under these circumstances, Starling's law quite clearly operated. In a similar investigation of the relationship between left ventricular end-diastolic fiber length, end-diastolic pressure and the force of ventricular contraction in patients with mitral valve disease and atrial fibrillation, studied at the time of operation, further evidence of the applicability of Starling's law of the heart to man was obtained.

As part of a continuing investigation of the pharmacology of digitalis glycosides, it was demonstrated both in dogs and in patients on cardiopulmonary bypass that these agents are potent vasoconstrictors. In addition, a venoconstrictor action in the dog was found. These observations, when taken together with the studies on myocardial contractile force in man, described above, permit a more rational explanation of the effects of digitalis on the nonfailing human heart. In a study of the effects of acute digitalization on left ventricular dynamics, it was shown that the elevated left ventricular end-diastolic pressure of myocarditis may be lowered, but that this did not occur in patients with aortic stenosis. It is suggested in the latter group of patients that the hypertrophied ventricular wall alters ventricular distensibility and in this manner elevates end-diastolic pressure; the latter then does not reflect the presence of myocardial insufficiency, as it does in other diseases. Finally, increased digitalis requirements were demonstrated when hypothyroid patients were rendered euthyroid or when euthyroid patients were rendered hyperthyroid. Since catechol amine depletion by reserpine blocked this antagonism between thyroid and digitalis, the increased digitalis requirements associated with augmented thyroid activity are believed to be related to increased sensitivity to endogenous catechol amines.

A preparation has been developed in the dog on complete cardiopulmonary bypass which permits the simultaneous determination of the capacity of the vascular bed and of the distensibility of the

venous bed. This preparation will permit a study on the effects of a variety of cardiovascular reflexes and drugs on these two important hemodynamic parameters.

In a continuing study of the factors which modify the distribution of blood it has been demonstrated in normal control subjects that exercise results in an increase in intrathoracic blood volume and that morphine apparently decreases intrathoracic blood volume.

Gerontology Branch

The program of the Gerontology Branch is concerned with 1) a description of physiological and psychological changes that take place with increasing age in humans and 2) investigations of the basic biology of aging.

HUMAN PHYSIOLOGY

Longitudinal Studies

Age differences in physiological and psychological characteristics of normal people still living successfully in the community are being evaluated by Dr. Shock, Dr. Falzone, and Mr. Norris. Subjects ranging from 32 to 99 years have agreed to return to the laboratory for retesting every 18 months for the remainder of their lives, so that age changes can be observed in individual subjects. Retest schedules began in October 1959, so that no serial analysis of individual records is possible. However, a preliminary comparison has been made of some tests on the first 100 subjects with previous results obtained on hospital subjects. The outside subjects are larger in size than the hospital group. However, they do not differ significantly in body composition or in basal metabolism from the hospital subjects. Their pulmonary function is better maintained than in hospital subjects and they fail to show an age decrement in concentrating capacity of the kidney as measured by 12 hours of water deprivation, although their 12-hour endogenous creatinine clearance falls with age at a rate similar to that of hospitalized subjects.

In addition to retesting subjects in the series, new subjects will be added to the program, with special emphasis on men over age 70. A number of new tests of intellectual functions and personality characteristics will be administered to all subjects. A special test of attitudes toward aging, developed in this laboratory, is being administered to these subjects.

Renal Studies

A method for estimating the glomerular clearance of unbound hemoglobin in the human has been developed by Dr. Lowenstein and Dr. Faulstick and hemoglobin/inulin clearance ratios of 0.028 to 0.065 have been found in preliminary experiments. The disappearance of the hemoglobin-haptoglobin complex from the blood follows first order reaction kinetics over the time period of 0–120 minutes. Age differences in this function are being assessed and the validity of this test as an index of the functional capacity of the reticuloendothelial system in the intact human is being assessed.

Comparison between the 12-hour endogenous creatinine clearance and short term inulin clearances have been made in the same subject by Dr. Oursler and Mr. Yiengst. Creatinine clearances are on the average 30 percent higher than inulin clearances, but the correlation is high, so that endogenous creatinine clearances can be used as an index of renal function where intravenous infusions and bladder catheterizations are impractical. Studies of age changes in renal function by serial measurements on the same individual will be continued. The studies of permeability of the glomerular membrane will be continued using infusions of dextran.

Body Composition

The helium chamber method for determining body volume has been perfected by Mr. Norris and Dr. T. Lundy. Estimates of body composition, based on density measurements as well as total water content, show a decrement with increasing age in community residing subjects that is of the same order of magnitude as hospital patients. Lean body mass, or body water determinations are being used rather than calculated surface area as a basis for the normalization of measurements such as basal metabolism.

Response to Standardized Exercise

Measurements of age differences in the cardiovascular, respiratory and metabolic responses to exercise have been continued by Mr. Norris and

Dr. Falzone. Maximum work output and rate of recovery of vascular and pulmonary displacements are lower in old than young subjects. Mechanical efficiency is lower in old subjects at high and low rates of work, but is essentially the same for old and young at intermediate rates. The factors involved in the reduced efficiency of the aged are being investigated.

PSYCHOLOGICAL STUDIES

Although an increase in reaction time with age has been previously demonstrated, Dr. M. Davidoff and Dr. G. Suci have shown a linear relationship between the rate at which information can be handled and the age of the subject. This relationship appears when stimuli are considered in terms of information theory. Further investigations of this phenomenon in different sense modalities will be followed. Other studies by Dr. F. Hugin and Mr. Norris show that slowing of responses with age appears in tasks that involve the central nervous system, so that the slowing is a reflection of central rather than peripheral changes in the nervous system. Dr. W. Surwillo is continuing his studies on the relationship between EEG frequency and spinal reflex time, to determine central effects on reflex times.

Previous studies, showing that short-term memory for visual comparisons is more easily interfered with in young than old subjects, have been extended to include auditory discriminations of time intervals by Dr. Davidoff. Other studies of memory for verbal material show that the poorer memory of older subjects can be improved by reducing the length of the verbal sequence to be recalled and increasing the redundancy or degree of relationships between the words in the series. Thus, rote memory is impaired to a greater degree than memory for logical sequences in older people.

BASIC BIOLOGY

Cellular and Comparative Physiology

The activities of the Cellular and Comparative Physiology Section involve (1) the description of cellular and organismic changes in humans and appropriate experimental animals during the aging process and (2) the measurement of the effects of environmental and physical manipulation on the performance and mortality of experimental animals.

Dr. Bodenstein has demonstrated clearly in his studies of regeneration in cockroaches that the capacity to regenerate lost parts (e.g. legs) is not lost even when the animal would normally no longer demonstrate such capacity. Adult cockroaches will regenerate legs to replace those amputated if supplied with growth hormone through transplantation of the prothoracic gland from a young (molting) animal. This demonstrates that the cells of tissues of adult cockroaches still possess the capacity to replace lost parts in the proper humoral environment.

Dr. Konigsberg has shown that contractile-striated skeletal muscles will differentiate from embryonic cells in tissue culture and that the process includes the following phases: (a) Cell division and multiplication in culture. (The cells which will differentiate into muscle are, at this stage, not distinguishable from other dividing cells in the culture.) (b) Cell fusion. During this phase, the cells form large multiple nucleated fibers or straps. Evidence from time lapse photography and electron microscopy makes it highly probable that these are indeed cells with cytoplasmic continuity. During this period, measurements of DNA per nucleus made in collaboration with Dr. Strehler indicate that there is no nuclear division following fusion. (c) Differentiation of contractile fibers from the multinucleate straps. During the early stages of this process striations are visible only in glycerinated preparations. Later they become visible even in nonglycerinated preparations observed under phase contrast microscopy.

These findings furnish an ideal tool for the study of differentiation, factors affecting it and the possible dedifferentiation and redifferentiation which have implications regarding the continued capacity of cells to furnish replacement parts in adults or aged vertebrate animals, provided that the factors limiting this process are known and modifiable *in vivo* (as they are, for example, in the roach studies).

Drs. Mildvan and Strehler have continued their study of the chemical and physical properties of heart age-pigment granules. Approximately 75 percent of the weight of the particles consists of an insoluble material with chemical and infrared

absorption characteristics consistent with protein. Further elucidation of its nature and of the adherent as incorporated pigment is being undertaken by Drs. Hendley and Strehler.

Dr. Strehler has continued his study of the effect of environmental factors on the longevity of *Drosophila melanogaster*. It appears clear that the aging of *Drosophila* is not a result of denaturation—since aging does not possess a high activation energy. This conclusion follows from the fact that flies that have survived high temperature shocks sufficient to kill half of them, are not aged as measured by their subsequent mortality behavior. Similarly, it has been shown that aging in *Drosophila* is not the result of mutation since exposure of animals to 4500 R actually doubled their life expectancy. Heavy water, on the other hand, in 20 percent or 40 percent concentration, reduced the longevity by about a factor of two.

Studies with *Campanularia flexuosa* hydranths have demonstrated no decrease with age in the following physiological functions: food catching ability, rate of ingestion of food, rate of digestion, rate of egestion, and amount of food that can be handled. Low temperatures extend the life span in a fashion similar to that observed with *Drosophila melanogaster*. X-radiation even in enormous doses (50,000 R) does not inhibit the continued development of hydranths which has already begun. Moreover, the animals receiving moderate doses of radiation lived twice as long as their controls—in agreement with the results on *Dorsophila* outlined above.

Nutritional Biochemistry and Tissue Enzymes

The determination of various enzymatic activities as well as DNA and protein nitrogen in the liver, kidney and heart of ten 1-, 3.5-, 12- and 24-month-old rats failed to establish a simple classification of enzymes into groups which follow similar age changes. Dr. Barrows has found that although the concentrations of the various enzymes in the tissues of 1-month-old rats were different from those of older animals, the only change which could be associated with senescence was the higher cathepsin activity in the liver and kidney of the aged rat. No evidence for an impaired protein synthesis in senescent rats was found by the depletion-repletion method and only slight differences were observed over the age span of 3.5 to 24 months.

A preliminary age study carried out on young and old rats subjected to unilateral nephrectomy showed similar hypertrophy of the remaining kidney in both young and old rats. Whereas the increase in total DNA, d-amino-acid oxidase, and alkaline phosphatase approximated the increase in organ weight, the total succinoxidase and pyrophosphatase showed a greater increment. The degree of hypertrophy estimated by any of these measurements failed to indicate any age differences.

Future experiments will include further studies on oxidative phosphorylation in order to find a system which will be adequate for an age study.

Intermediary Metabolism

One of the major research activities has been concerned with the mechanism of oxidative phosphorylation associated with α-ketoglutarate oxidation. The oxidative reactions in the electron transport chain immediately concerned with the synthesis of primary high energy compounds have not been identified. Preliminary work led to the hypothesis that the critical step might be reduction of a disulfide compound to a product with vicinal dithiols and simultaneous phosphorylation of one of the thiols utilizing the strain energy of the cyclic disulfide. Transfer of phosphate to an acceptor would lead to a dithiol compound. Studies to test the hypothesis are in progress but have not yet provided definitive conclusions.

In α-ketoglutarate oxidation, the primary high energy compound is an unidentified acyl enzyme complex. The present aim is to identify the complex and to study the mechanism of its formation. Resolution of the α-ketoglutaric dehydrogenase complex and purification of the components was necessary in order to permit use of stoichiometric amounts of highly purified enzyme for direct isolation of the acyl enzyme complex. One of the resolved components has been purified and shown to be a flavoprotein with flavin adenine dinucleotide as the prosthetic group. This flavoprotein which catalyzes the terminal transfer of electrons from the reduced thioctyl to DPN seems to be identical with Straub diaphorase.

There are claims in the literature that the morphology and oxidative properties of mito-

chondria change with age. It is of considerable interest in this connection to determine whether mitochondria have a defined life span at the end of which the entire unit disintegrates or whether components within the mitochondria turn over at different rates. It is proposed to label three different components of mitochondria—lipids, proteins, and cytochrome C—and follow the decrease in the labeled component with time. The results may be useful in deciding between the two alternative possibilities.

Biophysics

Evidence has been found suggesting that the amino group on the adenine ring of ATP is involved in the interaction of ATP with the muscle enzyme, myosin, and that this interaction is accompanied by a conformation change of this enzyme. Studies in this laboratory have shown that Cu^{++}, Cal^{++}, and Zn^{++} interact with myosin in a manner superficially similar to that of PCMB (parachloromercuribenzoic acid, a sulfhydryl reagent). Furthermore, these metals and PCMB are found to interact with myosin during the course of incubation with myosin. ATP appears to prevent some of this time dependent interaction.

Cultures of a bleached variety of *Euglena gracilis B* have been established and it is hoped that these organisms will prove useful as a tool for biophysical and biochemical studies on the effect of "age" on single cell systems. Preliminary results have shown that such cultures may be kept well over one month at constant cell density. Experiments have now begun to study the effects of aging under various well-defined conditions on subsequent growth. Since these organisms are sensitive to steroid hormones, Dr. Buetow has tested their response to vitamin D, and has found that growth appears accelerated by this vitamin. This is the first time an effect of this vitamin has been found in other than a mammalian system.

After the biochemical characterization of the mitochondria from *Euglena* has been completed, studies will be undertaken to determine the effects of aging on a variety of mitochondrial functions, such as stability, turnover, permeability, etc.

Molecular Biology

Important findings in the elucidation of the structure of catalase are that the four heme groups are symmetrically placed in the molecule, and that they can be reversibly removed from the molecule without affecting their site of attachment to the protein.

A method is being developed for detecting the position of nucleosides in a nucleic acid chain by selective complex formation with metal ions.

Catalase that has been cleaved into quadrants, each containing one heme, has no anomalous rotatory dispersion, indicating that the heme is symmetrically surrounded by protein; intact catalase, on the other hand, containing four hemes, has an asymmetric iron atom. The heme has been removed from catalase, yielding the apoenzyme; the latter can aparently be recombined with hemin to reform the catalase molecule.

Horse hemoglobin is split along different axes by acid and base treatment. Human hemoglobin, like horse hemoglobin, has asymmetric iron in the reduced, oxidized, and oxygenated forms.

Vitamin B_{12} exhibits anomalous rotatory dispersion due to the presence of an asymmetric cobalt atom. The rotatory dispersion curve is unaffected by substitution for cyanide on the cobalt atom, and it is not greatly affected by reduction of cobalt. The rotation is markedly influenced, however, by changes in the three-dimensional structure of the molecule.

A correlation has been made between the electronic configuration of transition metal ions and their ability to catalyze the aconitase and enolase reactions. It has been shown that nickel, cobalt, and iron catalyze the aconitase reaction in the absence of enzyme. The mechanism of formation of the Schiff base intermediates in transamination reactions has been further elucidated by the finding that a carbinol amine intermediate is not formed in such reactions.

NATIONAL INSTITUTE OF ARTHRITIS AND METABOLIC DISEASES

BASIC RESEARCH

INTRODUCTION

For the NIAMD to fulfill its assigned mission, its scientists are impelled to conduct research over a wide spectrum, ranging from the purest and most basic to the applied and immediately practical. Much has been written about the distinctions between these types of research and the purported merits of the one over the other. It is not our purpose at this time to enter into these controversial matters, other than to clarify the reasons why it is that both extremes, as well as all the intermediate shades between the practical and the pure, are legitimate areas of inquiry for scientists at this institute.

The benefits of applied research and development are likely to be immediate and immediately apparent. A new treatment for a disease, a new diagnostic procedure, a new prophylaxis, is a justification in itself, and the quest therefor requires no additional defense. Such research is characterized by the fact that the end is in view from the outset, and the motivation of the investigator is in major part this end in view. The research, if successful, has immediate consequences of assistance to the patient and to the doctor. A disease is more precisely diagnosed, more effectively treated, and more uniformly prevented than formerly. If the problem attacked is of sufficient magnitude, such research may markedly benefit the public as well as the private health of the Nation and the world.

It is, however, the history of science that nearly every practical advance is sooner or later rendered obsolete by yet another succeeding development. The new drugs of yesterday are supplanted by the new drugs of tomorrow, the diagnostic tests of yesterday are often only of historical interest today. In this sense, the results of applied research, although possibly of enormous contemporary importance, are largely to be evanescent. It is true of course, that the applied research of today may serve as a basis for future developments. This however is a by-product and insofar as it occurs, the earlier research should be regarded as basic in character.

The outstanding characteristics of basic research are its purpose and its motivation. Its purpose is simply to add to man's knowledge and understanding. No end other than this is in view. The motivation of the scientist engaged in such research is quite simple. It becomes increasingly clear that curiosity about nature is the major driving force of our most productive investigators in biomedical laboratories. In contrast to the immediate, often transient, effects of applied research, the fruits of pure research have a permanence about them. In a changing world there are few things so likely to endure as are the results of good basic research. Their importance lasts through the ages and may at any time give birth to further extension, both pure and applied.

It is in the nature of science to be both recorded and cumulative. All scientists depend heavily upon the researches of their predecessors, and doubtless this is what Newton had in mind when he wrote, "If I saw farther, it was because I stood on the shoulders of giants." The additions to man's knowledge, accomplished by the scientist of today, are the foundations upon which scientists of the future, both pure and applied, must build. In these terms, practical benefits from pure research are essentially inevitable, even though not necessarily anticipated when the research was undertaken.

We review with pride our research accomplishments for the calendar year 1959. The significance of the more applied aspects of our program is self-evident. Fully as significant are the results of the more basic aspects of our program. The conduct of basic research requires no apologies. It is a good thing to do for its own sake and we are happy to be in a position to support so many

fundamental investigations in natural sciences at NIAMD. The enduring fruits of basic research are the highest rewards of the scientific career.

The Blessing (and Curse) of Bigness. NIH is probably the largest concentration of science and scientific manpower in biomedical research in the world today. This fact carries with it certain advantages as well as some disadvantages. Furthermore, some of the benefits are more imaginary than real. Because of its great size, NIH can economically support vast central services such as an animal-production facility, extensive shops, and a fine library. It is economically feasible for NIH to engage in the purpose or construction of costly equipment which might be beyond the reason of a smaller institution.

It is noteworthy that, in the past, but a very small fraction of the major accomplishments of NIAMD have been directly dependent upon these purported advantages. To a far greater extent these accomplishments have depended upon the ideas and skills of the individuals who comprize the scientific and clinical staffs. The fraction of prominent research conducted at Bethesda which would have been impossible elsewhere for want of physical plant is not great, and this is also true of this year's results, reported in what follows.

Furthermore, there is a necessary price paid for the benefits of bigness. This is to be seen in the increased difficulties in communication, at all levels. The investigator in so large an institution loses contact with the administrator responsible for budget and policy decisions. He finds it increasingly difficult to maintain intimate contact with the shop that builds his equipment or the agency which renders some essential service to his project. Most important, with almost 1,000 scientists on the campus, each individual scientist can be familiar with only a small fraction of what is going on in other laboratories.

There is, on the other hand, one blessing of bigness, which, for certain investigators, is overriding. Among the many scientists about Bethesda, virtually all research disciplines are represented. Very high degrees of skill are to be found in physics, mathematics, chemistry, the biological sciences, and medicine. In the present era of research, the discovery that there are unplowed fields between established disciplines, that these fields contain pay dirt, has favored multidisciplined approaches to problems of interest. Some, but certainly not all of our more effective investigators, have realized and taken advantage of this situation. They have increased the scope of their effectiveness by the establishment of collaborations with others of diverse background and experience. Such collaborations are likely to be exceptionally productive. It often appears that the whole is far greater than the sum of its parts.

It must be borne in mind that not all scientists are naturally collaborative. Some of our best scientists, like most artists, work most effectively by themselves. Others prefer to acquire satellite scientists about them. Still others prefer to explore interdisciplinary areas in conjunction with scientists of other skills. It is to the last group that the size of NIH is a particular blessing.

Recruitment and Adjustments of Program. A number of additions to staff have been made. In the clinical area, a group interested in metabolic diseases of infancy and childhood has been collected. A part of the interests of this group is in cystic fibrosis, a pediatric problem of growing importance. A start has been made also in the area of diseases of the gastro-intestinal tract. In the laboratory area, it has been in the physical-mathematical specialties where most recruitment has occurred. Personnel in these specialties are in low supply and in high demand, making recruitment particularly difficult. A number of people of high ability have, however, been found and brought to Bethesda.

The recent retirement of Dr. Ralph Wykoff, and the imminent retirements of Dr. Ralph Lillie and Nathan Eddy, have created problems. For each of these vacancies, candidates have been considered and tentative solutions reached. It is hoped that before next year's report, a happy resolution of each of these problems will have been reached.

Laboratory of Nutrition and Endocrinology

One of the primary goals of the laboratory is to investigate the biochemical, physiological, and histological changes that occur in animals associated with dietary or endocrinological alterations. Such studies should eventually indicate how the nutrients and hormones carry out their essential functions.

NUTRITION

Factor 3. One of the more important scientific contributions made by this laboratory was the recognition that selenium is the active component of Factor 3. Rats developed a dietary-liver necrosis on a diet devoid of vitamin E, cystine, and Factor 3. In 1957, it was shown that the necrosis could be prevented by the addition of 4 to 6 μg. of selenium (selenite) to 100 gm. of diet. The naturally occurring form of Factor 3 is 3 to 5 times as active as an equivalent amount of inorganic selenium. The chemical properties associated with Factor 3 activity have been elucidated from studies of synthetic organoselenium compounds. One of these compounds, a racemic diselenocarboxylic acid, is half as active as Factor 3 on the basis of equivalent selenium contents.

Earlier work showed that most of the activity of cystine in protecting rats against dietary liver necrosis was traceable to contamination with a small amount of biologically active selenium. More recent work shows that the sulfur amino acids when added to the necrosis-producing diet delay the onset of the liver disease but do not prevent it. The addition of selenium-free cystine, homocystine, or methionine reduced to one-tenth the level of vitamin E required for the prevention of dietary-liver necrosis. When selenite replaces vitamin E, the sulfur amino acids reduce the level required for the prevention of liver necrosis by 30 to 50 percent.

Studies of the metabolic disturbances associated with dietary-liver necrosis showed that liver slices of rats with necrotic livers are unable to maintain a normal rate of oxidation. However, the mitochondria prepared from such livers show no decline in respiration when incubated with various members of the tricarboxylic-acid cycle. Succinate was the exception in that the mitochondria showed a respiratory decline when DPN was added to the system. Homogenates of the above livers show a respiratory decline similar to that of the liver slices with α-ketoglutarate or succinate as substrate. Dietary Factor 3 (as selenite) is without effect on these systems suggesting that Factor 3 and vitamin E participate in different pathways of intermediary metabolism.

The mitochondria from the deficient rats show more succinate cytochrome-c reductase and oxalacetic-decarboxylase activity than those from vitamin E-supplemented animals. A possible explanation for this anomalous finding is that the mitochondria from the deficient animals may permit greater access of substrate to enzymes due to structural change. Suggestive evidence for the presence of changes in the mitochondria comes from the increased swelling of the deficient mitochondria when suspended in a hypotonic medium. The addition of adenosine monophosphate to the preceding system overcomes the increased swelling of the deficient mitochondria.

Further study of the "swelling" of mitochondria confirmed observations of others that oxidative phosphorylation prevents it. The confusion existing in the literature as to the action of 2,4-dinitrophenol (DNP) in this system was resolved when it was shown that this compound could both prevent and accelerate swelling under different conditions. In media where phosphorylation is not possible, DNP protects; in a phosphorylating medium where adenosine monophosphate is the acceptor, DNP accelerates swelling; when adenosine diphosphate is the acceptor, DNP prevents swelling. A possible explanation for the peculiar behavior of DNP is that it acts on adenylate kinase at the surface of the mitochondria.

Vitamin E. Closely allied to the dietary-liver necrosis is exudative diathesis which develops in chicks fed a diet similar to that used in the rat work. In 1957, when the activity of Factor 3 was shown to be associated with selenium, it was observed that trace amounts of this element would prevent or cure exudative diathesis. Furthermore, when selenium was added to a vitamin E-free ration on which exudative diathesis was routinely produced, the animals now developed encephalomalacia. Thus, it became possible to explain, on the basis of the amount of selenium in the diets, the appearance of different vitamin E-deficiency syndromes reported by various investigators.

Additional work showed that chicks could be reared to adulthood on diets free of vitamin E provided the level of unsaturated fatty acids therein is very low. These vitamin E-free chicks grew at a normal rate and, following artificial insemination, layed eggs which were shown to be fertile.

Since no tocopherol could be detected in the tissues of these birds, it is suggested that vitamin E plays no role in the intermediary metabolism of chicks.

Other workers observed alterations in the serum proteins of vitamin E-deficient chicks suggesting that these changes might contribute to the edema seen in exudative diathesis. Studies in this laboratory show a decrease in albumin: globulin ratios in the blood of the deficient chicks but these changes were not great enough to account for the edema. The most prominent changes occur in the chicks showing spontaneous recovery where, after the recovery, increases appear in α_2-, α_3-, β- and γ-globulins.

As work progressed on the vitamin E-deficiency in chicks, it became obvious that some of the older reports on the pathological changes attributed to this deficiency were complicated by a concomitant deficiency of vitamin A. The latter deficiency would be hastened by a simultaneous dietary vitamin E deficiency. Work over the past few years has shown that in vitamin A deficiency, chick brains show scattered pyknotic neurons located in the optic tactum and Purkinje-cell layer of the cerebellum. In vitamin E-deficiency, large necrotic areas occur in the cerebellum and occasionally in other parts of the brain. A combined vitamins A and E deficiency produced many subcellular areas, especially in the frontal lobe of the brain.

A few years ago, a report appeared which suggested that vitamin E played an integral role in enzymatic oxidation. This was based on the observation that extraction of a cytochrome reductase preparation with a fat-solvent reduced the enzymatic activity which could be restored by the addition of Vitamin E. Work in this laboratory showed that the first action of the solvent is to combine with proteins thereby disrupting electron transport. Vitamin E appears to desorb the solvent. The vitamin is not alone in showing this effect especially with aged preparations since several hydroxylated antioxidants such as Santoquin and 5-pentadecyl resorcinol are also highly active.

Previous work on the metabolism of vitamin E has been handicapped by the analytical procedures which result in a poor separation of the vitamin and interfering substances. A new technique has been developed which permits excellent chromatographic separation of vitamin E by means of a column of basic zinc carbonate, aluminum oxide, and celite.

Vitamin B_{12} and Folic Acid. Some years ago, it was shown in this laboratory that the poor growth secured with normal chicks fed a diet containing large amounts of fat and minimal amounts of methionine, could be overcome by adding vitamin B_{12} to the diet. With this diet it was observed that a vitamin B_{12} deficiency had no effect on carcass composition in so far as protein, ash, and moisture were concerned. This finding suggests that the report of others on the importance of vitamin B_{12} in protein synthesis does not explain the physiological function of the vitamin in so far as the chick is concerned. It has been shown that the diets used in the above work must be deficient in methionine—a similar diet deficient in arginine does not show growth inhibition when fat is added. Apparently there is a specific vitamin B_{12}-methionine relationship in the chick which is sensitive to high levels of fat in the diet.

The vitamin B_{12}-deficient chick is similar to the rat in that each species excretes large amounts of formiminoglutamic acid in the urine. Vitamin B_{12} supplementation of the deficient chicks for several days produced a reduction in the excretion of this histidine metabolite. Supplementing the deficient diet for one day with methionine produced an immediate drop in formiminoglutamic acid excretion which was followed by levels of excretion greater than those seen in the pre-supplementation period.

Earlier work in this laboratory showed that rats deficient in both folic acid and vitamin B_{12} excrete large amounts of formiminoglutamic acid. The urinary excretion of this compound is increased when histidine is fed to the deficient rats. If ethionine (a methionine antagonist) is fed to these rats, the excretion of formiminoglutamic acid is reduced but the excretion of urocanic acid is increased. The ethionine inhibits the synthesis of urocanase, a deficiency of which blocks the metabolism of histidine at a step prior to the block induced by the deficiency of folic acid or vitamin B_{12}.

Although there is an apparent interrelationship between vitamin B_{12} and folic acid, it is not a direct one. Vitamin B_{12} is not involved in the activation of folic acid since rats deficient in the former can still convert folic acid to tetrahydro-

folic acid and related compounds which are assumed to be the metabolically active forms.

Additional evidence on the role which vitamin B_{12} may play in physiological processes comes from the purification of a protein which contains the vitamin firmly attached to it. This complex is required for the synthesis of methionine from homocysteine and serine by extracts of *E. coli.*

Although it is assumed that folic acid occurs as the citrovorum factor, evidence has been accumulated in the laboratory which suggests that the naturally occurring form of folic in the liver has not hitherto been characterized. Extracts containing unknown forms of pre-folic acid that can be converted to citrovorum factor by liver enzymes can be prepared from horse liver without enzymatic treatment. One of these pre-folic acid compounds has been partially purified. It can be converted successively to tetrahydrofolic acid and then to citrovorum factor by two separate enzyme systems. The first of these systems requires catalytic amounts of flavin adenine dinucleotide and a suitable hydrogen acceptor. The second requires formylglutamic acid and a liver enzyme preparation which forms citrovorum factor (N–5 formyltetrahydrofolic acid).

Germ-Free Animals. Germ-free animals appear to be the only means whereby unequivocal answers can be secured to such problems as the contribution of the flora in the gastrointestinal tract to the nutrition of the host. Studies are under way on the mechanism whereby antibiotics and large amounts of ascorbic acid in the diet reduce the animal's requirement for the B vitamins. Unless conventional rats receive pantothenic acid in their diets, they do not grow. When pantothenic acid is replaced by either 0.5 percent ascorbic acid or 100 mg. percent penicillin, the rats grow almost as well as those receiving pantothenic acid. There has been some controversy as to the exact mechanism whereby ascorbic acid and the antibiotics spare the animal's requirement for the B vitamins. When the above work was repeated with germ-free rats, no sparing effect of vitamin C or penicillin was seen, indicating that the action of the latter compounds was mediated through the intestinal flora.

A severe vitamin K deficiency develops in germ-free rats fed rations free of this vitamin. Conventional rats can maintain a normal blood-clotting time without any dietary source of this vitamin. On a low-fat diet, the vitamin K-deficient, germ-free rats died in 30 days. Increasing the fat in the ration decreased the survival time. A normal clotting time develops within 24 hours after the germ-free rats become contaminated on removal from the tank. It has been shown that sulfaquinoxaline functions not only as a bacteriostatic agent but also as a vitamin K antagonist. When germ-free rats are fed vitamin K-free diets containing sulfaquinoxaline, they develop hemorrhages within 2 weeks instead of the 4 weeks required on the basal diet.

Another method of studying the contribution of the intestinal flora to the rat's nutritional requirements is to work with animals having a cup over their anuses in which the feces are collected. Such rats excrete almost one and a half times as much feces as the rat that has access to its own feces. These "cupped" rats develop signs of a folic-acid deficiency when a diet low in this nutrient is fed. Rats that have access to their feces show a normal blood picture on such a diet, suggesting that the folic acid synthesized by the bacteria in the gastrointestinal tract of the rat is absorbed only after the animal consumes its feces. The above explanation is limited by the observation of a change in the intestinal flora as a result of the "cupping." The *Lactobacilli* count in the feces of rats with tail cups was only 0.1 percent that seen in their feces prior to cupping.

Protein. Studies have been initiated to determine whether the dietary deprivation of protein preferentially changes the activities of certain components of an enzyme system. Preliminary observations suggest that protein deprivation for 30 days in the rat produces only a slight decrease in succinic dehydrogenase, no change in the cytochrome-b-cytochrome-c_1 complex, a 70 percent reduction in cytochrome oxidase, a slight reduction in succinate-cytochrome-c reductase, and a 40 percent reduction in the activity of the overall succinic-oxidase system when measured by one method and no change when measured by a second method.

Obesity. Weanling rats fed the high-fat, obesity-producing diet show an increase in carcass fat over the lean controls as early as the fifth week

even though the body weights of the two groups are very similar. The livers of the obese rats are about 50 percent heavier than those in the lean controls but the percentages of protein, fat, and water are not changed. Similar findings hold true for kidneys and hearts. The adrenal glands in the obese rats are larger and have a higher percentage of neutral fat and vitamin C than the lean rats while the concentrations of cholesterol and phospholipid are the same.

The obese rats appear to absorb vitamin B_{12} to a greater extent than the controls as shown by higher plasma and liver levels. These increases occur in the obese rats even though the dietary levels of the vitamins are the same. Another peculiarity of the obese rat is the marked proteinuria which occurs in both males and females. These proteins are mainly albumin and β-globulin. The proteinuria is reduced by the addition of an inert filler (Solkafloc) to the high-fat diet.

Guinea Pigs. Work has been completed on the tryptophan requirement of the guinea pig which shows that even though maximum rates of growth are secured with 0.03 percent L-tryptophan in the ration, cataracts develop. Eyes appear to be normal only when the dietary tryptophan level is raised to 0.1 percent. The above studies will be facilitated by a newly developed diet in which the protein is replaced by an amino-acid mixture that permits as good growth as does the protein diet.

Rabbits. It has been assumed that rabbits do not require any dietary source of thiamine. That this may not be strictly so is suggested by work in which 3- to 4-week-old rabbits are put on a thiamine-free diet. On this diet, the animals grow almost as well as the controls receiving supplemental thiamine. However, about 40 percent of the rabbits on the deficient diet show locomotor difficulties after 3 or more months of feeding. The urinary excretion and fecal liver and brain content of thiamine were lower in the animals on the deficient diet than in the controls.

ENDOCRINOLOGY

Glucose-Tolerance Factor. An interesting observation made during the past year was the recognition that trivalent chromium is an integral part of the glucose-tolerance factor (GTF). A single dose of 20 to 50 mg. per 100 gm. rat will increase the removal rate for intravenously injected glucose from 2.8 to 4.8 percent per minute. The activity of chromium is confined to the trivalent form. The potency varies from compound to compound with the very stable coordination complexes of chromium being practically inactive. When adipose tissue from rats on a GTF-supplemented diet was incubated in a medium containing insulin it removed almost twice as much glucose as did the tissue from the deficient animals. The addition of 0.1 μg. of trivalent chromium to the flask containing the deficient tissue increased the uptake of glucose and its incorporation into fat threefold as shown by means of labelled glucose experiments. Preliminary evidence suggests that in stabilized diabetic rats (alloxan), trivalent chromium reduced the fasting blood-sugar level from 250 to 125 mg. and abolished ketonuria.

Experimental Diabetes. Diabetes produced in the rat by removal of 99.5 percent of its pancreas results, within 2 hours of the operation, in elevated blood levels of glucose, ketone bodies, and triglycerides. The increases in the latter two substances occurred before diabetic blood-sugar levels were reached. The increase in blood lipids was accompanied by excessive accumulation of fat in the liver and kidneys.

The level of fat in the liver of the diabetic rat is linearly related to the level of blood-ketone bodies. Removal of various endocrine glands showed that glucocorticoid hormone is the only one required for the development of fatty livers and ketosis in the diabetic rat. Growth hormone has no effect and ACTH is ketogenic only if the adrenal glands are intact. The adipokinetic and ketogenic actions of glucocorticoids can be overcome with insulin. The peripheral utilization of D(-)-β-hydroxybutyrate is reduced 60 percent in the pancreatectomized rat when insulin is withheld. Acetoacetate utilization is also reduced but to a lesser extent. On these bases, it appears that ketosis in the insulin-deficient rat results from an increased production and a decreased utilization of ketone bodies.

An elevated blood-glucose level appears to be the primary factor regulating the secretion of insulin from the pancreas. Peripheral blood of

dogs during fasting contains about 37 microunits of insulin per ml. of plasma. Administration of glucose increases the output of insulin by the pancreas five- to ten-fold. Pituitary hormones and the antidiabetic drugs such as tolbutamide do not stimulate the pancreas to release additional insulin.

Pituitary Hormones.—Purification of the thyroid-stimulating hormone (TSH) has progressed to the point where it is evident that activity is associated with several different proteins. The fraction prepared from TSH-producing pituitary tumors in mice has been shown to be free of exophthalmogenic and luteinizing activity. The thyrotropic effects of the mouse as well as human TSH preparations can be neutralized by anti-sera to bovine TSH which suggests a lack of species specificity in TSH. When chicks are treated with TSH, their thyroid glands show a reduction in sialic acid (a constituent of thyroglobulin) which parallels the depletion of iodine.

Preliminary studies show that in pigeons, from which the *pars distalis* of the hypophysis was removed, treated with insulin, the weight of the adrenal gland increases markedly over that of the hypophysectomized controls.

Studies are under way to perfect an assay for ACTH levels in the blood. This is based on the measurement of corticosterone in adrenal-vein blood of hypophysectomized rats following the injection of the hormone. As little as 50 microunits of ACTH produces a significant increase in the output of corticosterone by the adrenal gland.

Steroids. Analytical methods have been developed which have permitted the measurement of Δ^5-17-hydroxypregnenolone, Δ^5-pregnenetriol, and dehydroepiandrosterone in urine. Six patients with adrenal carcinoma exreted no hydroxyprenenolone but large amounts of both pregnenetriol (3 to 50 mg. per day versus 0.2 mg. in controls) and dehydroepiandrosterone (6 to 90 mg.) Pregnenetriol is thus established as an abnormal steroid metabolite characteristic of adrenocortical malignancy.

Midbrain Lesions and Endocrine Activity. The effect of the central nervous system on metabolic and endocrinologic reaction was studied in dogs with transections of the upper midbrain. These dogs show a depression of the hypothalamicopituitary activating system whereas in animals where the transection was not successful, the opposite effect was noted, namely a marked increase in urinary corticoids following a stressful experience. Spinal-cord transection in the dog results in a marked increase in urinary creatine excretion. By means of isotopically labelled compounds, it has been established that the source of the increased urinary creatine is muscle creatine.

Laboratory of Pharmacology & Toxicology

Shock and Infection. The problem of delayed deaths following extensive burns is receiving major attention. In the Peru Project, where evaluation of oral saline solution and plasma therapy for burn shock is being carried out, over half of the late deaths are due to *Pseudomonas* and *Staphylococcus* septicemias. Antibiotic therapy is not effective, and studies in burned mice indicate that a lowering of host resistance is important in the genesis of these infections. In mice, gamma globulin has been highly effective prophylactically and for the past two years it has been used on alternate cases in the Peru Project. The results have been encouraging, in that a significant decrease in septicemias occurred in the treated group.

Since *Pseudomonas* is the principal infection, a highly potent rabbit immune serum has been developed. This is 800 times as potent as gamma globulin in mice, and is effective therapeutically as well as prophylactically. This is being tried in established *Pseudomonas* septicemias in the Peru Project.

In the Peru Project, serum albumin is also being compared with plasma in the treatment of shock. Apart from comparing its efficiency in shock, it will also reveal whether the absence of antibodies will predispose to these septicemias.

In the field of experimental leprosy, conditions for the growth of *Mycobacterium muris* in tissue culture of monocytes have been established. Cultures can be maintained 1 to 2 months, and afford a means of *in vitro* study of the organism, and of assay of chemotherapeutic agents.

Calcium in Nerve and Muscle Function. Further evidence has been obtained that the calcium

ion may be an essential factor in muscle contraction. Caffeine contraction of striated muscle, in concentrations low enough not to cause membrane depolarization, increases Ca^{45} influx and outflux threefold. Increases are also seen after potassium depolarization, but it has been shown that caffeine affects calcium sites in the membrane distinct from those affected by membrane depolarization.

The behavior of veratrum alkaloids on a monomolecular surface film of stearic acid has been studied in an attempt to correlate physico-chemical behavior with pharmacological action. The pharmacologically active alkaloids penetrate and interact with the film, and orient both horizontally and vertically at the interface. The inactive alkaloids show only weak penetration and orient only horizontally.

Amine Metabolism. The biosynthesis of spermidine by purified enzymes from *E. coli* has been accomplished, according to the scheme:

I. Methionine+ATP→adenosylmethionine +PP+P
II. Decarboxylation of adenosylmethionine
III. Putrescine+II→spermidine+thiomethyladenosine

Enzyme I has been purified 2,000-fold and requires only magnesium. Enzyme II has been purified 20-fold, and requires magnesium. Enzyme III has been purified 1,000-fold, with no cofactor demonstrable. All of the products have been characterized. Spermine and spermidine are widely distributed in plant and animal cells and it is believed they have an important function. This elucidates their formation, as well as that of thiomethyladenosine.

Decarboxylated adenosylmethionine has been prepared by chemical synthesis, and the synthetic product shown to be active as a substrate for III.

Glutathione-Polyamine Conjugate. A high percentage of the glutathione in *E. coli* cells was found to be present as a conjugate with spermidine. It has been characterized by its behavior on ion-exchange resins and paper chromatography, and by identification of the amino acids and spermidine after hydrolysis of the isolated compound. When spermine, which is not normally present in *E. coli*, is added to the medium, a similar conjugate is formed with spermine.

Acetylation of Polyamines. Taking advantage of the differential absorption spectra of dinitrofluorobenzene derivatives of primary and secondary amines, a method has been developed which is useful for the assay of various amines, including the acetyl derivatives of the various polyamines. Monoacetyl putrescine and two isomeric forms of monoacetyl spermidine have been isolated from *E. coli* cells. They have been further characterized by behavior on ion-exchange resins and paper chromatography, and by acetate and amine assays on hydrolyzed samples.

Metabolism of Histidine and Related Compounds. Histidine is an essential amino acid and enters into many important metabolic relationships. Carbon-2 of the imidazole ring enters the "one carbon" pool which involves folic acid and vitamine B_{12} in its metabolism. Five steps have been shown in this degradation:

I. Histidine→Urocanic acid+NH_3
II. Urocanic→formimino - glutamic acid (Figlu)
III. Figlu+tetrahydrofolic (THF)→FormiminoTHF+glutamic acid
IV. FormiminoTHF→5-10 methenylTHF+NH_3
V. 5-10 methenylTHF⇌10 formylTHF

Enzymes have been purified from bacteria or animal tissues which catalyze each of these steps. Enzyme II has been purified 100-fold and III and IV, 1,000-fold. The kinetics and requirements of the latter have been characterized. The reversibility of V at neutral pH is important since 5-10-methenylTHF by enzymatic reduction is in the pathway for biosynthesis of serine and methionine. An enzyme has been purified from rabbit liver which carries out the following reaction:

$$\text{Imidazole acetic acid} + \text{1-pyrophosphoryl-5-phosphoryl ribose} \xrightarrow{\text{ATP}} \text{imidazole acetic acid ribotide}$$

This is the first demonstration *in vitro* of the mechanism of the riboside formation. The ribosides of histamine and imidazole acetic acid have been prepared by chemical synthesis.

Sialic Acid. A highly sensitive and more specific method, using thiobarbituric acid, has been developed for sialic acid. A histochemical method for staining sialic acid in tissues has also been

developed. Using these methods several observations have been made: species differences in salivary-gland content of sialic acid; a hormonal regulation of the amount of sialic acid in vaginal tissues (10-fold increase in pregnancy); an increase in thyroid cancer tissues; large amounts of n-glycolyl and N-acetyl neuraminic in fish eggs. The sialic content of the thyroid gland parallels its thyroglobulin content and is diminished by thyroid-stimulating hormone.

Cholesterol Synthesis. The enzyme, mevalonic kinase, has been purified 100-fold from rabbit liver. Methods for identifying its products—phosphomevalonic and ADP—have been developed. Requirements for the reaction include SH compounds, Mg, Mn, and phosphate.

Gramicidin J. To elucidate the formation of this polypeptide antibiotic, *Bacillus brevis* has been grown with C^{14} amino acids. Another compound closely related to this antibiotic has been synthesized enzymatically, and its structure is being determined.

Enzyme Activity and Molecular Structure. It has been possible to degrade acetylated trypsinogen by pepsin and obtain fragments that retain a high degree of proteolytic activity. Such a fragment has been further degraded to 10 amino-acid residues by leucyl peptidase. By another procedure it has been shown that, by treatment with bromsuccinimide, a differential destruction of the "specificity determining structure" and the "catalytic site" occurs. The localization of enzyme activity to specific sites on the protein molecule is of considerable importance.

Sulfur Amino Acids. Further knowledge of the metabolism of sulfur amino acids has been obtained from purified enzyme systems isolated from yeast. The reduction of methionine sulfoxide to methionine in the presence of TPNH was characterized. The enzyme system could be separated into three fractions, two of which catalyzed the nonspecific reduction of disulfides. In addition to these three enzymes, a new nonspecific disulfide reductase was isolated which reduced glutathione.

The sulfur amino acid, felinine, is excreted in large amounts in the urine of feline species. By S^{35} and C^{14} labeling, it has been shown that a cholesterol precursor, mevalonic acid, is involved in its biosynthesis.

Antibody Localization. By tagging antibodies with fluorescent dyes (Coons technique), a streptococcal antigen and the bacterial hyaluronidase have been followed in tissues after streptococcal infection in mice. By means of tagged antibodies to myosin, actin and sarcoplasmic proteins have been localized in the Purkinje cells of the conducting bundle of the heart.

Pharmacology of Iodate. A sensitive method has been devised for the detection of iodate in the urine.

Laboratory of Biochemistry & Metabolism

Carbohydrate Metabolism. The rate of degradation of glycogen by alkali under various conditions has been studied. Among the principal products of such degradations a number of mono- and polysaccharinic acids have been found. The most abundant of the monosaccharinic acids has tentatively been identified as iso-saccharinic acid. In other studies, the mechanism of action of a rat-liver transglucosylase has been studied. Its action does not involve phosphate compounds. The existence of a glucosyl enzyme intermediate is proposed.

The finding that progesterone, testosterone, and androsterone stimulate the oxidation of D-galactose *in vitro* has been further analyzed. The site of action of these hormones has been localized at the level of the uridine diphospho-galactose-4-epimerase reaction. This is one of the enzymatic steps by which galactose is converted to glucose and utilized in tissues. It happens that the epimerase enzyme requires DPN and is inhibited by DPNH. Accordingly, one mechanism by which progesterone could stimulate the epimerase reaction is by lowering the level of DPNH (and raising DPN). It has now been found that some DPN-linked aldehyde dehydrogenase reactions in liver are inhibited by low concentrations of the hormone. Thus, by its effect on aldehyde dehydrogenase, progesterone helps adjust the levels of the coenzymes DPN and DPNH which, in turn, affect a critical step in galactose utilization.

A sensitive, highly specific, and comparatively simple assay for galactose-1-phosphate in erythrocytes has been devised, that should be of value to physicians treating galactosemic patients. Treatment of three prepubertal galactosemic subjects with progesterone enables them to oxidize about 10 percent of a tracer dose of D-galactose-1-C^{14} to $C^{14}O_2$. An improved purification of UDP galactose-4-epimerase (discussed above) from galactose-adapted yeast was devised and the purified enzyme shown to contain tightly bound DPN. The yeast enzyme, like that from liver, failed to incorporate tritium into hexose nucleotide from either tritiated water or tritium-labeled DPNH. The role of DPN in the reaction thus remains to be clarified.

Studies on inositol biosynthesis in the rat continue to support the hypothesis that a six-carbon precursor (not glucuronic acid) is cyclized in some manner. This contrasts with the yeast system, where a two-carbon and a four-carbon unit combine to form this cyclic alcohol. Further, it has also been established that cleavage of inositol to glucuronic acid observed in rat-kidney extracts is not sufficiently reversible to account for inositol biosynthesis in the whole animal.

Studies of the metabolism of insulin-I^{131} by surviving rat liver have been carried out under a variety of conditions. The results of such studies have implicated the cell membrane as a possible determinant of the specificity of capture and degradation of the hormone by the intact organ. The results suggest that degradation of insulin-I^{131} by intact liver may proceed in the following way: (1) binding of insulin by the cell membrane; (2) transport of insulin to the site of insulinase activity; (3) degradation of insulin.

A new pathway for the metabolism of uronic acids in bacteria, outlined in the last report, has been intensively studied and purification of all of the reactions achieved. This work is completed and has been submitted for publication.

Preliminary studies on mucopolysaccharide formation have demonstrated the presence of an enzymatic system in mouse-skin homogenates capable of synthesizing chondroitin sulfate B, an iduronic acid containing polymer. Studies are under way to elucidate the biosynthetic mechanisms involved in the conversion of glucose to this new uronic acid. Concurrent studies in rat kidney have revealed that L-iduronic acid is readily metabolized. At least two enzymatic steps have been demonstrated, one of which has been purified about 200-fold. This represents the first report of an iduronic acid metabolizing system.

Continuing work on ascorbic-acid metabolism in collaboration with NHI has led to the identification of the two pentonic acids, L-lyxonic and L-xylonic acid, arising from diketogulonic acid. A second enzyme acting upon diketogulonic acid has recently been discovered which catalyzes the formation of 2-ketogulonic acid. The fate of this new intermediate, as well as its possible role in the mechanism of action of ascorbic acid, is being explored.

An investigation has been undertaken of a new group of rare sugars, the 3,6 dideoxy hexoses, which are responsible for the immunological specificity of the O antigen of *Escherichia* and *Salmonella.* A rapid and sensitive assay technique has been developed for the determination of these compounds. Preliminary results indicate that these sugars are formed from glucose without rearrangement or inversion of the carbon skeleton.

Nucleic Acids: Structure and Metabolism. Increasing emphasis in this field is being placed on the matter of secondary structure, the holding together of polynucleotide strands by hydrogen bonds. Theories of genetic duplication and specific nucleic acid synthesis depend on these hydrogen-bond interactions in which only certain base pairs participate.

A number of recent projects contribute to our understanding of such interactions. By means of infrared spectra in D_2O solution the tautomeric forms of the nucleotide components of nucleic acids are being studied. This question is fundamental to the structure of nucleic acids because the possible modes of hydrogen bonding are determined primarily by this structural feature of the component nucleotides. During the past year it has been found that polyinosinic acid exists in the keto form in aqueous solution and polycytidylic acid probably in the amino form, and that these tautomeric structures are maintained in the helical interaction product formed by mixing the polymers.

A fundamental question is how large a polynucleotide has to be in order to interact and help to form a double helix. It has now been found

that simple trinucleotides can line up in a chain and become attached by hydrogen bonds to a polymer, thus forming two chains bound together. For example, the trinucleotide pApApA will interact with the polymer, polyuridylic acid.

The action of the bacterial enzyme, polynucleotide phosphorylase, is governed by the possibilities of hydrogen bond interaction. Thus, polymerization of ADP is inhibited by poly U and vice versa; polymerization of CDP is inhibited by poly I and vice versa. This reflects the fact that poly A+ poly U, and poly I+poly C represent strong secondary interactions. Every permutation has been tested, and the enzyme activity is strongly suppressed only by pairs where hydrogen bonding between bases is possible. This could be the basis for specificity in RNA synthesis.

An interesting finding from studies in collaboration with NIMH is that soluble RNA, so-called S-RNA, the acceptor for amino acids in protein synthesis, is phosphorylized very slowly by polynucleotide phosphorylase and reaction stops when 20–30 percent of the soluble RNA has been converted to nucleoside diphosphates. Experiments now indicate that it is the nature of the secondary structure of the chains or hydrogen bonding that determines their resistance to degradation. Soluble RNA has a terminal phosphate endgroup at the beginning of the chain and the other end of the chain is called the "nucleoside end." The nucleoside end of the molecule consists exclusively of adenosine. Now it has been found that, in rabbit liver S-RNA, all of the chains begin with a guanylic-acid residue. A new nuclease has been discovered and purified from extracts of *Azotobacter agiles*, and its mechanism of action delineated. It promises to be very useful in studies of S-RNA.

As stated above, S-RNA is widely believed to be involved in a scheme for protein biosynthesis. For example, in *E. coli*, S-RNA and a soluble enzyme have been found necessary to incorporate certain amino acids into a particulate fraction. This system differs from previously described systems in that it is not inhibited by chloramphenicol or lecithinase A. It also requires ATP, Mg^{++}, inorganic phosphate, and a sulfhydryl-containing compound.

Steroid Metabolism. The work on steroids has followed two separate courses. The first has dealt with the metabolism of these compounds and the second with their mechanism of action as hormones.

As for their metabolism, considerable attention has been given to the reduction of the 4–5 double bond of hormonally active molecules. There are two sets of specific, TPNH-dependent enzymes for catalyzing this hydrogenation: the microsomal 5α reductases, and the soluble 5β reductases. There are, therefore, for each steroid, two separate 4–5 reductases. The microsomal enzymes respond to various physiological stimuli like thyroxin by increasing in activity. These two sets of similar proteins are being examined to determine if any biosynthetic or genetic relationship exists between them.

The 11-hydroxylation of adrenal steroids, an important reaction in the biosynthesis of steroid hormones, and a good model reaction for all hydroxylations, has also been investigated. Three enzymes, TPNH, and an unknown cofactor are required for this reaction. One form of the metabolic disease, the adrenogenital syndrome, may be associated with loss of one of these enzymes.

There have been several separate studies on the mechanism of steroid action: The first on steriods as inhibitors of DPNH-cytochrome c reductase. Here, the steroids act between flavoprotein and cytochrome c and their effect can be overcome by α-tocopherol or other lipids. Also it has been shown that TPN, produced from TPNH in the course of steroid double-bond reduction, can regulate such processes as glucose-6-phosphate oxidation in tissues containing both the shunt pathway and the steroid reductases.

In another study, intracellularly generated tritium labeled DPN, namely diphosphopyridine nucleotide-4-T, was used to evaluate the concept that hydroxysteroids are involved in liver transhydrogenase activity. To this end the several hydroxysteroids were tested for possible effect in catalyzing the approach to equilibrium of the DPN-DPNH/TPN-TPNH couple. No evidence came forth to suggest that, in liver cells, hydroxysteroids functioned in a transhydrogenase capacity.

Gene-Enzyme Relationships in Histidine Biosynthesis. Work by Hartman (Johns Hopkins University) on mapping of the genes of histidine

biosynthesis in *Salmonella* mutants has shown that they are all in a cluster on the chromosome. Biochemical analysis of these mutants has now revealed that the sequence of the histidine genes on the chromosome linkage map corresponds to the sequence of the enzymes they control in the biosynthetic pathway.

It has further been shown that histidine alone controls the rate of synthesis of the various enzymes of its biosynthetic pathway; if the histidine pool is increased there is repression, and vice versa. A major finding is that histidine affects the synthesis of each of the enzymes of the pathway to the same extent. It is as if histidine (or a derivative) had a specific affinity for the histidine section of the chromosome and could "turn off" these genes when the internal histidine concentration rises.

Role of Polyamines in the Neutralization of Bacteriophage DNA. It has been established that the cations putrescine^{++}, spermidine^{+++} and Mg^{++} neutralize the DNA of T4 bacteriophage obtained from *E. coli* grown in minimal medium. The cations in T4 phage have been shown to be a function of both the composition of the pool of cations in the host bacterium at the time of phage assembly and the affinity of each species of cation for the phage nucleic acid.

Enzyme Induction. γ-Hydroxybutyric acid was found to induce the formation of γ-hydroxybutyric acid dehydrogenase at low inducer concentrations. Higher concentrations of γ-hydroxybutyric acid were highly effective inducers, not only of γ-hydroxybutyric acid dehydrogenase, but also of β-hydroxypropionic acid dehydrogenase. The question whether the same genetic unit (cistron) contains the information necessary for the synthesis of a protein subunit which may be an integral part of both enzymes was studied. There was no evidence of shared genetic information.

Enzymatic Utilization of Model Compounds. Studies on the biosynthesis of γ-aminobutyrate from pyrrolidine and putrescine and the subsequent utilization of this compound have been studied at the enzyme level. Of particular interest has been the study of the kinetics of one of the reactions involved in γ-aminobutyrate utilization, namely, its transamination with α-ketoglutarate, forming succinic semialdehyde and glutamate. The data had suggested that transamination occurred by way of a series of binary complexes of enzyme and each substrate. Further support for this concept has been obtained by the dissection of the transamination into two exchange reactions.

In a study of a novel aldehyde dehydrogenase oxidizing malonic semialdehyde, both DPN and CoA were found to be involved, resulting in the direct formation of CO_2 and acetyl-CoA. Free malonyl-CoA was not formed.

Coenzyme Studies. Synthesis of thiamine has now been shown to involve the initial formation of thiamine monophosphate rather than the free vitamin, as claimed by others. The synthesis involves three enzymatic steps: The phosphorylation of the pyrimidine to the corresponding pyrimidine pyrophosphate; the phosphorylation of the thiazole to thiazole monophosphate; the condensation of these derivatives to form thiamine monophosphate with the elimination of pyrophosphoric acid.

Laboratory of Pathology and Histochemistry

Hematologic and Genetic Studies. Continued investigation of factors influencing erythropoiesis has revealed a correlation of plasma erythropoietine with the grade of marrow cellularity and an accelerating effect of erythropoietine on red-cell maturation. Attempts to assay erythropoietine by use of short-term tissue cultures have not been successful. Studies using tritiated thymidine have shown that erythropoietic cells do not enter mitosis *in vitro*. A second regulator of erythropoiesis has been postulated to account for some of the results obtained in recent experiments.

Studies on the genetic control of hemoglobin structure have been continued. A molecule of hemoglobin, which is composed of a pair of alpha chains and a pair of beta chains, dissociates reversibly in acid into the two unlike pairs. These pairs are exchanged between molecules when a mixture of two different hemoglobins is dissociated and recombined. With use of this tech-

nique it has been shown that the two types of chains, alpha and beta, are controlled by different genetic loci. The reactivity of hemoglobin SH groups toward each other can be greatly increased by combination with nitrobenzene. Tryptic digestion and paper electrophoresis followed by paper chromatography has shown that fraction 23 of hemoglobin I contains tryptophan whereas this fraction of normal hemoglobin does not. Search for other amino-acid differences between these hemoglobins is underway.

Studies have been instituted on human karyotypes in relation to localization of specific genes. Sex-chromosomal variations in clinical and latent hermaphroditism and chromosomal variations in neoplasia and in male meiosis are also being investigated.

Experimental Arthritis. Studies of the experimental arthritis induced in rats by *Streptobacillus moniliformis* have demonstrated that a focal osteomyelitis proceeds to periostitis and synovitis by local invasion and thence to joint involvement. The organism has proved infectious for mice, and is progressively less lethal after intravenous, intraperitoneal, and subcutaneous inoculation. Grossly evident joint lesions appear in as little as 1 day and persist for as long as 3 months.

Blood from infected rats shows a positive Bentonite flocculation test and sensitized sheep-cell hemagglutination reaction similar to that seen in human rheumatoid arthritis. These reactivities have been produced in sera of rabbits immunized with formaldehyde-killed cultures of infective strains of *S. moniliformis*. Immunochemical studies indicate a small molecular size for the active fraction, contrasting with the large molecular size of the human rheumatoid serum factor. The organism apparently must be grown on human ascites fluid to be antigenic, but ascites fluid itself does not contain the antigen in question.

Studies on degenerative joint disease of mice have continued. Genetically, susceptibility to osteoarthritis behaves as a recessive; studies designed to determine the number of genes involved will be completed shortly. At the time of this report, the findings suggest that there is a single gene factor. The sources of different susceptibility of various inbred strains of mice were found not to reside in the differences in rates of skeletal aging (epiphyseal development), thyroid function, obesity, or response to high fat diets.

The swellings of the paws of rats following injection of Freund adjuvants were demonstrated as not representing arthritis but a periarthritis. Evidence was presented that such swellings arose from dissemination of adjuvant from the depot site to the periarticular tissue.

Two interesting, genetically determined, spontaneous diseases of mice have been observed for the first time during the course of these studies: (1) a polydipsia that leads to lethal hydronephrosis in males of strain STR/N. (The findings suggest that there is a primary disturbance of thirst mechanism to account for the syndrome), (2) a pelvic inflammatory disease in male STR/I N mice that is related to the seminal function of this strain.

A paper on the pathogenesis of ochronotic arthropathy, based on anatomic, electromicroscopic, and *in vitro* chemical studies of the combination of homogentisic acid and articular cartilage is in preparation. Other reports on the pathology of human rheumatic diseases published during the last year have dealt with joint involvement in sarcoidosis and the genesis of rupture of extensor tendons at the wrist in rheumatoid arthritis.

Infectious Processes. A staphylococcal endocarditis was produced experimentally by an intravenous injection of staphylococci in dogs rendered susceptible by prior surgical induction of aortic insufficiency. Endocarditis could be prevented by penicillin given within 8 hours after injecting the bacteria. If treatment was delayed 24 hours or longer, relapses often occurred after cessation of treatment. The endocarditis was arrested by treatment of relapses, but some animals died of acute heart failure due to valvular deformities and insufficiency. After delayed therapy, nearly all dogs developed a diffuse proliferative glomerulonephritis which resisted therapy and occasionally showed evidence of developing into a chronic glomerulonephritis. These findings support the concept, recently questioned, that a chronic glumerulonephritis is a sequel to an acute nephritis. This experimental method should prove a useful tool in studying staphylococcal infections resistant to antibiotic therapy and in the study of glomerulonephritis.

The pathologic process engendered by inoculation of a virulent strain of *Trichomonas vaginalis* into mice by the intraperitoneal route has been characterized but the mechanism of the resulting liver-cell destruction remains to be elucidated.

Stress and Endocrine Effects. Repeated exposure of dogs to a 30,000-foot-simulated altitude resulted in the development of nonlipid arteriosclerotic changes in the aorta and occasionally in the coronary arteries in a substantial portion of the dogs. This new experimental method of producing arteriosclerosis supports the concept that anoxemia may be a cause of arteriosclerosis.

Studies conducted in collaboration with NHI, showed that adrenergic blocking agents prevent or diminish fat deposition in the liver occurring after the administration of CCl_4, ethionine or ethanol. The adrenergic blocking agent, phenoxybenzamine, also inhibits a rise in serum lactic dehydrogenase as well as a rise in serum transaminases and fatty myocardial changes reported previously in dogs given large doses of norepinephrine of epinephrine. However, phenoxybenzamine or reserpine does not prevent a similar rise in serum-enzyme levels and the fatty changes bordering myocardial infarcts produced in dogs by coronary ligation. Since reserpine depletes the myocardium of norepinephrine, these findings do not support the hypothesis that ventricular tachycardia is due to epinephrine and norepinephrine, liberated from the infarcted myocardium.

Histochemical Studies. A combined critical review and an original study of the histochemical reactions of human gastrointestinal carcinoid tumors has been completed. The routine use of ferric ferricyanide and a stable diazotate is recommended to get more data on functional activity of these tumors. A new metal chelation reaction with enterochromaffin is perhaps suggestive of an *o*-diphenol structure, or *o*-secondary aminophenol.

Studies have been continued on the localization of oxidative or dehydrogenative enzymes using tetrazoles as histochemical indicators. The presence of both TPN- and DPN-linked 17β-estradiol dehydrogenases in placenta has been demonstrated with distinctly different topochemical distribution patterns.

Studies with NIDR on aminopeptidases in human tumors have been extended. Some epithelial tumor types possess this peptidase activity in the tumor cells themselves. More numerous epithelial tumors exhibit proteolytic activity in adjacent stroma, independent of proliferation of stroma or inflammation. A third category of tumors including sarcomata invade without evident proteolytic activity.

A chromogenic substrate specific for trypsin was synthesized and this type of activity has been demonstrated in mast cells, though not in tumor stroma. The diazotisation procedure recently developed in this laboratory for demonstration of tyrosine in protein has been used to show high tyrosine concentrations in hypophyseal alpha cells.

Mucins and chemically related substances in rodents have been studied by means of a variety of histochemical procedures. Methylation-demethylation sequence procedures have been used for the distinction of sulfate from carboxyl acid mucins, the former being methylated and converted to neutral mucins, the latter being esterified, saponified, and restored by acid to the original status. $S^{35}O_4$ incorporation has been compared with alcian-blue staining and thiazin uptake at pH 1.5 and 3.0, and aldehyde fuchsin staining. Sulfated mucins stain at the lower pH, some are nonreactive to alcian blue and to periodic acid Schiff stain. Sialic acid mucins generally do not stain with thiazins at pH 1.5 but do at pH 3. They take alcian blue and are digestible with *V. cholerae* and *Cl. perfringens* sialidases with liberation of recoverable sialic acid. Topographic distribution studies indicate a good deal of specific localization of the several types of mucopolysaccharides.

Studies of the histochemical reactions of neuromelanin in man have allowed the identification of this material in *substantia nigra*, *locus caeruleus*, and *nucleus dorsalis vagi* of *Macaca mulata*. The eosinophilic granules which are mingled with neuromelanin granules in man owe their eosinophilia probably to ϵ-amino groups of lysine and hydroxylysine, and seem to include a phenolic substance other than tyrosine, histidine or tryptophan.

Studies have been made on the rat preputial gland, assessing response of the two separate demonstrable secretion products to hormonal stimuli.

The histochemistry of the gland secretion products has been reported.

Renal Architecture. Thru collaboration with NCI, electron microscopy has been applied to the study of the counter-current vascular bundles of the renal papilla. The structure of these vessels proved to be essentially identical to that of the vessels in the well-known *retia mirabilia* of the toadfish swimbladder. Continuous basement membranes are displayed in both ascending and descending vasculature.

Glutaminase has been localized to the proximal convoluted segment and the distal straight segment of the rat nephron. The distribution and amount of glutaminase found in the distal tubule cannot readily account for the ammonia excretion in the renal papilla of the rat, and therefore some other mechanism for this excretion must be found.

Chlorophenol red is apparently excreted in the proximal convoluted segment, and is resorbed, at least partially, in the proximal, straight segment. Whether the resorbed dye is returned to the venous circulation has not been determined. This might account for incomplete clearance.

Germ-Free Animals. Studies in germ-free animals have been directed to the pathogenesis of liver cirrhosis. Choline-deficiency liver cirrhosis in rats develops quite typically and within the usual time range. It has been alleged by other workers that the lesion is attributable at least in part to intercurrent infectious processes.

Human Pathology. Studies of the geographical and racial differences in human pathology have been continued. Autopsy material collected in Japan by the Atomic Bomb Casualty Commission is being studied for degenerative cardiovascular diseases among the Japanese of Hiroshima and will be compared with similar published data for American and European peoples. Along with NINDB, this laboratory also is participating in a pathologic and experimental study of the neurologic disorder in man and animals produced by the eating of fish caught in Minamata Bay in Japan. Japanese workers have reported that this disease may be an organic mercurial poisoning from industrial wastes. From pathologic material obtained through the diagnostic services provided to Indian hospitals, this laboratory has noted a dietary hemosiderosis and an obscure hepatic alteration in American Indians. A pathologic study of sarcoidosis in American Indians of Oklahoma is being supplemented by cooperative epidemiologic studies conducted by the University of Oklahoma Medical School.

An outbreak of pneumocystic carinii disease in a Korean orphanage has demonstrated a non-European focus of this disease. Promising results have been obtained by induction of this disease in rats.

The histopathology preparation laboratory provides services needed by two sections of LPH, and continues to give aid and consultation on technical problems to scientists in many other laboratories. During the past year this laboratory prepared a total of 49,181 sections: 16,555 routine, 18,254 special stains, and 14,372 spare slides; from 2,193 surgical specimens, 103 human autopsies and 5,484 animals. Animals were derived as follows: 3,346 from DBS, 1,137 from NIAMD and 1,001 from other Institutes. This total does not include the large number (approximately 19,000) of sections prepared by a large variety of procedures within the Histochemistry Section.

Laboratory of Physical Biology

The Laboratory of Physical Biology comprises a broad spectrum of fundamental biological and related research, much of it independently executed but invariably arising from interrelated problems which attract the interest and curiosity of many scientific disciplines. The approaches to the analysis and elucidation of the diverse factors impinging on living systems vary from the investigation of the role of structure in function, from molecular aggregation to histology, to the analysis of the energetics of the interaction of environment and organisms. The past year has seen the addition of staff in biophysics particularly in the section of photobiology and, with its cleavage, to form a new section on bionucleonics to attack separately the problems of high-energy radiation interaction with biological systems. Problems of space have become increasingly acute and, despite cooperation of sister laboratories off campus in housing some of our staff, we look forward to re-

lease of space indirectly through the current building program of other Institutes.

In view of the very diverse nature of investigations coming under the planned experimentation of this laboratory, the following segments have been summarized somewhat arbitrarily and no significance is to be attached to the order of presentation. In no case is it possible to include all the results of the year's work or to give individual credit for particular findings. The bibliography of the laboratory staff and the directory of the laboratory will show how ably this heterogeneous staff works both cooperatively and independently.

In the area of photobiology, studies on basic photosynthesis have shown that the organization of pigment-protein molecules into a functional network may well explain the extraordinary effectiveness of this "machine." It was shown that a partially reversible change in the bleaching of chlorophyll is dependent on oxygen and that the site of this action is related to the lamellar chloroplast structure. If one digests lipoid from the chloroplast, the still intact layers are free to separate in fan-like fashion. On the other hand, protein digestion causes the layers themselves to collapse. Other studies relating structure to function in this area show that anomalies in the scattering of radiation by pigments account for only a small fraction of the wavelength change describing the difference between free pigment and the same material *in vivo*—an indication that this wavelength shift may relate to the extent of macromolecular organization of structure. Findings on the structure of porphyrins are particularly interesting in that specific stoichiometric binding is exhibited by copper porphyrin to bovine serum albumin whereas there is a contrasting lack of such binding in β-lactoglobulin. In other physical examinations it is evident that specific electrochemical properties characterize such biologically functional structures—in particular, the demonstration by nuclear magnetic resonance that the conjugated porphyrin molecule carries "ring currents" producing local magnetic fields within the molecular structure due to the induced circulation of the π electrons.

In the allied field of the basic mechanism of vision the way in which energy is absorbed and transferred is under study and in the cooperative work with the Naval Medical Research Institute good agreement with theory has been shown on the minimum electrical current that a photo receptor must produce (about 1,000 charges/photon) to convey information to the brain. This value has been measured as 750 electrical charges per incident photon in photo-receptors of the squid. The underlying physicochemical bases for these biological functions are of direct interest to chemists who relate such properties to triplet and metastable states of the molecules and their aggregates. Thus, pigments having photodynamic properties are studied for clues to mechanism of energy economy. For example, a red pigment from a fungus has been found to exhibit an usual pattern of conjugation and to have photodynamic properties, in particular, a destructive photo-oxidation which may be able to furnish energy to useful endothermic reactions in the organisms.

It has been shown in this section that the control of energy through wave-length specificity for unit molecular action and resonance phenomena is of great importance to the economical and specific production of certain essential chemical and biological substances. Thus, only ultraviolet light will produce the conversion of 7-dehydro-cholesterol into vitamin D_2 (calciferol), cause erythema, bactericidal action, and mutations in various organisms. The section has carried on quantum measurements of ergosterol transformation in a variety of monochromatic irradiations and conducted flash photolysis studies to reduce the incidence of thermal changes during irradiations. An extensive reexamination of monochromatic irradiation data on the reaction of precalciferal and tachysterol which tends to show through flash photolysis that a dark reaction exists in this direction following irradiation.

In extension of these interests the section is looking into the problem of genetic changes produced by resonant energy mechanisms for specificity to wavelength as contrasted to the high-energy effects of radiation. Current studies are of a cooperative nature on chromosomal alterations as related to lethality of dose.

At the high-energy level, studies are just beginning to be set up for elucidating the effects of neutrons and X-radiation in nonlethal dosages partly by study of molecular interactions and

partly by cytological changes which can be observed. Staffing and equipping of this endeavor have been major problems.

The physicochemical study of living systems may require the examination of basic nonliving models which exhibit and perhaps elucidate the complex membrane-system integral with biological function. In the section on macromolecular biophysics this type of investigation has laid a broad groundwork leading to the rational interpretation and prediction of properties of ion transport through various types of membranes with definable characteristics. Thus, in the current work, porous membranes of highly specific ion-transfer characteristics have been prepared reproducibly and their mechanisms analyzed and related to theory. This has been extended to oil membranes of similar characteristics as a step toward the more complex living-tissue interfaces. The behavior of various components of these membrane-solvent-ion systems has been shown to include the concept of membrane hydrolysis which appears to act against theoretically derived estimates of membrane potentials at low electrolyte concentrations. Further studies demonstrating the inability of the "Donnan equilibrium" concept adequately to describe the conditions obtaining in conventional aqueous concentration cells are being carried on and from a theoretical analysis of the problem it can be seen that such preparations must be viewed as dynamic "two-ionic" cells to which the theory of the dynamic aspects of polyionic potentials recently developed here must be applied.

Studies in molecular biophysics have proceeded also to the analysis of the structure of protein crystals by electron microscopy of very high resolution and the use of a minimum assumption model to reproduce the observed phenomena. The crystal structure of Rothamsted tobacco-necrosis protein has been determined in this manner and is in close agreement with the crystal structure found using X-rays. The photography of molecular separations in crystals of organic compounds of molecular weights of about 500 to 700 has been continued and it has been determined that the image obtained is not a direct one but an interference pattern produced by phase changes in the electron waves passing between and through the planes of properly oriented crystals. It is of significance to the analysis that the images appear not in the principle focus but above and below it in a predictable pattern. Further development of microspot X-ray microscopy to photograph diffraction patterns using long wavelength X-rays (up to 10 Å) has resulted in achieving increased dispersions on the recording plate over those possible by former and currently available devices and permitting the easy determination of large molecular plane spacings in crystals. The work of the section has made it possible to image more accurately and to interpret the fine structure of biological and organic molecular structures appearing in high-resolution electron micrographs.

The section on physical biochemistry is devoted to an intensive analysis of the various manifestations of the relationships between structure and function at the molecular, cellular, and organ level in biology. Its staff continues to analyze the mechanical, chemical, and energetic factors in the special protein complexes which account for the unique behavior of muscle, of blood clotting, and of a number of allied systems in which the interconversion of energy and structure takes place through highly specialized properties of discrete parts of oftentimes huge molecular aggregates. The concepts of enzyme-substrate relationships are met in this area and have proved useful in analysis, characterization, and prediction of action. A continuing study of the manner of polymerization of muscle proteins has been carried on as well as related studies on the behavior of fibrinogen and the essential alterations in producing netted structures of special attributes which produce the gross effects.

In X-ray studies of glycerol-treated muscle fiber it was shown that α-keratin patterns disappeared in contractions induced by ATP, indicating a type of "melting" of the ordered filaments which leads to the assignment of two roles for ATP: one, the initiation of contraction in which no appreciable energy is lost by ATP and, secondly, the furnishing of energy ultimately by acting as the restorative agent to bring the contracted structure to its original oriented state. Other studies of muscle-fiber relaxations were carried on through the use of polyphosphate in the presence of ATP. Studies of the detailed structure of myosin are under way as a necessary prerequisite to the understanding of its function in the process of contraction. Comparative myosin studies have been made from

widely different species as well as from various tissues of the same organism and, in the case of the mammalian heart, it has been shown that the conduction bundle tissue which functionally is similar to nerve tissue still comprises protein constituents which are characteristic of muscle, thus supporting the inference of common physicochemical bases for action tissue.

Studies on actin characterization indicate a great similarity to myosin and support the hypothesis that actin is a part of the larger myosin entity. The process of the transformation of globular actin to fibrous actin has been followed by optical rotation studies and microcalorimetric measurements and shown to be due to a gain in order with energy derived from ATP.

Structural studies on other proteins including salmine and various micelle-forming substances such as soaps and detergents are being carried on to determine the role of specific structure and order of structural components with the hypothesis that such relationships may determine specific capacities especially with regard to the transmission of genetic information content. The relations of sulfhydryl content of proteins are also under study with special reference to the correlation of reversible dissociation of hemoglobin and the Bohr effect as functions of pH and sulfhydryl changes. Studies on the metabolism of collagen in various conditions are continuing with peripheral studies of substances appearing as metabolites and detectable by chromatographic procedures.

In the correlated studies on proteins in this section the enzymatic nature of the processes by which proteins manifest their biological functions is under intensive investigation. Through successful purification of thrombin it has been made possible to study their kinetic-molecular properties. It has been shown that the Laki-Lorand factor liberates a single peptide from fibrinogen through the action of thrombin This specificity of thrombin was studied by the preparation of peptides of arginine which demonstrate the different effects of various structures on the splitting role of the reaction. An esterase activity of thrombin was demonstrated which seems to be exhibited toward all basic amino acids.

Carboxypeptidase A has been intensively studied with the finding that binding cobalt on the zinc-bound enzyme enhances its activity. Carboxypeptidase B has been isolated in a highly purified form from pig pancreas and seems to be a zinc metalloprotein strikingly similar to the A form although its specificity is markedly different. Trypsin has been shown to bind a second site of DFP^{32}. A peptide of 19 amino acids is isolable which is different from that released from the first site of binding at the site of inactivation. A complete structural characterization is being undertaken. A similar definition of sequence has already been carried out for the peptide A liberated from fibrinogen during the clotting process. New studies on enzyme complexes have been initiated and attempts to fragment these complexes with preservation of activities is underway. Associated studies in immunochemistry have been carried forward with the observation that serological reactions of rats and rabbits to killed *Streptobacillus monoliformis* are largely due to immunization with gamma globulin from human ascites fluid as corroborated by immunization with cultured organisms in media containing human ascites fluid.

In the section on physiology, which supports a wide range of function studies, the work on nonmammalian forms has been centered about several different phyla. The cyclic gonadal differentiation and its controlling factors in freshwater hydra are being studied in order that this useful preparation may yield basic information on cellular responses and perhaps ultimately be available as a biological index of the effect of various environmental factors, including various forms of radiation on biological rhythms. Numerous possible factors such as CO_2 concentration and population density have been shown to be noncritical in their effect.

A further section of study is devoted to insect metabolism and constitutes the continuing core of interest with regard to respiratory phenomena in metabolic cycles of unusual interest. The finding of high citrate levels in the blood of five species of insects examined to date led to the hypothesis that this is a biochemical peculiarity of insects but it has been shown that it is not the result of a blockage of later stages of the TCA cycle since the requisite enzymes are demonstrable. The *in vitro* oxidation of citrate, alphaketoglutarate, malate, fumarate, and pyruvate has also been demonstrated by insect mitochondria. Further studies will follow organic-acid metabolism during vari-

ous stages of development and a study of amino-acid hormones and protein synthesis using lysine C^{14}.

The study of the relation of oxygen tension and temperature to respiration of fly larvae and pupae has been completed and is being compared to the adult stage. It has been shown that larval respiration is not limited by physical dimensions as contrasted to limitation in pupae and that temperature dependence of larval respiration shows a discontinuity between 10–15° C. Central-nervous-system control or its absence does not affect oxygen uptake over the range of 0–41° C. in day-old flies.

Other studies on trauma indicate a difference between diapausing types of moth pupae and those which have no diapause. The study of the triggering of the flash of fireflies was continued this year with the finding that the photogenic tissue exhibits action potentials preceding flashes, that the latent period is separable into two components of which the first and longer one can be bypassed by intense stimuli, and that a variety of agents, including eserine and veratrine, can disrupt the lantern's coordinating mechanism to cause asynchronous activity.

Proceeding to physiological studies of higher forms, the section's work on hypoxia has continued with the examination of altitude tolerance in chickens, rats, rabbits, and dogs. While the work is of a comparative character, the particular species used have certain differences in reaction which lend themselves to the elucidation of basic factors. As in previous years the studies show hematopoietic stimulation in mammals, but the interest of the staff is held by results indicating that body temperature in rats affects altitude tolerance and that such subtle factors as restraint of the animals has a marked effect on body-temperature maintenance. It was also shown that dogs with experimental aortic insufficiency, mitral insufficiency, have an unsuspected and remarkably high altitude tolerance despite these drastic cardiac disabilities. Further findings of lipid atherosclerotic plaques in the hearts and aortas of dogs exposed to altitude suggest that hypoxia may play an important role in the etiology of this disease.

Studies of the mechanism of circulatory reaction of sensitive species to synthetic macromolecules have been continued in the section and deal with dextran and polyvinylpyrrolidone effects in rats and dogs, respectively. It was found that conscious rats showed no reaction to dextran if a proper combination and dosage of antihistamine and antiserotonin drugs were administered in confirmation of independent results elsewhere on anesthetized rats. Since *H. pertussis*-inoculation of rats increases their susceptibility to exogenous histamine and serotonin, and since dextran is a histamine-and serotonin-releasing agent in rats, *H. pertussis* was given to rats subsequently given dextran. Susceptibility remained unchanged but counts of mast cells (the most important source of histamine and 5-hydroxytryptamine in rats) decreased in the target areas of dextran sensitivity after inoculation and before reaction tests, thus tending to show that the presence of mast cells is not prerequisite for the reactions. Other studies on the effect of insulin on dextran reactions, for which other investigators have reported both enhancing and mitigating roles, show that it acts in either manner depending on the route of administration and the dose of both dextran and insulin. Dextran at low doses tends to induce fatal convulsions in rats receiving nonconvulsive doses of insulin. There is evidence in dye diffusion and signs of thirst for an effect of insulin through its known action to increase cellular permeability to carbohydrates.

Finally, the section has continued to devote some study to environmental factors affecting pulmonary ventilation in human subjects. It was shown that time-lag between rise in alveolar pressure and the resultant mouth airflow is correlated with frequency of the breath and the effort exerted. The enhancement of this effect with denser gas mixtures than air and its mitigation with light mixtures has been demonstrated in accord with theory.

Laboratory of Chemistry

Analgesics. With a view to developing a new approach to compounds of the benzomorphan type, investigations on the dihydropiperidine systems have been initiated, directed towards selective alkylation at the 2 and 3 positions. Thus, research has continued in this family of neuropharmacologic agents (of which phenazocine is the presently most notable example) and includes

further study of the physical dependence capacity of several of the more active members (particularly optical isomers). A significant number of these, ranging in potency from 5 times that of morphine to twice that of codeine, appears to be ineffective in suppressing morphine abstinence in the monkey at relatively high (subtoxic) doses.

In addition, a new series of benzomorphans (9-hydroxy derivatives, analogs of the potent oxymorphone and oxycodone derived from thebaine) have been synthesized and found to have interesting pharmacological properties. It is of special interest that the synthetic sequence we have devised for these compounds provides us with steric control in the formation of the carbinol grouping. Thus it is possible to obtain in excellent yield the racemate corresponding in configuration to oxycodone (OH and iminoethano groups *cis*) or to completely reverse this stereochemistry to obtain the opposite racemate and a configuration not known in the morphine group. The charge on the nitrogen atom appears to be the major directing influence, although steric hindrance may also be a consideration.

Research is being pursued toward devising a phenazocine-like structure containing an ether bridge characteristic of morphine, and toward simple neurotropic agents based on acetyl choline.

As for the present status of phenazocine, the Smith, Kline & French Laboratories have applied for and expect to obtain, shortly, a new drug license in the hope of putting phenazocine on the market by December 1. Five other U.S. firms have been licensed to manufacture phenazocine (at least four are going ahead with pilot-plant synthesis), but foreign rights will not be exercised by DHEW. A publication for world-wide dissemination of all pertinent data has been recommended. Finally, completed, short-term addiction studies at the Addiction Research Center, Lexington, Ky., and scattered clinical observations indicate less addiction potential for phenazocine than for morphine.

Carbohydrates. The chemical synthesis of 2-deoxy-D-ribofuranose nucleosides starting from the sugar itself was achieved for the first time. A new pathway to the synthesis of the component parts of deoxyribonucleic acid, the central substance in the mechanism of heredity, was thus made available

2-Deoxy-D-ribofuranose 1-phosphate, the intermediate in the enzymatic synthesis and scission of deoxyribonucleic acid, was synthesized by chemical means for the first time.

A wholly new pathway to the synthesis of nucleosides, involving 1-thiosugars was discovered.

A simple method for the preparation of 3-deoxy-D-glucose and 3-deoxy-D-mannose from 2-deoxy-D-ribose was developed. These 3-deoxysugars are of some importance in current studies on the mechanism of insulin action.

An eight-carbon sugar, D-*glycero*-D-*manno*-octulose, has been discovered in the avocado and in a *Sedum* species. This is the first known occurrence of an octose in nature.

Metabolites. In connection with fundamental studies in peptides and proteins, selective cleavage reactions have been elaborated with tryptophyl, tyrosyl, and methionine peptide bonds. Such studies obviate the great need for rapid methods for the establishment of the primary structures of proteins. They form part of a concerted effort to put available sequence data of amino acid combinations on punched cards and subject them to information analysis.

Thus, one out of more than 560 peptide bonds in human serum albumin (molecular weight around 70,000) was selectively split by a purely chemical method. Bovine and avian albumins were subjected to similar cleavages and revealed species differences in the amino-acid sequence following the two and three molecules of tryptophan per molecule of protein. This new chemical method allows the rapid classification of albumins and is a simple test for the discovery of genetic and species changes in proteins.

The use of this chemical cleavage method has allowed new insights into the structure of the protein of tobacco mosaic virus and revealed the presence of three tryptophan units in the molecule (molecular weight 18,200), followed by alanine, lysine, and threonine. The *try-lys* and *try-ala* bonds show normal reactivity and cleavage, the new *try-threo* bond is unreactive in the large A-protein and reactive in the new C-terminal peptide containing 16 amino acids. This lack of reactivity is attributed to secondary and tertiary effects.

The application of the N-bromosuccinimide cleavage to the vasoconstrictor peptide angiotensin has led to cleavage at the tyrosyl-peptide bond preceding the special amino acid which changes from species to species.

The cyclic antibiotic gramicidin A, containing >40 percent tryptophan (prepared in a pure form in collaboration with the Rockefeller Institute), is resistant to enzymatic hydrolysis and not suitable for conventional chemical cleavage; it has now been selectively split by the new method and preliminary sequence data have been obtained.

It has been demonstrated that the imidazole ring undergoes smooth oxidative cleavage with N-bromosuccinimide to a keto-aldehyde, formic acid, and two moles of ammonia. In the course of efforts to protect the imidazole ring from cleavage it was found that the ring may be selectively acylated with various sulfonyl halides at pH 5.5 to give sulfonyl imidazoles which are resistant to oxidative cleavage but are reconvertible to imidazoles under relatively mild hydrolytic conditions. This newly discovered phenomenon has advanced the chemistry of histidine along several fronts: (1) A novel protective group for the synthesis of histidyl peptides has become available, (2) the imidazole ring may be selectively and *reversibly* blocked in enzymes to determine the contribution of histidine to active catalytic sites, (3) the imidazole ring can be protected in the course of oxidative degradation of tyrosine and tryptophan-peptide bonds and can be subsequently recovered.

In a collaborative study with the NHI an approach to a topographic neurochemistry of the brain has been started. Only certain regions of the brain, such as hypothalamus and caudate nucleus, but not cerebellum, have been found to contain the specific enzyme, dopamine-β-oxidase, that converts dopamine to norepinephrine. Boiled brain tissue produced only a "norepinephrine-like" fraction identified as the "isographic" and isomeric 2,4,5-trihydroxyphenethylamine, an isomer of trisnormezcaline, arising not by enzymatic hydroxylation of the side chain, but by non-enzymatic nuclear hydroxylation. The existence of this new isomer of norepinephrine, having all of its chromatographic properties, has necessitated the reevaluation of a large body of previous studies on the biosynthesis of norepinephrine in other laboratories.

On administration of radioactive dopamine to animals, up to 1 percent of radioactive trihydroxyphenethylamine can be isolated from urine. This is a minimum conversion value since only 1 percent of administered trihydroxyphenethylamine is recovered from urine. The new metabolite, as preliminary pharmacological studies indicate, is more active in certain systems than dopamine. Its endogenous formation remains to be shown.

It has now been found that the new enzyme involved in the inactivation of catecholamines, *i.e.* catechol-O-methyltransferase, converts *p*-substituted catechols not only to *m*-O-methyl, but also *p*-O-methyl derivatives. The ratio of *m*- and *p*-O-methylation is dependent on the electronegativity of the *para* substituent, and the pH at which enzymatic methylation is carried out. For example, 3,4-dihydroxyacetophenone is methylated enzymatically to the extent of 40–60 percent at the *p*-hydroxyl. Dopamine is converted to 10–15 percent of the *p*-O-methyl derivative. The same conversions *in vivo* lead to lower ratios of *para*-methylated products, because enzymatic demethylation by a microsomal TPN-requiring system proceeds faster with *p*-O-methyl than with *m*-O-methyl catechol ethers. A novel type of "transmethylation" is the *in vivo* conversion of a *p*-O-methyl to a *m*-O-methylcatechol, namely, the interconversions of acetovanillone$\rightleftarrows$acetoisovanillone, a type of compound recently isolated from adrenal extracts. Epinephrine and norepinephrine gave 10 percent "paranephrine" and "norparanephrine" *in vitro*, but not *in vivo*.

In collaboration with LPT, the effect of the selective oxidation of tryptophan on the enzymatic activity of trypsinogen, acetyltrypsinogen, an enzymatically active fragment of trypsinogen and trypsin has been explored. The marked difference in reactivity of tryptophan in trypsin and trypsinogen is ascribed to differences in their secondary or tertiary structure. Enzymatic inactivation (trypsin) or less of activatability (trypsinogen) was studied as a function of the oxidative modification of tryptophan. Such partially inactivated enzyme preparations still had their active phosphorylation sites intact. At least one tryptophan residue is needed for activity. This demonstrates that an intact phosphorylation site *per se* is not sufficient for enzymatic activity but that

additional sites, such as an intact indole nucleus, are necessary. This points to a possible separation of the sites of activity and specificity which may be put to further use for the study of enzyme mechanisms.

A rapid synthesis of kynuramine from tryptamine has made possible the observations in the NHI that kynuramine is probably not another biogenic amine, but that it is an excellent substrate for monoamine oxidase. The aldehyde produced by this enzyme undergoes *intra*molecular cyclization to the end product 4-quinolone before any oxidation to the acid takes place. Thus, kynuramine is an ideal substrate for the rapid routine assay of monoamine oxidase activity in tissues without getting interference from DPN.

A cooperative study with the Laboratory of Clinical Biochemistry, NHI, has demonstrated the competitive inhibition of O-methyltransferase. The potentiation of epinephrine by pyrogallol had been interpreted 30 years ago as an antioxidant effect. It has now been shown that competitors of methyl transferase, such as pyrogallol, catechol, glycocyamine, 3,4,5 - trihydroxyphenethylamine (trisnormezcaline) inhibit the inactivation of norepinephrine by methylation to the extent of 40–100 percent. By contrast, the half-life time of norepinephrine is not affected by typical monoamine oxidase inhibitors such as marsilid.

The study of the kinetics of the decarboxylation of substituted *p*-hydroxycinnamic acids has adduced evidence for the intermediate existence of quinonemethines as the species undergoing decarboxylation, an observation of significance for the evaluation of similar metabolic processes.

New staining reagents for the histochemical detection of proteolytic enzymes have been developed in collaboration with the Laboratory of Pathology. By these techniques, a new trypsin-like enzyme has been found to occur almost exclusively in mast cells, which differs from trypsin in not requiring activation.

A new tool, nuclear magnetic resonance spectroscopy, has been applied to several problems of structure determination during the year. It has revealed the position of the double bond in dehydroproline, the position of the hydroxyl in an isomer of hydroxyproline, the location of the acetyl groups in partially acetylated derivatives of deoxystreptamine, the course of bromination of certain indoles, the location of the bromine in brominated lysergic acid diethylamide, and the absence of ring-chain tautomerism in various tryptamine derivatives.

A novel bound form of the neurotropic γ-aminobutyric acid in brain has been discovered in the Laboratory of Clinical Biochemistry, NHI. A collaborative study led to its identification as homocarnosine, *i.e.*, γ - aminobutyrylhistidine, which was also obtained by synthesis.

Steroids. Three new crystalline substances have been added to the list of compounds isolated from fecal lipids. Two of them may be hydroxylated fatty acids and the third a new sterol.

A fascinating collaborative project of the Steroid Section with an NHI microbiologist culminated in the identification of *acrasin*, a factor or hormone involved in the differentiation of slime mold, as a derivative of stigmasterol. This success was possible only on the basis of previous improvements in the identification of steroids on a micro scale in connection with studies on fecal lipids.

In the reinvestigation of the nonsaponifiable fractions of the tapeworm, it was found that in *Taenia taeniaeformis* and in *Moniezia sp.* cholesterol constitutes by far the most prevalent unsaponifiable substance (98 and 85 percent).

In order to study the biogenesis of sapogenins, slices of *Dioscorea*, the Mexican yam, the major source of the Western Hemisphere's supply of partially synthetic cortical steroids, have been incubated with radioactive mevalonic acid. The latter has been converted into four radioactive products whose identification is in progress.

In work toward the elucidation of the structure of pennogenin, it was found that the lithium hydride reduction of 22,26-oxido-$\Delta^{17(20)}$-cholestene-3β,22-diol-16-one yields 22,26-oxido-$\Delta^{17(20)}$-cholestene-3β,16ζ-diol (I) and not $\Delta^{17(20)}$-22-isoallospirosten-3β-ol as formerly believed. The Δ^5 series of compounds also gave analogous results. The catalytic reduction of I leads to the formation of the hitherto unknown 17α-cholestane side chain. 17-Isocholestane would be of value as a reference compound.

Solasodine, the aglycone of a number of widely occurring solanum alkaloids, has now been successfully degraded in very good yields (65 per-

cent) to the acetate of pregnadienolone, the starting material from which most of the biologically active steroids are obtained. The method consists in the acid catalyzed rearrangement of O,N-diacetyl solasodine to the pseudo derivative, followed by oxidation and solvolytic cleavage of the acylester side chain. A semicontinuous procedure has also been developed. This process has attracted much attention in the Eastern hemisphere where solanum alkaloids (and not sapogenins) serve as starting material for partially synthetic cortical steroids.

The stereochemistry of the six- and five-membered (C/D) ring juncture in steviol and isostevoil, the aglycones from stevioside, one of the most potent natural sweetening agents occurring in a Peruvian shrub, has been established. Furthermore, the two epimeric dihydrosteviols from stevoil and isosteviol have been converted in an eight-step degradative process to (-)α-dihydrokaurene and (-)β-dihydrokaurene, two naturally occurring terpenoid derivatives from New Zealand. With the exception of a few minor points, the structure of stevoil and isosteviol has been established.

In continuation of the search for corticoid- and sex-hormone antimetabolites and carcinostatics, Δ^4-androstene-9α-thiocyano-3,11,17-trione has been converted to the corresponding 9α-thiocarbamide and 9α-thiol. Similarly, 9α-thiocyanocortisone has been converted to the corresponding 9α-thiocarbamide. A new route to 11β-mercaptocorticoids has been opened through the synthesis of 3,9α - epoxy - 11β-thiocyano-5β-pregnan-3β,17α,21-triol-20-one.

In connection with studies on the chemical conversion of steroidal hormones and vitamins to possibily physiologically active derivatives of anthracene, two new isomers of anthroergosterol and anthracholesterol have been obtained from the corresponding $\Delta^{5,7,9(11)}$-trienic sterols by treatment with *p*-toluene sulfonic acid in chloroform. Their chemical structures and relationship to the Nes-Mosettig rearrangement are being studied.

In order to refine the methods for routine analysis of clinically important steroids the separation and quantitative estimation of a number of 17-ketosteroids by gradient elution chromatography have been elaborated and further work is continuing in this direction.

In cooperation with NCI, rat adrenal tumor is being analyzed for adrenal cortical hormones.

Office of Mathematical Research

General. The Office of Mathematical Research has the broad objective of contributing to theoretical biology as a biological subscience; producing concepts and theoretical apparatus for the rational analysis and quantitative interpretation of biological problems; and furnishing consultation, aid and advice to the subject-matter scientists of NIH. Much importance attaches to stimulating experimental biology by invoking new points of view, producing theories of practical predictive value, and formulating deductive models which can give purposeful direction to further experimentation. But it is held to be equally important to contribute to sound "philosophy," to deal with abstract problems on their proper mathematical ground and to operate at a level of generality not necessarily dictated by immediate and obvious applications in biology, medicine, and public health.

Research. Work has continued on the development of mathematical and computational methodology for the analysis of tracer data. Programs have been written for parameter estimation in compartmental systems. These programs, available to any investigator, are sufficiently general to cover a broad spectrum of models and to accept, as input, experimental data in any one of a variety of forms. Methods have been developed for obtaining the variances and covariances of such estimates so that the significance of observed changes, introduced for example by a given treatment, can be assessed. These programs can be applied to any problem in the realm of "linear kinetics." In particular, they have been applied to C–14-labeled glucose data on human subjects and in a comprehensive continuing study of patients having various thyroid abnormalities and subjected to various treatments. In both instances suggested additional experimentation has come out of the mathematical analysis. Work has been underway and will continue on the problem of taking into quantitative account, in the fitting of data on a given compartment, information which is available on one or more other compart-

ments, studies of consistency checks, and the exploitation of redundant data in the uniqueness question.

The problem of the spread of electric current from a neuron soma into branching dendritic trees has been formulated and solved. Analysis of current experimental data indicates that motoneuron dendrites play a dominant role in determining motoneuron properties and this is in direct contrast to what had been assumed in the absence of an appropriate mathematical solution. Significant progress has been made on the general problem of membrane-potential spread over soma and dendrites in response to synaptic current generation with an arbitrary time course and spatial distribution. The theory permits distinction between synaptic potentials generated predominantly in the dendrites and predominantly on the soma. Recent experimental techniques and several ambiguities in the current theories of synaptic excitation are being analyzed within the framework of these theoretical results. The above general analysis has also led to practical estimation procedures for motoneuron time constants and soma-dendrite conductance ratios. A procedure has been devised and programmed for the computation of external potential fields around a neuron. Application of this program will enable the mapping of isopotential contours and quantitative assessment of certain approximations which have been standard in neurophysiology.

Work has continued on the problem of specifying the area under the curve in terms of the coefficients of the differential equation of which that curve is a solution. Special cases of the general theorem have found extended application in the analysis of the inhibition of thrombin formation by soybean trypsin inhibitor, in the estimation of rate constants in systems for which no analytical solution of the rate equation is known, and is the choice of kinetic models based solely on final yields. This same problem has appeared in the stochastic theory of absolute reaction rates and it has been shown that the results employed there, subject to severe restrictions in fact, apply to a very general class of matrix. Work has continued on matrix theorems as applied to linear analysis in general, relaxation time analysis of systems close to equilibrium, and the relation between irreversible thermodynamics and chemical kinetics.

Other Activities. This office has continued to furnish extensive consultation and collaboration to other scientists at NIH. During the past year, such activities have involved members of the Research Associate Program. A member of this office is giving the mathematics course in that program and at their request is supervising an applied mathematics seminar for the Research Associates. Members of this office have served on committees (as well as in less formal advisory roles) for NIAMD, NINDB, NIMH, Office of Research Planning, DRG, DGMS, and Heart Control Program, PHS. Members of this office presented an entire symposium on Mathematical Models in Biology (jointly sponsered by the Biometrics Society and AAAS). Further external interests in the activities of this office are indicated by the following invited one hour lectures: Two at an IBM Symposium on Computers in Medicine, one for the American Mathematical Association, and one before a joint symposium sponsored by the American Statistical Association and the Biometrics Society.

Concluding Comments

The vigor of a research organization is in good part determined by the flux of scientists in and out. Its status in the community of such institutions is likewise measured by this flux. Throughout the year, a sizable number of visiting scientists have been in attendance in the laboratories and clinical facilities of NIAMD, these ranging from senior and world-renownel investigators to select and exceptional younger scientists, hopefully leaders of the future. Among the more distinguished of these visitors have been Drs. Gustafsson of Lund, Hestrin of Jerusalem, Pitt-Rivers of London, Tjio of Saragossa, Watson of Minneapolis. Their terms of service at Bethesda have ranged from 1 to 12 months, and both the visitors and the permanent staff have profited enormously from these arrangements. In addition a considerable number of very able guest workers have elected to spend part or all of their available time at NIAMD. The avidity with which such guests and visitors seek out this institute is taken as an indication of the excellence of our staff and our facilities.

By way of compensation to the scientific community, a number of NIAMD scientists have been provided with the opportunity to work and spend time in other laboratories in America and abroad. The 11 members of our staff who have spent significant periods of time in other laboratories during the past calendar year have worked at such centers of scientific activity as Woods Hole, Paris, Copenhagen, Mill Hill (London), and Osaka. We believe this exchange to be extremely fruitful, bringing to Bethesda foreign ideas and techniques. Also it serves to familiarize our foreign colleagues with recent scientific progress in this country. We further believe that this flux of persons to and from Bethesda is almost as essential to the continued vigorous pursuit of our mission as is the flux of fluids across cell membranes to the continued survival of the organisms we study.

Another essential feature of organic survival is growth, and, in the necessarily intellectual atmosphere of NIAMD, growth connotes learning and teaching. Over and above the process of mutual education which is inevitable when many scientists work together, a consciously designed teaching program is now well under way. The first class of research associates graduated in June 1959; the third class was admitted at that time. This comprizes four new members, young physicians desirous of getting concentrated training in research. Many more applications are filed by candidates than can be accommodated, and the quality continues to be the highest. The program is popular both with the associates and with their preceptors. Additional activities have been the provision for seven fellows in the COSTEP program and an increasing interest in the laboratory and academic graduate education of secondary-school science teachers. In the latter capacity, we have housed, for brief periods, four high-school teachers and four students. NIAMD scientists collaborated with the staff of NCI in an intensive 1-day program designed to give high-school teachers an appreciation of contemporary biomedical research as practiced at Bethesda.

From these activities it will be seen that, in addition to fulfilling its committed obligation of producing publishable research, NIAMD has, in limited degree, assumed the responsibility of producing men. It may be that in the long light of history, the men which it produces will be as important a contribution to the community at large as is the scientific research which its scientists conduct.

*CLINICAL INVESTIGATIONS**

The Clinical Investigations Unit has grown conservatively and according to plan in several functional areas: clinical research program; number of patients and varieties of diseases studied; scientific publications; and number of staff scientists, visiting scientists and guest workers. This growth has been encompassed without any structural (laboratory space) expansion.

One new branch, Pediatric Metabolism, and two new groups, gastroenterology and epidemiology of arthritis and rheumatic diseases have been added. Pediatric Metabolism Branch, under the supervision of Dr. Paul A. di Sant'Agnese, will study three childhood diseases: fibrocystic disease of the pancreas, glycogen storage diseases and celiac disease. The programs of the other new groups are described below.

A total of 389 inpatients was admitted during the 12-month period from December 1, 1958, to November 30, 1959, an increase of 55 over a similar period last year. The total patient days were 16,942, an increase of 690 over the preceding year. In the admissions and followup, 1,520 patients were examined and studied, an increase of 325 over the past year. The average inpatient stay at the Clinical Center was 43 days. The bed capacity increased from 59 to 65 during the current year. An average census of 79 percent was maintained. As in the preceding three years, the inpatients on the service of the National Institute of Dental Research were accommodated on NIAMD units.

Clinical and laboratory investigations related to the diseases studied at NIAMD have resulted in 72 publications in scientific journals, monographs, annual reviews, and medical textbooks. During the past year, Dr. J. Edward Rall was given the Arthur H. Flemming Award, Dr. Joseph J. Bunim

* Prepared by Joseph J. Bunim, M.D., Clinical Director, NIAMD.

556044—60——9

was President of the American Rheumatism Association and Cochairman of the Pan American Congress on Rheumatic Diseases. Dr. Bunim was appointed Clinical Professor of Medicine at Georgetown University. Dr. Paul A. di Sant'Agnose was given an award by the National Cystic Fibrosis Foundation.

At the request of the Bureau of Medical Services of the USPHS, members of the Clinical Investigations staff gave lectures and conducted ward rounds at the following Public Health Service Hospitals: Baltimore, Seattle, Staten Island, and Savannah.

Clinical investigation on selected ambulatory patients was conducted by staff members at three "outside" specialty clinics in regional hospitals: Dr. Roger L. Black at the Arthritis Clinic of D.C. General Hospital, Dr. Kurt J. Bloch at the Arthritis Clinic of Georgetown University Hospital, and Dr. James B. Field at the Diabetes Clinic of Georgetown University Hospital.

Our staff scientists have derived considerable benefit from the association of distinguished visiting scientists and guest workers, who have come to work here during the past year: Dr. Cecil J. Watson, Professor of Medicine, University of Minnesota; Dr. Rosalind Pitt-Rivers from the Medical Research Council, Mill Hill, London; Dr. Anne-Marie Hofer from Frankfurt, Germany; and Dr. Samuel Rose, Professor of Physiology, University of Melbourne, Australia; Dr. Panu Vilkki, a visiting Fellow from Turku, Finland. The guest workers were: Dr. John Worthington, Jr., sent here by the Mayo Clinic; Dr. Kingsley Mann, sent here by the Upjohn Company; and Dr. Evelyn Hess, Fellow, Empire Rheumatism Council of England.

Arthritis and Rheumatism Branch

Research in arthritis and connective-tissue diseases has taken new direction as a result of recent studies done at NIAMD and other institutions, and of new immunological and genetic concepts advanced in this country (Lederberg, Billingham), Australia (Burnet), England (Medawar), and Sweden (Grubb). Some of the recent observations and provocative ideas that have opened new trails of investigation can be mentioned only for orientation in this brief report. The rheumatoid factor, a macroglobulin (19S), has been isolated in other laboratories and at NIAMD, and its chemical composition and physical properties defined. We are in a better position now to wrestle with the important questions: Is the rheumatoid factor a true antibody and, if so, what is the specific antigen? Is the rheumatoid factor, whatever its biologic nature may be, a by-product or does it have pathogenetic implications? Evidence has been collected which strongly suggests a close relationship between rheumatoid arthritis and systemic lupus erythematosus (S.L.E.). The L.E. factor, consisting of light (7S) gamma globulins, is composed of multiple antibodies to several components of cell nuclei. This suggests that S.L.E. may be another example of an autoimmune disease in man. The L.E. factor is found more frequently in serum of patients with rheumatoid arthritis than in any other disease except S.L.E. itself. Patients with congenital and acquired agammaglobulinemia develop rheumatoid arthritis far more frequently (25%) than does any other human population. Since gamma globulin is deficient in these patients, both the rheumatoid factor and the L.E. factor are, of course, absent. But many relatives of these patients have either the rheumatoid factor or L.E. factor or, less frequently, rheumatoid arthritis or systemic lupus erythematosus. Sir Macfarlane Burnet and Joshua Lederberg have separately postulated that "each immunologically competent cell, as it begins to mature, spontaneously produced small amounts of antibody corresponding to its genotype; that the stereospecific segment of each antibody globulin is determined by a unique sequence of amino acids; and that the cell making a given antibody has a correspondingly unique sequence of nucleotides in a segment of its chromosomal DNA—its 'gene for globulin synthesis.' The mature cells proliferate extensively under antigenic stimulation but are genetically stable and therefore generate large clones genotypically preadapted to produce the homologous antibody. These clones tend to persist after the disappearance of the antigen, retaining their capacity to react promptly to its later reintroduction."

Clinical research in the Arthritis & Rheumatism Branch will include attempts to determine immunological reactivity in patients with rheumatoid arthritis and connective-tissue diseases and by epidemiological studies to ascertain whether ab-

normal serum proteins in patients and their kinships are genetically controlled.

STUDIES OF SJÖGREN'S SYNDROME

This syndrome consists of a triad of keratoconjunctivitis sicca (KCS), xerostomia and rheumatoid arthritis (r.a.). However, any two of the three features justify the diagnosis of Sjögren's syndrome. Twenty-one patients with this relatively rare syndrome have been intensively studied during the past year. The diagnostic criteria of KCS and xerostomia have been established and verified in all our patients by staff members of the Ophthalmology Branch of NINDB and NIDR respectively who actively collaborated in this project. About one-third of the patients had Sjögren's syndrome without arthritis, a few patients had systemic scleroderma (a connective-tissue disease) but not rheumatoid arthritis, and the rest had Sjögren's syndrome with classical r.a. Yet every patient but one (even those without r.a.) had the rheumatoid factor and two had the L.E. factor. All 21 patients had hyperglobulinemia, 75 percent hypergammaglobulinemia, 60 percent had antinuclear antibody demonstrated by immunofluorescence, and 25 percent had positive direct Coombs' test. Biopsies of the salivary glands done in 10 patients showed in each case typical histopathological changes consistent with Sjögren's syndrome. These lesions bear a striking resemblance to the histological alterations of the thyroid gland in Hashimoto's disease which is believed to be an autoimmune disorder.

Our studies reveal that Sjögren's syndrome is a variant of r.a. in which an exalted immune reactivity is exhibited. Prospective investigation will consist of two types of study: (1) An attempt to find an antibody to the patient's tissue components and (2) using the 21 patients as propositi, kinships will be studied by the *epidemiology group* to determine the pattern of abnormal serum proteins and the occurrence of rheumatic diseases in these families.

EPIDEMIOLOGY OF RHEUMATOID- AND OSTEOARTHRITIS

This study was initiated intramurally by the Arthritis and Rheumatism Branch of NIAMD during the past year. The prevalence of both types of arthritis was studied in the following population groups: Alaskan Eskimos (457), Alaskan Indians (182), U.S.A. whites (436), and English whites in Wensleydale (489). The diagnosis was based on three kinds of evidence: (1) clinical findings (history and physical examination), (2) X-rays of hands and feet, and (3) serological examination for rheumatoid factor by the bentonite flocculation test and the sheep-cell agglutination test.

The prevalence of osteoarthritis in Eskimo males and females in each decade was less than in U.S.A. whites. The difference was statistically significant in the North and South Eskimo males above age 40 ($p=.001$) and in the North Eskimo females of the same age group. The difference was not significant in the South Eskimo females. Analysis of the data on the other population groups has not yet been completed.

MECHANISM OF ACTION OF STEROIDS AT MOLECULAR LEVEL (*IN VITRO*)

DPNH Oxidation. Low concentrations of various hormonally active steroids and stilbesterol inhibit DPNH oxidation (but not TPNH oxidation) by enzyme preparations from a number of sources (noncompetitive with DPNH). The effect is catalytic and not related to steroid metabolism. In addition to variations in sensitivity from organ to organ, there is a 1,000-fold variation in range of potency among the active steroids. In kidney, the Ki (or half-maximum inhibitory concentration) for stilbesterol is 8×10^{-7} M. This means that a measurable effect can be observed with concentrations as low as .02 gamma/cc. Cholesterol and tetrahydro E are ineffective. The effect has been studied in brain, spleen, muscle, heart, liver, thymus, and kidney of the rat. Beef pituitary is also under study.

Extension of studies to include preparations from *E. coli*, *B. subtilis*, and yeast (*S. fragilis*) revealed a similar inhibition of DPNH oxidation, and this could be correlated with known effects of steroids on growth. The effect was also demonstrated in preparations from Ehrlich and S–37 mouse ascites tumor.

Further refinement of studies has revealed the site of inhibition to be DPNH-cytochrome C reductase (specifically between flavoprotein and cytochrome b), a major link in the chain of hydrogen transfer in the cell's oxidative reactions. Interestingly, the inhibition is competitively reversed by tocopherol. This suggested a need for investigation into a possible relationship between steroid and vitamin E. Examination of the tissues of vitamin E-deficient rats, however, did not reveal differences in DPNH cytochrome C reductase or the degree of steroid inhibition.

EFFECT OF CERTAIN COMPOUNDS ON URIC-ACID SYNTHESIS IN MAN

The metabolic origin and disposition of uric acid in gouty patients has been studied further by administering isotopically labeled precursors of uric acid along with labeled uric acid itself and following the incorporation of label into urinary uric acid in normal and gouty subjects. A substantial portion of the patients with gout show an excessive synthesis *de novo* of uric acid as measured by the extent of incorporation of glycine-1-C^{14} into urinary uric acid. Additional patients can be shown to be producing excessive amounts of uric acid if the glycine incorporation data is corrected for the dynamics of the urate pool. This increased production does not show up in the urinary uric-acid excretion values because of its extrarenal disposition. There still remains a portion of the gouty patients who show no demonstrable difference in the extent of glycine-1-C^{14} incorporation into urinary uric acid from that of normal individuals.

A pharmacological agent which suppresses the excessive uric-acid synthesis found in some gouty subjects has been studied further. 6-Diazo-5-oxo-L-norleucine (DON) which has been shown in this laboratory to suppress uric-acid synthesis in two gouty subjects has been administered to a total of seven gouty patients. Two patients who showed no drop in serum uric acid or in urinary uric-acid excretion nevertheless showed a substantial reduction in the incorporation of glycine-1-C^{14} into urinary uric acid. This suppression of purine biosynthesis was evidently masked by the large urate pool in these subjects. Undesirable effects of DON consisted of duodenal ulcers in two patients and ulcerations of the oral mucosa in five of the seven patients studied. Routine use of this drug for suppressing the uric acid production in gouty patients appears to be imprudent. It is conceivable, however, that more specific inhibitors of purine biosynthesis might carry with them a more favorable therapeutic index.

An experimental tool for studying the homeostatic control of purine synthesis in the human has been found in the action of a drug, 2-ethylamino-1,3,4-thiadiazole, a nicotinamide antagonist which has been used experimentally in the treatment of cancer. Other workers noted that its use in the human resulted in an increase in both serum urate values and in daily urinary uric acid. The origin of this increased uric acid, whether from cellular breakdown or *de novo* synthesis, was not clear. We have been able to confirm this finding and furthermore to show that the increased uric acid production is the consequence of an increased purine biosynthesis induced by 2-ethylaminothiadiazole. The extent of incorporation of glycine-1-C^{14} into urinary uric acid in a non-gouty individual was brought up to the range observed in gouty subjects by administration of 2-ethylaminothiadiazole. Furthermore, its effect was completely prevented by administration of large doses of nicotinamide. This drug was found to have a comparable effect on urinary allantoin and uric-acid production in the guinea pig and *in vitro* studies of its action are now underway.

NEW URICOSURIC AGENTS IN GOUT

Studies on drugs for the management of problem cases of gout have been continued. Previous work in the National Heart Institute on a group of compounds chemically related to phenylbutazone had shown that antirheumatic activity could be correlated with chemical structure and that uricosuric activity could be related to the acid association constant (pKa) of the drug. A urinary metabolite of phenylbutazone, oxyphenbutazone, had been shown to possess potent antirheumatic activity, but very little uricosuric activity. Upon the introduction of a keto group into the side chain of oxyphenbutazone, the resulting compound (G–29701) was found to possess potent uricosuric activity, but little antirheumatic activity. Again the uricosuric activity was cor-

related with an increased acidity of the compound with the pKa dropping from 4.6 for oxyphenbutazone to 2.3 for keto-oxyphenbutazone. There was a corresponding decline in the biological half-life from 72 hours to 8 hours, which prevented the high-serum levels needed for an antirheumatic effect with the parent compound. This drug has been given to nine patients for short periods of time and has been very well tolerated, with no toxic side effects to date. Since the other potent uricosuric agents now available have a considerably shorter biological half-life, on the order of 3 hours, the 8-hour half-life of keto-oxyphenbutazone gives promise of providing a more sustained uricosuric action with less frequent administration of the drug.

The antagonistic action of salicylates on the uricosuric effect of zoxazolamine and sulfinpyrazone has been further studied and found to exist at even low doses of salicylates. By contrast, acetaminophen, which is the active metabolic product of acetanilid and acetophenetidin, gives no such antagonistic action, while at the same time providing an analgesic action.

ENZYMIC AND CLINICAL STUDIES ON INBORN ERRORS OF METABOLISM OTHER THAN GOUT

Alcaptonuria and Ochronotic Arthritis

Objectives in studying patients with alcaptonuria have been several: (1) to determine the exact nature of the metabolic defect in this condition, (2) to study the hereditary pattern of this disease, and, if possible, to develop a test which will detect the heterozygous state in relatives of alcaptonurics carrying the trait, (3) to study the formation and deposition of the pigment derived from homogentisic acid and to determine how it produces the pathological changes in the connective tissues, particularly the joints, (4) to study the cause of the arthritis nearly always associated with this condition, and (5) to attempt various means of treatment of this metabolic disease.

Nature of Defect in Alcaptonuria. Quantitative analysis of the enzymes involved in tyrosine metabolism has been made in liver and kidney homogenates from autopsy specimens of another patient with alcaptonuria. Again it has been possible to show that alcaptonuric tissues differ from the normal only in having no detectable homogentisic-acid oxidase activity. Thus, it has been clearly demonstrated in two families that the defect in this metabolic disease consists of a deficiency of homogentisic-acid-oxidase activity in the liver and kidney.

Inheritance of Alcaptonuria. The recent suggestions of Milch and Milch that this disease is inherited as a dominant trait with incomplete penetrance, rather than as a simple autosomal recessive inheritance, is not supported by an examination of the pedigree of one of our patients. Upon careful questioning it was disclosed that there had been a consanguineous marriage which was not mentioned in earlier interviews. With this complete information, a simple recessive inheritance adequately explains the expression of the disease in this family.

Experimental Ochronosis and Arthritis in Guinea Pigs. As part of the study on the mechanism by which the accumulation of homogentisic acid leads to the development of ochronotic arthritis in alcaptonuria, the distribution of homogentisic acid in the tissues of guinea pigs has been measured at different times by a specific enzymatic method after the intraperitoneal injection of this acid. Very low concentrations were found in the muscle, liver, and the other organs, but values almost as high as in plasma were present in the cartilage and skin. This unusual predilection of homogentisic acid for the connective tissues is in agreement with the deposition of the ochronotic pigment in the same areas. Further studies are being made of the nature and syntheses of the pigment and its relationship to the associated arthritis.

Enzymatic Synthesis of Homogentisic Acid (Role of Vitamin C in Tyrosine Metabolism). Detailed studies of the enzyme system of liver which catalyzes the formation of homogentisic acid from p-hydroxyphenylpyruvic acid (the keto acid of tyrosine) are being continued. Vitamin C is involved in this enzymatic reaction and scorbutic guinea pigs have a defect in tyrosine metabolism and excrete p-hydroxyphenylpyruvic acid when fed this amino acid. The metabolic defect is corrected by vitamin C, but until recently

it was not known how this vitamin maintains normal tyrosine metabolism. Some insight into the mechanism came in studies with purified liver enzymes. It was found that ascorbic acid and 2,6-dichlorophenolindophenol had the property of protecting one of them, p-hydroxyphenylpyruvic acid oxidase (the enzyme which catalyzes the oxidation of p-hydroxyphenylpyruvic acid to homogentisic acid) from being inhibited by its substrate. In the presence of ascorbic acid, oxidation continued; in its absence, the oxidation slowed down and stopped. Recently we have been able to demonstrate the way the vitamin acts *in vivo*. Scorbutic guinea pigs were found to have as much p-hydroxyphenylpyruvic acid oxidase as normal animals, but when the scorbutic group was injected with p-hydroxyphenylpyruvic acid, over half of their liver p-hydroxyphenylpyruvic acid oxidase was inactive one hour later. In contrast, injection of the substrate did not inhibit the oxidase in normal guinea pigs. It appears that ascorbic acid acts *in vivo* to protect the oxidase from inhibition as was found in the enzyme studies *in vitro*.

Method for the Estimation of Homogentisic Acid in Synovial Fluid and Other Tissues. The specific enzymatic method to measure small amounts of homogentisic acid in plasma has been modified to make it suitable for the analysis of homogentisic acid in tissues. The method has been utilized in studies of the distribution of homogentisic acid in the tissues of patients with alcaptonuria and in studies of the distribution of this acid in guinea pigs.

Phenylketonuria

Early Diagnosis of Phenylketonuria in Newborn Infant. A sibling born in a family with known phenylketonuria has a one in four chance of being affected. The new enzymatic method for blood phenylalanine developed in this laboratory, particularly the micro modification, makes it reasonable to make the diagnosis within a day or two after birth, and such analysis should be done in newborn infants in families with known phenylketonuria. We are presently doing serial analyses on blood samples on an infant with this background in order to make the diagnosis and start the special diet as soon as possible if this child should have phenylketonuria.

Effectiveness of Diet Low in Phenylalanine in Preventing Mental Retardation in Phenylketonuric Children. Studies of the effectiveness of the low phenylalanine diet in treating phenylketonuric children require repeated measurement of the level of blood phenylalanine and tyrosine. The variable response to the diet, particularly in older infants, may be largely due to the greater difficulty in maintaining a low level of blood phenylalanine in this group. We have been analyzing the blood each month of several children being followed at the Retarded Children's Clinic, Georgetown University Hospital, to evaluate the effectiveness of the diet.

GENETIC POLYMORPHISMS IN MAN AND OTHER ANIMALS

Many biochemical traits in humans and other animals are genetically determined. Some of these may be classified as genetic polymorphisms; that is, the existence in a population of two or more easily distinguished forms of a trait, the lesser of which could not be maintained by recurrent mutations alone. The normal hemoglobin-sickle-cell hemoglobin system, most of the blood groups, and other systems to be described below, fall within this classification. From studies in lower animals and theoretical considerations, there is reason to believe that selection may operate to maintain these traits in the incidences found and in some cases the selective forces may be related to disease. It is of interest to determine if populations living under different environmental conditions and prone to different diseases have different incidences of these genes. In addition, studies of the distribution of these traits can sometimes provide information on genetic relations between separated population groups. A field trip to the Central Pacific was made in the winter of early 1959.

Haptoglobins

These are a family of serum proteins which bind hemoglobin. There are three major patterns in humans and these are under genetic control. Some rare types have been discovered by an NIAMD scientist and other workers. Bloods were collected from Micronesians living in the Marshall Islands. The prevalence of type 1–1 was found to be high in the Rongelap people,

although not quite so elevated in the smaller number of sera studied from the other atolls. Several individuals with no haptoglobin were found; this would presumably be of physiological importance under some conditions of red-blood-cell breakdown. In two cases it was found that individuals who had no haptoglobin in 1959 had small amounts of haptoglobin in 1957. This implies a phenotypic change during the course of the two years.

Monkeys, chimpanzees, and baboons were found to have only one of the haptoglobin types and, presumably, one of the genes. This implies that the polymorphism originated in human populations and has been perpetuated by forces of selection present in humans but absent in lower primates. The haptoglobins have also been studied in a variety of animals and interesting species differences have been observed.

Gamma Globulin groups

The agglutination test for "rheumatoid factor" has been used as a diagnostic method for rheumatoid arthritis. It has recently been shown by Grubb that the sera of some humans will inhibit this reaction, and that the inhibiting material travels in the gamma globulin fraction. This inhibiting property is determined by an allelic pair of autosomal genes, Gm^a and Gm^b. A third gene, Gm^c, which may or may not be at the same locus, has also been detected by Steinberg. In a preliminary study of African, Eskimo, Alaskan Indian, and Micronesian populations, it has been shown that the Gm^b gene appears to be absent from these populations. Studies on the Gm^c gene are in progress. Furthermore, it was found that there is a striking variation in the gamma-globulin levels in these populations. Thus, some apparently normal Africans have total gamma globulins three times that of normal white Americans. The significance of this finding in relation to immunity may be of importance.

Urinary β-aminoisobutyric acid (BAIB) excretion

It has been shown by Gartler and others that the excretion of BAIB is in part under genetic control. Some persons with leukemia and other cancers are also high excretors, but the genetic role in these cases is not clear. Individuals who excrete large amounts of BAIB are rare in European populations. Approximately 200 urine samples from Micronesians were studied. It was found that nearly 90 percent of these were high excretors as compared to 10 percent high excretors in white American populations. Some of the Micronesians studied had been subjected to fallout in 1954 following the detonation of a nuclear device on nearby Bikini atoll. It has been shown that radiation can increase the urinary BAIB output. However, it is unlikely that this is the explanation of the present findings; there was no difference between the exposed and unexposed groups, and there was a high prevalence of high excretors in a small Micronesian population, from a nearby atoll with nearly normal levels of radiation. An alternate explanation is that a focus of the high excretor genes is present in Oceania or Southeast Asia. Studies to determine if this is so are in progress.

Thyroxine-binding proteins of serum

The serum proteins which bind thyroxine have been studied. Four separate binding bands have been detected, representing a much more complicated pattern than had been suspected. These bands have been correlated with those seen on paper electrophoresis, by the use of two-dimensional paper-paper and paper-gel electrophoresis studies. Variations in the patterns in various disease conditions have been studied, and some significant alterations noted. Species differences in binding patterns have also been found.

A variation in the position of the fastest moving band (the thyroxine-binding pre-albumin) has been found in *Macaca mulatta*, and this may represent a polymorphism. In order to determine if this is so, studies on monkey families are contemplated. The relation of these variations in protein binding to differences in thyroid physiology could be the subject of further study.

BIOCHEMICAL STUDIES IN DISEASES OF THE GASTROINTESTINAL TRACT

Recent advances in the understanding of disease processes in terms of biochemical abnormalities at an enzymatic level have made the time opportune to explore intensively the gastrointestinal tract from this point of view. It is the aim of the newly established Gastroenterology Unit to pursue such studies at the level of laboratory investigations of metabolic pathways in tissue extracts as well as at the clinical level.

The metabolic processes that transpire in the cells of the mucosal lining of the stomach, intestines, and gall bladder are far from completely delineated, and consequently their interrelations with the physiologic activities of these areas are not fully understood. Although the biochemical reactions that occur within the liver and pancreas have been studied in greater detail, there remain many gaps in our knowledge of these organs, too. It is the long-term purpose of this unit to investigate pathways of metabolism of these tissues, using at first material from animal sources and eventually biopsy specimens from human subjects with and without diseases of these tissues. It is then intended to apply the information gained in these studies to the further investigation of the functions of these organs, in animals and humans, and to investigations of hormonal regulation of their functions.

Initial efforts are directed toward disease of the small intestine. Within this organ food is digested, and the digestion products are then transferred across its wall. The diseases of man in which these processes are impaired are known collectively as the "malabsorption syndome." Patients with this syndrome are being studied, as is also the process of absorption *in vitro*, with the aim of correlating the information obtained by these two approaches in order to understand more fully the mechanisms underlying the human diseases.

Also being studied are families is which gastrointestinal diseases occur as genetically determined traits, in an attempt to discover proviously undetected biochemical aberrations as causal factors in the diseases.

Pediatric Metabolism Branch

The Pediatric Metabolism Branch will devote most of its efforts to the study of cystic fibrosis of the pancreas. Other disorders leading to intestinal malabsorption in children and diseases due to glycogen storage will also be the object of investigation.

PROPOSED INVESTIGATIONS IN CYSTIC FIBROSIS OF THE PANCREAS

Cystic fibrosis of the pancreas, despite its name, is a generalized, hereditary disease of children and adolescents in which there is a dysfunction of exocrine glands. The fully manifested patients have chronic pulmonary disease, pancreatic deficiency, abnormally high sweat electrolytes, and at times cirrhosis of the liver.

Cystic fibrosis was recognized as a separate disease only 20 years ago. At that time most patients died in infancy of bronchopneumonia. With the advent of effective antibiotic agents and with increasing awareness of the disease, hundreds of patients are being seen in leading medical centers in this country and abroad. The significance of the planned researches becomes apparent when one realizes that this generalized disease is responsible in the pediatric age group for the great majority of patients with chronic lung disease, for virtually all cases of pancreatic deficiency, and for about a third of children with cirrhosis of the liver and portal hypertension. Cystic fibrosis is now recognized as one of the most common chronic diseases of children and a leading cause of death in this age group. It is estimated that about 25 percent of relatives of known patients have some manifestation of the disorder and that 5 to 15 percent of the population are carriers of the recessive gene. There is reason to believe that cystic fibrosis bears a relation to at least some cases of chronic lung disease in adults.

Recent studies, many of them carried out under the direction or collaboration of the Branch Chief, have shown that there are three defects in exocrine secretion in cystic fibrosis that need further explanation: (1) An abnormality of mucus production, affording a reasonable explanation for the pulmonary, pancreatic and hepatic symptoms of the disorder. (2) An abnormally high concentration of electrolytes in eccrine sweat, mixed saliva, and tears, a finding which has had great importance in changing our concept of the disease. The "sweat test" (analysis of perspiration for sodium and chloride levels) has become the generally accepted method of diagnosis for cystic fibrosis, because of its simplicity and reliability. It also affords a useful tool for further studies of the pathogenesis and genetics of the disease. (3) An increase in the parotid secretory rate, which has no clinical consequences but much theoretical interest.

These exocrine glands, different in function and in the products they elaborate, are thus affected in different ways. The basic defect has not been uncovered as yet, but whatever its nature, it appears to be genetically transmitted. It is in order to explore this problem further with the ultimate aim of finding the etiology of the disease and therefore a better and more logical approach to treatment of the disease that the following investigations are planned:

Chemical Studies of Mucus Structure. Such studies may contribute to elucidation of the physico-chemical mechanisms involved in the higher viscosity of mucus in patients with cystic fibrosis and of their tendency to denaturation and precipitation which may be one of the major pathogenetic factors in this disorder.

Studies of Intestinal Absorption. Preliminary investigations by means of neutral fats and fatty acids labeled with I^{131} have indicated that pancreatic deficiency may not be the only factor involved in the intestinal malabsorption of cystic fibrosis. These leads are to be pursued further. Accurate balance studies of fat and nitrogen metabolism have never been performed in the recently recognized patients with normal or reduced pancreatic function. Sufficient data are not available even in patients with complete pancreatic deficiency. In addition, the effects of varying dosages of pancreatic extracts have not been carefully assessed. It is planned to carry out further studies along these lines.

Treatment of Pulmonary Involvement. The pulmonary involvement dominates the clinical picture and determines the fate of the patients with cystic fibrosis. Therapeutic trials of antibiotic drugs, of enzymes, and physical methods in the treatment of this complication are planned.

PROPOSED STUDIES IN INTESTINAL MALABSORPTION IN CHILDREN

Many of the techniques perfected for the study of cystic fibrosis can be applied to further investigation of other diseases leading to intestinal insufficiency in children. The recently developed fat absorption tests by means of fats tagged with I^{131}, the oral small intestinal biopsy, and the recognition of wheat and rye gluten as a noxious factor in the diet of many pediatric patients with malabsorption offer new tools for investigation of this very confused field.

PROPOSED INVESTIGATIONS IN GLYCOGEN-STORAGE DISEASES

Congenital and usually familial errors of carbohydrate metabolism lead to a group of disorders characterized by accumulation of glycogen in various tissues and organs leading to enlargement and dysfunction of the structure involved and, frequently, to death.

In recent times by the application of chemical techniques several different diseases have been defined, each one characterized by the absence of an enzyme necessary for completion of the carbohydrate cycle. Much further definition is necessary as to the metabolic consequences and clinical manifestations of these conditions. This represents a very dynamic field for investigation and other syndromes will undoubtedly be recognized as studies are pursued.

Clinical Endocrinology Branch

Interest in the Clinical Endocrinology Branch has continued to center around carbohydrate metabolism and general aspects of the biochemistry of the thyroid gland. The work encompasses both clinical and laboratory investigations and in many instances studies on patients follow more or less directly from results obtained in the laboratory.

CARBOHYDRATE METABOLISM

Insulin. A considerable amount of work has been done on assays of insulin and insulin inhibitors in human plasma, the mechanism of insulin resistance, and routes of insulin metabolism. As a result of modifications introduced in the rat-diaphragm assay, it has now been possible to assay human serum for insulin-like activity and a value in the normal individual of 0.01 milliunits per ml. has been found. This is somewhat lower than most figures in the literature but is in good accord with estimates made from kinetic studies.

556044—60——10

The metabolism of insulin in rat-liver perfusion experiments and by liver homogenates has been investigated. It has been shown that insulin is bound in a particulate form in the liver before it undergoes proteolysis. The binding of iodo insulin is competitively inhibited by crystalline insulin and not by ACTH, growth hormone, or other proteins. Hence, this appears to be a specific type of bond. At 3° C. it has been shown that binding of insulin to subcellular particles proceeds normally but proteolysis is markedly slowed, making this preparation particularly useful. Interestingly enough, a liver homogenate which will hydrolyze insulin is unspecifically inhibited by a variety of proteins and peptides. These data in general demonstrate that an important and early step in the metabolism of insulin consists of its binding to subcellular particles.

Work on the effect of insulin on human white-blood cells has been commenced. It has been shown that leukocytes from both normal and diabetic individuals metabolize glucose. The uptake of glucose is increased in both cases by insulin although the formation of carbon dioxide is unaffected.

Carbohydrate Metabolism. The relative importance of the hexose monophosphate shunt in the whole animal and in intact humans has been studied in collaboration with Dr. Berman of the Biomathematical Panel. A consistent mathematical analysis of the data obtained after administration of variously labeled glucose has been made. The comparison involves the rate of metabolism of C^{14} from carbon-1 labeled glucose versus carbon-6 labeled glucose. It has been shown with this technique that in the normal human about 10 percent of the glucose is metabolized via the shunt pathway.

Work has continued in collaboration with Dr. Topper on the metabolism of galactose and agents which affect it. It has been shown that ethanol inhibits the metabolism of galactose in the normal individual. In individuals with galactosemia, progesterone markedly enhanced the metabolism of galactose. Another study utilizing human leukocytes has shown marked differences between normal and galactosemic leukocytes in their ability to metabolize galactose. Preliminary data suggest that this test is sensitive enough to detect individuals with the galactosemic trait but without the disease.

THE THYROID

Proteins and Proteolysis. A study of the molecular properties of thyroglobulin has been extended. It has been shown that the denaturation of thyroglobulin as observed by its insolubility at its isoelectric point obeys first order kinetics. Between pH 7 and pH 9, this rate varies but little with pH but changes rapidly with temperature and an activation energy of 160,000 kilo calories per mole has been calculated. Above pH 11, the rate increases rapidly with increasing pH and the temperature coefficient decreases by about one half. The dissociation of thyroglobulin into subunits by alkali which was reported last year was postulated to involve a significant activation energy. This has been confirmed by the finding that an increase in temperature accelerates the rates of dissociation and changes the equilibrium to favor dissociated products. Evidence so far suggests that dissociation precedes denaturation. Somewhat surprising has been the observation that the dissociated unit (the 12S material) and the denatured molecule (8S) both appear to behave as globular proteins when examined by sedimentation and viscosity. The minimum changes in optical rotation attendant upon the production of these molecular species tend to confirm this picture.

Investigations have continued on two other species of iodoproteins found in both normal and abnormal thyroid tissue. The particulate iodoprotein (P1) which is particularly high in certain strains of transplantable thyroid tumor has been further characterized. Contrary to the results reported last year, the particulate iodoprotein is not located in nuclei. Radio-autographs have shown that nuclei in general are free of radioiodine and differential centrifugation has demonstrated that the iodine is located in two different sized particles—one about the size of mitochondria and the other the size of small microsomes.

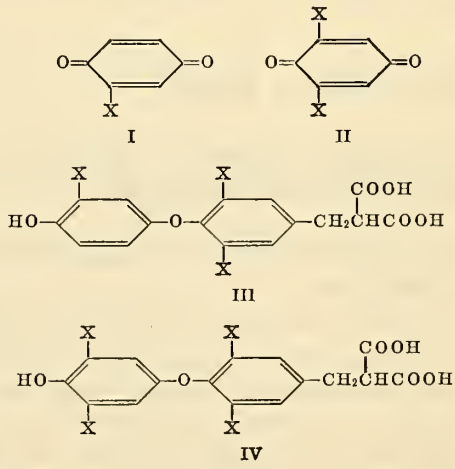
It has now been found that the particulate radioiodine can be solubilized by very brief treatment with either pepsin or trypsin. Fifteen minutes at 24° C. solubilizes from 80–90 percent of the radioiodine. Chromatography of the solu-

bilized material on diethylamine ethyl cellulose reveals a single major peak of protein and radioactivity and several smaller peaks. Amino analysis of the main peak shows a preponderance of glutamic and aspartic acids, satisfactorily accounting for an acid isoelectric point. The solubilized material when studied in the ultracentrifuge appeared to be of relatively small molecular weight and sedimented slowly. In electrophoresis, the material appeared to be homogeneous and with a mobility at pH 8.6 similar to that of thyroglobulin. The results of analysis of P1 for iodoamino acids have been reported previously as has evidence suggesting that it is synthesized independently of thyroglobulin.

Proteases in the thyroid gland have been the object of some recent study. Preliminary experiments have shown that two proteases with pH optima of 3.7 and 5.4 can be identified. They have been purified by column chromatography about 500 fold and reasonably well separated.

Work on thyroxine-binding proteins in animal sera has continued in collaboration with Dr. Blumberg of the Arthritis & Rheumatism Branch. The thyroxine-binding proteins have been studied with starch gel electrophoresis. By this technique, four proteins which bind thyroxins have been found and utilizing two-dimensional (paper-starch gel) electrophoresis, the bands seen in starch gel have been identified with the proteins separated on paper. It has been shown that prealbumin is identical with the first band on starch gel and the α-globulin thyroxine-binding protein seen in paper is identical with band 4 on starch gel. Studies using ammonium carbonate as a buffer for paper electrophoresis have shown that the capacity of the interalpha thyroxine-binding protein (TBG) has a capacity of approximately 0.2 μg thyroxine per ml. This is identical with the figure previously found when the analysis was done in barbital buffer. Prealbumin which is revealed in ammonium carbonate has been shown to have a capacity of approximately 1.5 μg thyroxine per ml. Further studies have shown that the thyroxine–binding protein which is elevated in pregnancy and depressed upon treatment with testosterone is identical with TBG as seen in ammonium carbonate and with band 4 as identified on starch gel electrophoresis. In collaboration with Dr. Beierwaltes of the University of Michigan, an extremely interesting family with congenital familial elevation of TBG has been studied. The propositus and one of the three children who are perfectly euthyroid have serum thyroxine levels from two to three times normal. They also have TBG levels from four to five times normal. These data are consistent with the previous theory from this laboratory that, in the interaction of thyroxine and TBG, it is the level of unbound thyroxine which governs the rate of metabolism and the effect of this hormone.

Iodoamino Acids of the Thyroid. An interesting reaction of monoiodotyrosine with certain heavy metals which results in the formation of a double zone on chromatography has been shown. The precise mechanism of this reaction is not yet clear. Further studies have continued on the synthesis of partially and completely hindered analogs of thyroxine. The tertiary butyl quinones I and II required in these syntheses and which have never previously been described here have been prepared. In addition, substances III and IV not previously known have been synthesized. Both of these later two were shown to be biologically active in tadpole metamorphosis.



Factors Affecting Thyroid Activity. An extremely interesting recent finding in this laboratory has been the demonstration that within 5 minutes, thyrotropic hormone accelerates the metabolism of C–1 labeled glucose. Under normal circumstances in thyroid slices a substantial portion of glucose appears to be metabolized via the hexose monophosphate shunt. TSH appears to increase the metabolism of glucose via this pathway.

This is a specific effect not manifested by a variety of other pituitary hormones and not shown by TSH on other tissues. The effect of TSH has further been studied in phospholipid synthesis in the thyroid. As has been known from previous studies, TSH in thyroid slices relatively rapidly increases the incorporation of P–32 particularly into phosphatidyl inositol. Incorporation of phosphate into phospholipids in cell-free homogenates of the thyroid has been difficult to demonstrate in more than tracer amounts. However, it seems that α-glyceryl phosphate is incorporated in a homogenate system and work is currently under way studying these reactions.

Further work on thyroid biochemistry has been concerned with the activity of isolated thyroid cells produced by trypsinization of thyroid slices. Cells prepared in this manner incorporate iodine but do not appear to synthesize thyroglobulin and the major iodoprotein made is particulate in nature. Interestingly enough serum albumin or thyroglobulin added to the cells is iodinated. Catalase completely inhibits iodination and TSH accelerates it. When tyrosine is added to the cells, it is iodinated with the formation of free MIT. The mechanism involved in these reactions is currently under study.

Metabolic Diseases Branch

CLINICAL HEMATOLOGICAL RESEARCH (STUDIES IN BLOOD DISEASES)

Biochemistry and Physiology of Initial Stages of Blood Coagulation

The combined work of many investigators has thus far indicated that at least five and possibly eight different coagulation factors interact during the initial stages of blood coagulation to produce thromboplastic activity, the activity which converts prothrombin to thrombin. The numerous factors have been identified primarily because each factor is responsible for a different congenital hemorrhagic disease (the hemophilias and hemophilioid states) yet there is remarkably little information concerning the biochemistry of thromboplastin formation or of its activity.

This section's studies have shown that two of the factors involved in thromboplastin formation—antihemophilic globulin (AHG) and Factor V—can be irreversibly inactivated *in vitro* by agents which strongly bind calcium (e.g. ethylenediamine-tetra-acetic acid (EDTA)) and that it is possible to make animals artificially deficient in these factors by exchange transfusion with blood treated with EDTA. This accomplishment has permitted for the first time an evaluation of the turnover rates of these factors in normal animals with acutely induced deficiency states. Combined studies in animals with induced deficiencies and in patients with congenital deficiencies have shown that the half-life of AHG, of Factor V, and of two other thromboplastic factors—plasma thromboplastin component (PTC) and Factor VII is in the order of 6 hours or about 10 times shorter than the half-life of other clotting factors not involved in thromboplastin activity (prothrombin and fibrinogen). These types of *in vitro* and *in vivo* studies provide information concerning the metabolism and interaction of clotting factors during the initial stages of blood coagulation which are pertinent to the development of better methods of treatment of hemophilioid conditions.

Stemming from observations (see 1958 report) that calcium is an integral part of the AHG and Factor V molecules, these NIAMD investigators have been measuring the calcium content of different plasma fractions in order to determine whether specific clotting factors can be detected and measured by means of their calcium content. Preliminary results indicate that it may be feasible to determine specific deficiencies of clotting factors by calcium determination alone and possibly to follow changes in the distribution of plasma calcium in different protein fractions during blood coagulation. These studies may prove to be helpful in relating molecular structure of these factors to their biochemical activity.

Clinical Studies of Unusual Coagulation Disorders

Combined clinical and laboratory studies of patients with unusal coagulation disorders continue to provide further understanding of diseases of hemorrhage and thrombosis. The following are examples of such studies made during the past year:

1. The mode of inheritance of Factor VII deficiency was studied by surveying the family of

a Navajo Indian patient with this disease. This necessitated a field trip to collect blood samples and to obtain a careful family protocol on the Arizona reservation. Our finding that the disease is non-sex-linked and dominant with variable penetrance is in agreement with the one other genetic study of congenital Factor VII deficiency.

2. Study of several patients who developed unusual hemorrhagic manifestations while on dicumarol drugs, although adequately controlled clinically as determined by prothrombin time values, showed that they had, in addition to the usual Factor VII and prothrombin deficiency, a deficiency of PTC and an abnormality in the thromboplastin generation test which suggested the lack of an additional factor as well. There have been conflicting reports in the literature concerning the factors which may occasionally become deficient during dicumarol therapy. The present studies indicate that PTC as well as Factor X are affected and that these factors remain depressed long after prothrombin and Factor VII return to normal after discontinuing dicumarol.

3. During the course of evaluating new chemotherapeutic agents, the cancer chemotherapy group of the NCI found that the drug 4-aminopyrazolo-pyrimidine (4–APP) produced marked prolongation of the prothrombin time in patients treated with this compound. Investigations of this abnormality by the NIAMD clinical hematology group showed that 4–APP produced an acute transient drop in prothrombin, Factor V, and Factor VII concentrations which could not be prevented by massive doses of vitamin K_1 and which could be attributed to hepatocellular damage. Apart from establishing the precise nature of the toxic effect of this drug, these observations are of research interest because 4–APP may prove to be an excellent agent for producing in laboratory animals controlled specific deficiencies of various blood-clotting factors.

Studies of Prothrombin Conversion

The transformation of prothrombin to thrombin by thromboplastin (biological activators) has been considered to be a relatively simple activation of a pro-enzyme. Because little if any change in the physical properties of prothrombin takes place, it has not been possible to conclude from physico-chemical studies that transformation of prothrombin into "biothrombin" involves formation of other prothrombin derivatives. However, kinetic studies of this transformation, which were carried out in association with the biomathematics group, have demonstrated that actually more than one prothombin derivative is formed in this step (see 1958 report). Further combined experimental and mathematical analyses of the prothrombin conversion system and of its inhibitions by proteolytic enzyme inhibitors have resulted in the following basic model for the reactions:

$$\begin{array}{ccccc} & k_1 & & k_2 & \quad k_3 \\ \text{Prothrombin} & \rightarrow & \text{Derivative} & \rightarrow & \text{Derivative} \rightarrow \text{Thrombin} \\ & \searrow k_8 & & \downarrow +\text{Inhibitor} & \\ & \text{Inert compound} & & \text{Derivative-Inhibitor} & \\ & & & \text{Complex} & \end{array}$$

The kinetic details of thrombin formation and of this unique form of competitive inhibition will be described in the report of the biomathematics group. The fact that such derivatives form accounts for a number of the puzzling attributes of prothrombin conversion in biological systems and implies that prothrombin contains several moieties which may have separate biochemical and physiologic functions.

Study of the Immunology of Blood Cell Deficiencies

"Auto-immunity" (antibodies formed in an individual which react with the individual's own tissues) has been implicated with increasing frequency as the basis of diseases involving cellular destruction. Some of the most incisive examples are hematologic diseases in which a single type of circulating blood cell is destroyed by a specific antibody which appears to react with one particular cellular antigen. Although these hematologic immune diseases are relatively well defined, there are a number of major questions which have not yet been answered: Do antibodies really develop against substances which have always been present in the individual? Do some antibodies formed against truly foreign antigens attach to cells, not by forming a specific antigen-antibody complex, but by a more fortuitous process of nonspecific adsorption on a receptive cell surface? Are certain somatic antigens essentially foreign

to antibody-forming tissues? Current studies of hematologic, auto-immune diseases have been directed at answering these and similar questions.

Following the finding by this group (see 1958 report) that the complex reactions which take place between quinidine, antibody, platelets, and compliment in quinidine thrombocytopenic purpura are the same as the reactions which take place between stibophen, antibody, red cells, and complement in stibophen hemolytic anemia, work has been continued with the rare antibody induced by stibophen in an attempt to resolve the question of antigen specificity. Drug purpura and drug hemolytic anemia are uniquely suitable for such studies because the type, the amount, and the rate of antigen-antibody complex formation can be controlled by a single factor, the concentration of drug. Using specialized radioisotopic techniques developed by this laboratory for measuring kinetics of antibody complex formation with 10^{-9} to 10^{-11} M concentrations of antibody, results so far indicate that the first step of the overall reaction is the attachment of drug to antibody. This is an important finding, for if the first step is combination of antibody with drug rather than cell with drug, the implications are that the cell-drug complex is not the antigen but that the antibody-drug complex may be nonspecifically adsorbed on cell membranes just as other non-antibody plasma proteins are adsorbed. These studies provide a clearer understanding of the basic immunoreactions which can result in cellular destruction *in vivo* and have numerous implications in a large group of diseases suspected of being due to sensitivity reactions.

Of interest, primarily in the field of immunochemistry, is the finding that stibophen-antibody-cell complexes fix only the second component of complement in a hemolytic reaction. The fixation of a single complement component (C'_2) by a hemolytic antibody is unique in immunology and continued study of this reaction promises to provide further information concerning the chemistry and significance of the different complement components.

Establishment of a New Syndrome. Studies of two patients with an unusual form of idiopathic thrombocytopenic purpura (ITP) have permitted differentiation of their disease from all other types of ITP and definition of a new syndrome. Both patients had a similar atypical onset of fulminating thrombocytopenia and were unique in that their plasma contained a complement-fixing antiplatelet antibody, a finding which has never before been described. The patients also showed a remarkable peculiarity after recovery in that the platelets which formed in both patients would not react with the previously isolated antibody of either case, in spite of the fact that all normal human platelets and the platelets of all animals tested thus far react with the antibodies. Further studies have been aimed at trying to differentiate the two major possibilities that the "recovery" platelets are coated with some substance (blocking antibody or otherwise) which prevents attachment of antibody or that a somatic mutation has altered the antigenic properties of the platelets. Of special interest and value was the finding that this life-threatening disease could be cured in a matter of hours by exchanging the patient's blood with normal blood in order to deplete the body of circulating antibody. The finding for the first time that ITP can be caused by a complement-fixing antibody is of special significance because up to now there has been no proof that ITP is an immunologic disease. The establishment of a new thrombocytopenic syndrome will help to clarify our understanding of the pathogenesis of an obscure group of diseases and will provide a rationale for further experimental approaches to effective therapy.

Study of the Normal and Abnormal Physiology of Formed Elements of the Blood

In studies of various patients with refractory anemias it has been shown that there is a direct correlation between plasma and urinary levels of erythropoietine and bone-marrow erythroid cellularity. The relationship holds even in patients whose hypercellular marrow is unable to deliver mature red cells to the circulation. Turnover studies indicate that in patients with refractory anemias the blood-cell-forming elements are being replaced at normal or accelerated rates and that the marrow responds normally to physiologic stimuli. Determination of the factor or factors which prevent normal maturation of effective oxygen-carrying blood cells will require extensive additional study.

STUDIES OF HUMAN TOTAL ENERGY METABOLISM

Change in Concept of a Basic Metabolic Phenomenon

Last year's Metabolic Chamber Study of the influence of cold environment on the metabolic effect of food (specific dynamic effect or SDE) produced such an unexpected finding that the experiments were repeated in additional young male subjects, using instrumentation with improved sensitivity. The added studies have confirmed the earlier observation that human beings differ greatly from dogs in the utilization of thermogenesis associated with eating for body-heat balance. The studies on dogs, performed by Rubner during the classic period of calorimetry investigation, had shown that food-induced thermogenesis would replace cold-induced thermogenesis and animals fed in the cold did not shiver. The current human studies, on the other hand, have shown summation of the two types of thermogenesis; the type from food did not replace that from cold. It will be necessary to modify the commonly accepted statements on cold-SDE thermogenesis interrelationships, which suggest that Rubner's work is applicable to all homeothermic species, and indicate inter-species differences.

Delineation With Fidelity of Moment-to-Moment Metabolic Changes Reveals Characteristic Features of Phenomena Obscured by Older Methods

The unique capacity of the metabolic chamber's instrumentation for tracing the patterns of fundamental physiological phenomena has been demonstrated in this study of the influence of cold on SDE. Not merely the degree of energy expenditure but the variegated changing form can be outlined by the chamber's system of continuous expired gas sampling for minimal changes in oxygen and carbon dioxide concentration, in conjunction with continuous recording of other physiological data such as body temperature at various sites, heart rate, etc. Various methods applied in the past to human-energy studies have all been based on interval sampling of expired air which totally obscures moment-to-moment metabolic changes. Although cyclic variation in oxygen consumption associated with shivering has been suggested in previous interval sampling studies, delineation of metabolic changes with fidelity which is provided by the chamber continuous-sampling procedure has made possible the following observations: (1) determination of the duration of and interval between various bursts of energy expenditure associated with shivering, (2) recognition of a sustained underlying increase in metabolism in the cold distinct from the periodic peaks associated with gross body shivering, (3) the finding of marked interindividual differences in the metabolic response to cold, both in lag before initiation and in magnitude attained, (4) definition of the duration and total amount of metabolic change associated with ingestion of food (SDE) and (5) accurate separation of the metabolic responses due to SDE and to cold, and recognition of an altered SDE metabolic pattern in the cold as compared with its form in a comfortable environment.

Instrumentation Research

Initiated for the purpose of altering industrial, continuous-flow-gas analyzers specifically for metabolic-chamber research (50-fold increase in sensitivity desired), efforts to improve the stability and sensitivity of the oxygen analyzer can now be considered an instrumentation-research accomplishment. Aided by advice from the NHI Laboratory of Technical Development, the oxygen instrument has been refined to the point where 0.02 percent changes in oxygen concentration can be accurately detected in air streams of 100 liters per minute. The carbon-dioxide analyzer was modified some time ago and has performed satisfactorily over the past 2 years, but steps are under way to improve this cell even further. The staff of the chamber has also developed a data-handling system to deal with the voluminous data generated on the strip-chart recorders and to facilitate calculations; paper tape from the system will be fed to the NIH-IBM computer facility. This system has just been installed and is currently under test.

MINERAL METABOLISM STUDIES

Expansion of the Concept of Altered Bone Metabolism in Osteoporosis: Importance of Nutritional Factors

Current isotopic and metabolic-balance studies of the patho-physiological processes of mineral

metabolism in various bone diseases have been emphasizing efforts to determine the pathogenetic factors underlying post-menopausal and senile osteoporosis. These investigations have progressed to the point where it seems evident that long-accepted concepts are inadequate and a new approach is needed.

The basis for the current studies is as follows: Gonadal-hormonal therapy of senile and post-menopausal osteoporosis, based on the concept of inadequate protein matrix formation, has failed to provide a fully satisfactory answer to a difficult and increasingly important clinical problem. Remineralization has virtually never been seen by X-ray following androgen-estrogen therapy. Recently, the possibility has been raised that the mineral—calcium—has some direct pathogenetic relationship to osteoporosis, a condition in which bones manifestly contain less calcium than normal. Dietary histories in the modest number of patients with osteoporosis studied by this Branch have indicated habitually low calcium intakes in the diet. In two large patient series recently reported dietary histories have also indicated significantly lower levels of calcium intake in osteoporotic patients than in non-osteoporotic individuals.

First indication that the accepted idea of diminished bone formation as the basis for osteoporosis might not be correct came from radioisotopic (calcium-45) studies in our laboratory to determine rates of calcium deposition in new bone formation. These have revealed apparently normal rates in seven patients with osteoporosis, raising the possibility that an important mode of demineralization in this disease is by increased resorption of bone rather than by diminished bone formation; this unexpected finding has been confirmed by a foreign investigator. Metabolic balance studies in five patients with osteoporosis indicate a positive relationship between calcium intake and calcium balance through a wide range of intakes (150 to 2,400 mg./day), a relationship not previously demonstrated. A widely held concern that high calcium intakes would lead to more frequent development of renal stones has not been supported by the metabolic data; urinary calcium levels increased only minimally as the intake was raised. These studies have not only indicated significant calcium storage by increasing calcium intake; they have also suggested that the calcium requirement may vary widely from one individual to another and hint that in patients who develop osteoporosis the requirement may either be higher than for others or that it may be higher than their customary intake over many years. Very recent balance studies have shown a relative resistance in certain patients, as shown by the very high calcium intake levels required in them to obtain positive balance. This suggests that in some patients there is a gastro-intestinal absorptive defect for calcium and has led to the planning of collaborative work with the NIAMD gastroenterology group to detect the possible presence of subclinical and/or selective mineral malabsorption in these patients.

Review of the data acquired to date has led to the following formulation: Bearing in mind the idea that the skeleton is a great storehouse of calcium to be called on when calcium is needed for homeostatic control of the miscible calcium pool and plasma levels, the concept is suggested that in osteoporosis the calcium stores of the skeleton may have been gradually drained to compensate for losses from the pool by mandatory excretion and by bone formation as trabeculae are continually remodeled. The implication is raised that the degree to which the skeletal stores have had to be called upon has been in proportion to relative insufficiency of calcium from dietary sources. The importance of endocrine hormonal factors is well recognized in the pathogenesis of osteoporosis. These studies direct attention to dietary mineral factors, thus expanding the concept of altered bone metabolism in this disease toward recognition of a dual set of influences, hormonal and nutritional.

NATIONAL INSTITUTE OF MENTAL HEALTH

AND

NATIONAL INSTITUTE OF NEUROLOGICAL DISEASES AND BLINDNESS

INTRODUCTION*

IDEALS PASS INTO GREAT HISTORIC FORCES BY EMBODYING THEMSELVES IN INSTITUTIONS
(Hastings Rashdall, 1895)

The safeguards of civilization and the purposes of civilized man are intimately dependent upon ideals that have become embodied in institutions. Parliamentary rule, trial by jury, English common law, the Federal Constitution, and many agencies of our Government (including the Department of Health, Education, and Welfare, and the National Institutes of Health) are bold and effective institutional expressions of ideals.

Behavior is the outward expression of internal values. It follows that fostering socially constructive and adaptive behavior among individuals, institutions, and nations, requires the discovery, communication, and internalization of appropriate values. The magnitude of this task seems unimaginable. Perhaps this task looms larger than it really should because of our extensive ignorance and relative neglect of the brain-mind as an instrument for social integration. Moreover, it is unlikely that we can attain a difficult objective without trying; and it is unlikely that we will effectively pursue what is conceived as unattainable.

As I have written in a previous Annual Report (1957): "At the root of the matter are as yet unsolved problems relating to the perception of actions and of shibboleths, the translation of ideas, the momentum of traditional concepts, the adhesive behavior of groups, the communication of ideals and goals. Many scientists have confidence that these problems can be solved, given time and effort. Our country is presently buying time; we can undoubtedly improve our effort. . . . There may be short cuts, but few are evident. We have to learn how signals enter the nervous system, how they are distorted by concurrent and antecedent events, how they relate to mechanisms of reward and punishment and emotional expression, how learning occurs, and what are the limitations of our mnemonic and behavioral response systems. These mechanisms have their anatomical, physiological, chemical, psychological and sociological manifestations. What more interesting or important labor than to be involved in the unravelling of these mysteries?"

For 200 years western civilization has been preoccupied with the development and distribution of energy sources and property. Is it conceivable that we could now dedicate ourselves more conspicuously to fundamental studies concerning behavior and particularly social behavior?

As in previous Annual Reports, the Laboratory Chiefs have provided comprehensive statements of research progress throughout the year. I have attempted here to continue as in the two previous Annual Reports an exploration of more general scientific issues. These tend to be overlooked in the immediacy and seeming urgency of our daily undertakings. Yet I believe they are truly pertinent to our ultimate best achievement.

> Raising the level of aspiration and action of society requires the widespread internalization of ideals. Clearly the formulation and dissemination of ideals constitute an indispensable social function. Ideals can shape social behavior through their incorporation as institutional traditions or other kinds of social convention: in this way they can gain the force of "unwritten law;" they can become more powerful than any statutory regulation. Most social behavior is governed in this way, through the popularization of ideals

*Written by Robert B. Livingston, M.D.

which then operate without need of law or administration. Most of our freedom of action and security depends upon conventions favoring altruism, faith and mutual trust. Yet, even widespread and powerful supports for constructive social comportment may be too idly held and fall into desuetude: ideals tend to "run downhill" and for this reason they need to be continuously sought out on a higher level and reinforced.

Ideals are not only important historic forces but they evolve historically. Hard-won human values are safeguarded and extended mainly through the evolution of ideals. For this to occur, ideals need to evolve at a rate that will match the changing forces of circumstance. *Ours is a period when ideals do not seem able to keep pace with social, economic, political, and scientific changes which appear beyond our understanding or control. It may be that every period of history has this aspect, yet ours is fortunately the only one we have to face.*

Although in "sophisticated" conversation we are inclined to deprecate altruism, faith, and mutual trust as "unreal" or "impractical," we actually live by these ideals. Mankind could not have lasted to this risky moment without having developed steadfast biological foundations for altruism, faith, and mutual trust. These functions are built into our chassis, so to speak. They are vital mechanisms which have earned for us our biological and social freedom. Coupled with awareness, such mechanisms can impel further achievements to enlarge human dignity and freedom; it seems necessary only to encourage a greater awareness of our opportunities. Improvement will be measured in "little pieces of the striving" in any single act directed toward the realization of higher ideals.

The purpose of this essay is to discuss certain ideals relating to the pursuit of science, to the interface between science and society, and to the contributions which science can make toward the development of worthier social purposes and means. It is my intention to show that:

1. The selection of worthier values in a democratic society depends upon ideals conceived by individuals, especially by individuals possessing training and experience in the dispassionate exercise of evaluative skills. In recent years this essential process of democracy has been eroded and given away.
2. In science there has been an unfortunate rejection of the importance of evaluative judgments, and of the need for scientists to contribute in a professionally broad and responsible way to the improvement of social purposes and means. Only through an effective and disinterested assumption of this responsibility by scientists will science itself escape from being a toy of technology, pitted against all manner of competitive special interests throughout technology.
3. From several directions science is revealing an ethic based upon scientific rather than religious or philosophical grounds. Through such findings, science may be enabled to provide an increasing power and guidance for "life, liberty, and the pursuit of happiness."
4. Science itself is a valued human enterprise which has much to offer society, both apart and beyond utility.
5. What seems most urgently required is first, a more successful interface between science and society, and second, a greater sense of professional responsibility and probity among scientists. This latter calls for the resumption of an idealistic spirit of craftsmanship, something akin perhaps, to the medieval notion of guild.

All this requires individual internal actions as well as administrative changes relating to conditions of scientific enterprise. Neither the individual internal actions nor the administrative changes will suffice alone. They are attainable, together, as a natural outgrowth of wider recognition and exercise of man's capacity for altruism, faith, and mutual trust.

Sources of Professional Responsibility

In a democratic community, where can responsibility be placed for formulating and improving ideals and purposes of society and its institutions? Does responsibility lie in the White House? In the Cabinet? In the Congress? In the Supreme Court? In the communications industry? In the marketplace? Among the citizenry at large?

Improved ways of handling society's problems need to be conceived and made available broadly

throughout society. Through the action of political and social leaders, evolving ideals will influence the development and improvement of society's purposes and means. Among the most important means available are institutions which themselves progress through becoming more closely approximated to the noble ideals for which they represent an embodiment. It is chiefly by this leavening of ideals that society can be improved, and chiefly by means of institutions that ideals can become "great historic forces." Each of us shares responsibility for choice of both ends and means in our society. Desirably, this responsibility is borne through the exercise of individual interpretations, formulated as conscientiously and rationally as possible. This is essential, by definition, in a democratic community. Individual default of conscience in this public responsibility is detrimental and morally reprehensible.

In addition to such broad-based individual responsibility there is also *professional responsibility*, borne variously by lawyers, physicians, teachers, scientists, civil servants, and others; professional responsibility which is far heavier than that borne by the citizens-at-large. This additional responsibility grows out of the professional skills and experience of the individual. Thus, as scientists, we bear a special social responsibility because of our first-hand knowledge of the nature and potentialities of science. This makes us responsible not only for the excellence of investigative work for which we are more or less directly responsible, but also for contributions of professional insight and effort toward achieving a worthier destiny for our social and institutional environments.

> Our responsibility in this more inclusive professional sense derives quite naturally from the facts: (1) that our own professional destiny is intimately bound up with the achievements of our immediate professional community, (2) that this group in turn has the capacity to contribute more effectively to the achievements of our society, and (3) that our responsibility for a share in making this go well in its entirety is neglected at our own peril, individually, institutionally and as a society.

Somehow organizational and societal bigness has induced a psychological dwarfing of the conception of the only proper role of the individual in a democracy. The town-meeting ideal has been lost to some degree, and individual responsibility, especially individual professional responsibility, has been eroded and given away. Responsibility has been eroded insofar as individuals and institutions reject or fail to allow for it. Rejection is of course encouraged by those whose ambition favors their own limited interest. Failure to allow for responsibility has two principal origins: one, a lack of faith in the willingness or capacity of individuals to bear such responsibility; the other, an unwillingness to cope with confusions that occur with the widespread exercise of responsibility. Responsibility has been given away insofar as those by whom it should be exercised have not conceived the need for it or because they prefer responsibility that is limted strictly to their own immediate work, or because they accept a view of themselves as helpless cogs, too ineffectual or too inadequately informed to be able to exert an effect on the massive and supposedly inflexible institutions of society.

To the extent that individual responsibility and especially individual professional responsibility is precluded or avoided, our institutions and society are made to depend on undemocratic procedures. This is perhaps not disadvantageous in itself in the short view, and under broadly responsible and objective leadership, but it inevitably entails two further substantial losses, both of which bear importantly upon our ultimate institutional and social accomplishment. First, there is a loss of the many conscientious and responsible intellectual contributions which otherwise could have been made toward a more desirable destiny. Second, there is a subtler but more influential loss of group identification and motivation which otherwise derives from the sharing of social responsibility. Finally, as John Stuart Mill said, an institution or society "which dwarfs its men, in order that they may be more docile instruments in its hands even for beneficial purposes, will find that with small men no great thing can really be accomplished."

Professional Evaluations Indispensable to Science and Society

It is often supposed that scientists deal exclusively with facts, and that they eliminate from

their deliberations considerations concerning values. It is supposed that facts can be assembled and systematized in relation to other facts, whereupon out of an examination of such relations will emerge general "laws of nature." This is a mistaken view. Actions stemming from this false supposition may be devastating. Scientists have been blocked or dismissed from responsible positions, in part at least on the assumption that their reasoning can and should exist in isolation from considerations of value. This primitive notion that scientists should be professionally obligated to reject value discriminations, or that they are ill-fitted by their scientific experience to make value discriminations, represents a completely inadequate conception of the scope and method of science. I believe that the practice of science requires certain value analyses that are indispensable to science and society. These include (1) evaluations intrinsic to the scientific method; (2) evaluations covering professional excellence in science, and (3) evaluations relating science to society.

Evaluations in Science. It is true that in the factual stage of inquiry scientists try to characterize their observations in a form as free as possible from personal bias and opinion; this is an ideal toward which all scientists strive. But facts, even when ideally established, are only bricks from which the structure of science is developed. By themselves, facts tend to be uninteresting. The really significant features of science are established from facts which are given meaning, i.e., value, through conceptual thinking.

From start to finish of any scientific problem, scientists are engaged in value discriminations, in committing themselves to choices which severely delimit whatever may be the ultimate value of their scientific accomplishment. The selection of a problem to study, choice of methods, development of conceptual and technical operational definitions, attempts to isolate facts from artifacts and from underlying assumptions, selection of those facts presumed to be objectively meaningful, interpretation of the factual data (which are inevitably compounded of theoretical interpretations and sense perceptions), and the representation of these data and interpretations for the purposes of meaningful communication—each step in scientific accomplishment requires value discriminations of a high order. A differential capacity for handling these difficult discriminations is the principal distinction between truly great and lesser scientists.

It can readily be conceded that only scientists are professionally qualified to make judgments concerning values that are intrinsic to science. Yet it is seldom remembered that the profession of science *demands a continuing exercise of value discriminations* and *provides disciplined experience in making such evaluations.*

Evaluations of Scientists. All of science and technology depends upon a small but indispensable population of creative scientists. Such men are professionally disciplined to deal with peculiar instrumental devices, with abstract thought at the limits of conception, and with certain general principles which guide their intellectual progression. What a scientist sees with his instruments and what he interprets from these revelations is by no means obvious. As I have written elsewhere, "A more adequate understanding of nature cannot be achieved in the abstract; it must be brought about through the consideration of materials with which the scientist is already familiar. Even the most gifted and energetic person must have achieved a certain mastery in the field of his pretended accomplishments. He must have a keen sense of what needs to be done to solve a given problem and a sufficient skill to do that. . . . Important scientific achievements thus seem to depend upon the fruitful combination of a group of essentially positive factors; some of these relate to the competence, self-discipline, and nimble imaginativeness of the scientist himself, and others concern his surroundings. Research in laboratories of the Federal Government will surely progress in the sense of advancing the frontier. And the rate of advancement may be speeded up somewhat by administrative hustling or by providing additional money or personnel in a given field. But saltatory advancement of concepts—the kinds of change in point-of-view that may alter the entire character and direction of scientific pursuit, the kinds of advancement that may cut short years of striving—these are not likely to occur except where circumstances are especially favorable for creativity. *In the long run, the reputation and credit*

of any laboratory will depend upon a few advances of this sort far more than upon the extension of studies that now seem entirely familiar."

The creation of worthier new concepts in science is impossible without intellectual nonconformity. What is considered to be "logical reasoning" evolves as a delayed consequence of scientific achievement; thus the steps in the formation of a new concept not only seem alien and eccentric and in conflict with common sense, but they frequently seem "illogical." It must be remembered that any concept is a "freely chosen convention" and nothing more: it is yielded through intuitive and nonlogical mental processes which are not under any satisfactory degree of voluntary control. Abstract ideas involved in the creation of a new concept need to be "played with" imaginatively, often over a period of years, before a truly new level of understanding is achieved. It is only after a new concept has been clearly differentiated that the logical processes of science and the disciplined testing against sense experience can be pursued.

The history of the growth of scientific concepts makes obvious a primary requirement in scientists of a high-level capacity for conceptual thinking coupled with a capacity not to hold any concept too dearly. Widespread acceptance of a new level of understanding in science is achieved through the examination of evidence that is made as free as possible from personal appeal, coercion, or "fashion" of thinking, and without recourse to authority external to the body of science. As a system of thought science is practically unique in not being imposed by coercion or persuasion, and in not being destroyed when found internally inconsistent; paradoxically, science becomes stronger and more coherent as its limited views are made manifest. The search for a Scientific Truth (which can never be realized) becomes ever more powerful as error is discovered in lesser "scientific truths."

Science, in contrast with many other callings, naturally creates a zeal for integrity; a lie in science cannot persist, for it will be found out through the continuing activities of science. Scientists are professionally indoctrinated to the practice of probity. They fail in this regard only insofar as they are persuaded to abandon their professional role in society.

Scientists alone are adequately qualified to evaluate a fellow scientist and his scientific performance. Since scientific accomplishments of high quality are the *sine qua non* for the existence of a scientific establishment, a code for the selection and promotion of scientists must be based upon scientific considerations applied by scientists knowledgeable concerning both the individual and his field of learning.

The profession of scientists is uniquely qualified to provide those discriminations which will foster genuine scientific excellence. They alone can prevent freedom, which creativity requires, from being used as shelter for inefficiency, superficiality or uncritical partisanship. Scientists are well aware that if their own profession does not provide these discriminative evaluations, they will have to be made by others who may lack the necessary qualifications. If this comes about (through casualness or default of the scientists concerned, or by direction of persons unfamiliar with the values and conditions essential to professional excellence), the resulting actions are certain to breed suspicion and controversy that will be deeply injurious to the internal order and to the external standing of the institution.

Evaluations of Science in Relation to Society. Science consists of a collection of information, a body of theory, and a methodology. All the disciplines of science share a dedication to certain general principles of inquiry and evidence: this forms the only basis for the unity of science. Science is one of the few creative, truly progressive, intellectual activities. Theoretical notions tested and found valid are of use in the pursuit of further understanding. It is this progressiveness which gives science much of its power. A further source of power derives from the scientist's internal discipline to seek simpler and more general expressions which can account for the vast schemes of nature.

Since World War I, science has become dominant in generating and directing the development of technology. In earlier years, the relations between science and empirical discovery were sporadic, with practice influencing theory more often than the reverse. Since World War II, science

and technology have been more and more lumped together. There is now developing a widespread concern within the Executive and Legislative Branches of the Government regarding the extent of tolerance to be allowed for the "tyranny" of which science and technology seem to be capable. The question of how, i.e., *by whom* and *by what criteria* science is to be evaluated, is acute as well as important.

> Several serious problems need to be addressed: How does science need to be distinguished from technology in terms of both its planning and realization? How can the intellectual content and power of the educational and research activities of science be strengthened? (How can the mutual interdependence of science and the humanities be more fully recognized and made effectual?) What branches of science need encouragement for the immediate and more distant scientific and technological advantages of society? (How can scientists best participate in the social value determinations this requires?) How can program developments in science be generated and encouraged more in accordance with professional scientific rather than simply political and economic conceptions of need and of research potential? In a science mart of limited resources, how can the tendency for competitive over-justification, for "tyrannizing with facts" by scientists be discouraged? (How can such forthright and natural traits as professional congruity and candidness be given greater encouragement?)

Few of the answers recommended for these problems have been put to any test. The problems themselves are not diminishing; they are getting worse as public demands and needs are expanding, and as competition beyond the control of our society is exerting an avalanche of pressures on our technology. These problems have been addressed in different ways and with perhaps greater degrees of success in some other democratic communities. The United States clearly has no monopoly on creativity in science and no surety for the best ways to utilize such talent in improving, safeguarding, and attaining a worthier destiny for mankind. We need speedily to bring these issues to more objective analysis and to work out ways for improving the interdependent working relations between science and society. To this end, there is an imperative need to stop eroding and giving away professional responsibility which belongs to scientists.

Power and wealth are actively sought; technology yields power and wealth; technology is dependent upon science. Yet because of vast discrepancies in their relative costs, there is danger that science, whenever lumped together with technology, will be conceived as riding on the coattails of technology rather than the other way around.

> We need to make a fresh analysis of the role of Governmental institutions bearing directly or indirectly on science: what sufficed for a realization of ideals for democratic Government in the late 18th and 19th centuries, when science was a negligible factor in the health, welfare and defense of society, may require revision now when science has become so prominent and indispensable. It is mandatory that any institutional revisions be performed with conscious deliberation and wisdom instead of crisis to crisis improvisation. Our Government will enjoy wisdom in its councils to the degree that it can understand and foster wisdom and can distinguish this from the cacophony of limited-interest appeals. *Decision making, in areas relating science to society, should be as carefully objectified and deliberated as decisions and interpretations in the field of law by our Supreme Court.*

It is evident that scientists are needed not only for the evaluations of science, of scientists, and of scientific performance, but also, for the difficult judgmental evaluations relating science to society. They are needed as full and responsible participants throughout the decision making processes involved in the conception and realization of society's goals. Although all of this seems patently true, when it comes to practice there are obstacles. Some of these arise out of the tendency to deprecate the scientists' training and capacity for making evaluative judgments. A second obstacle results from the expectation that scientific values are to be measured according to marketplace values; that what is scientifically "good" or "bad" is determined, as are so many other social values, by some kind of scale of popularity, through personal suasion, coercion, or appeal to external au-

thority. Some scientists may be persuaded into a degree of conformity to this expectation, especially when it is held rather uniformly by those controlling the supports of science. Responding to such an expectation, scientists try to express the goals of their scientific endeavors entirely in terms of technological and marketplace considerations. This in itself is a principal obstacle to the cultivation of high quality science. It is a barrier to understanding the nature and scope of science by a wider public.

The degree to which scientists make use of "a tyranny of facts" or other limited-interest techniques which violate professional probity is a measure of their failure to qualify properly for bearing their valid professional responsibilities. *It is not so much that the facts and concepts of science need translating for the public as that the purposes and systems of value of science need translating.*

The Evolution of Human Values

Evolution, as popularly understood, emphasizes conflict as the principal fulcrum around which evolutionary progress takes place. It deemphasizes altruism and cooperation as contributing importantly to evolution. The popular derivations from the teachings of Darwin and, indeed, of Marx and Freud as well, give us only half of our nature. Conflict cannot be put aside altogether, but as an instrument for evolution, conflict alone is like the odd half of a pair of scissors. An emphasis on conflict as the basis for individual or collective evolution reveals only half our opportunity, half our capability, and half our responsibility.

How this notion of the significance of conflict can have become so widely accepted in the face of commonplace evidence to the contrary, how it can have magnified the acceptance of conflict as a way to the solution of problems, and how it can have secured the social acceptance of conflict to the degree that it has is beyond my understanding. It is not that Darwin, Marx, or Freud accomplished this directly, because popular conceptions embrace only fragmentary parts of their contributions. *It has been simpler, and hence more popular, to believe that evolution proceeds predominantly through success or failure in conflict situations, and that an individual succeeds or fails according to his natural endowments, in which his potentialities for conflict are of paramount importance; that great social forces stem from conflict in which power, aggressiveness, and wealth are principal determinants; that the individual is merely a moving atomy of conflicts, each being the victim of instinctual drives, chiefly of a gross and disagreeable nature.*

A more valid thesis, I believe, is that altruism, faith, and mutual trust are built into our behavior just as surely as are the mechanisms for aggression and conflict. Each of these systems represents a vital force which has developed progressively throughout phylogeny. Each has played a central role in determination of freedom as well as survival and creative evolution of biological organizations.

> Plants, which must remain relatively fixed in relation to their environments, are capable of living off fairly homogeneously distributed raw chemicals. Biological discriminations upon which plants depend are concerned with relatively elementary chemical and physical factor. Animals, on the other hand, depend upon partly organized chemical substances which are heterogeneously distributed and which they must actively seek out. Animals must be able to discriminate objects in their environment which may provide suitable energy sources, and to secure them for their own and their progeny's use. Animals are characteristically mobile, built for action. The evolving nervous system has from the beginning been a system that is both selective and directive. Higher animals have more complex systems for discrimination and action, and a greater capacity to learn new discriminations and new actions.
>
> The ability to discriminate is intimately related to what we call appetite, feeling and emotion, and also with mechanisms concerned in the direction of action. Behavior is generated by motivations which in turn are shaped by biological systems of value, whether these are consciously manifest or not. Built into such differentiating-action-generating systems are vitally essential mechanisms for value discriminations affecting preservation of individuals and the species. *Survival*

> *and evolution could not have gone very far in the creation of complex forms of life without having biological foundations for altruism, faith and mutual trust, in addition to mechanisms for combat.* Cooperation and faith in some degree are absolutely essential for the reproduction and survival of most offspring, and are admittedly very far-reaching in yielding internal biological satisfactions. It must be remembered that neither aggression nor altruism requires deliberate conscious participation, even in those organisms capable of consciousness.

We act, and live, by faith: faith in ourselves; faith in the consistency of nature; faith in each other. Every perception and every overt act is based on faith. Action follows the state of the nervous system whether it be so-called "spontaneous" action, reflex action or action of the "will." The state of the nervous system (the brain-mind) is variously called an image, an idea, a feeling, an emotion, or a judgment which in turn is based upon comparative evaluations of various images, ideas, feelings, etc., built up and stored during previous experiences of the species and of the individual. Knowledge of the outside world (and of ourselves) largely evolves out of a cumulative experience which begins with our own "spontaneous" actions. Deliberated decisions—even decisions based on strong feelings of "will"—are largely founded on systems of experiential consistency and the projected faith derived from that.

To be determined by one's own nature is to be free. An educated man is said to be one able to foresee the consequences of his actions in the widest possible totality of their relationships (Kermit Eby, 1951). A wise man is most fully self-aware. He is sensitively empathic regarding the possible consequences of his actions considered in the widest context and in the longest view. A wise and resolute person has a store of stable and worthy ends, patience, and a style for engagement in action. Consciousness (and the contributions of education and wisdom to a conscious and resolute person) provides the fullest opportunity for the development and exercise of capacities for altruism, faith and mutual trust, which nonetheless remain just as much natural and vital mechanisms as are the most primitive acts of cooperation observed in lower animals.

Freedom to act purposefully with intelligent foresight of the probable consequences of action stands as moral freedom when the social consequences of the action are taken into account. These actions are also both natural and vital. They relate importantly to survival and embody the realization of vital satisfactions. "*Man's capacity for intelligently directed self-development confers upon him the ability to determine the pattern of his culture and so to shape the course of human evolution in directions of his own choice. This ability, which no other animals have, is man's most distinctive characteristic, and it is perhaps the most significant fact known to science*" (C. Judson Herrick, 1956).

> The brain-mind is an evolutionary tool like teeth and claws, but we can expect from it a much more creative performance. The brain-mind of man is highly developed with respect to its capacity for discrimination among objects of the environment in favor of suitable energy sources. The present uneven distribution of wealth and power throughout the world is largely due to the purposeful use of the brain-mind according to the cumulative methodology of science. Yet garnering and exploiting energy sources is not the greatest nor loftiest purpose of mankind. With all that the brain-mind has accomplished, it is still a very incompletely exploited instrument for contributing to the extension of freedom and to the further encouragement of man's natural capacities for altruism, faith and mutual trust.

For many years psychologists, sociologists, and social anthropologists have studied human capabilities and limitations in this regard. Recently Dr. Marion Yarrow and Dr. John Campbell have shown that children brought into newly formed children's groups display differences in initial relations which are predictive of their later social effectiveness within that group. Children who on first acquaintance find other children attractive come to be the best liked and most effective members of the group in terms of acceptance and influence. Children who are initially aggressive or angry in their reactions become typed in this characterization and are impaired in their perception

of others as well as being less effective in their later interactions within the group. More recently has appeared a growing insight into what may be the internal biological mechanisms underlying altruism, faith, and mutual trust. The work of Dr. Paul MacLean is particularly significant in this regard. He has localized two separate brain regions, one of which concerns behaviors leading to preservation of the individual and the other of which concerns behaviors leading to preservation of the species. Each of these regions incorporates mechanisms both for cooperation and for combat. In actuality, several disciplines basic to neurology and psychiatry are contributing to a system of ethics that can be developed without dogma and entirely on scientific grounds.

Scientific contributions relating to consciousness, perception, appetite, emotion, learning, memory, motivation, value discrimination, decision making, social awareness and responsiveness, and will are all pertinent to considerations of what we have to deal with in human nature. They are profoundly important in relation (as above) to "the perception of actions and of shibboleth, the translation of ideas, the momentum of traditional concepts, the adhesive behavior of groups, the communication of ideals and goals." At the very least, contemporary research indicates that human nature may be considered from a positive as well as from the more traditionally negative point of view. Such contributions, being cumulative, will provide a continuing improvement of our understanding of the potentialities of human behavior. These fields of science, although late to mature, portend to contribute more importantly than any other intellectual enterprise of man to his ultimate fulfillment in "life, liberty and the pursuit of happiness."

Science as a Human Value

Science itself has evolved as a valued human enterprise. Emphasis has been greatest in this country, and especially in recent times, upon science as valuable from a predominantly utilitarian point of view. Science has proven so useful in finding and exploiting suitable energy sources, that its contributions through technology to the standard of living is taken by many to be the chief social value of science. Yet those who have heard the "beep" of an earth satellite or have seen one crossing the sky, have experienced an inevitable cultural thrill through their own perceptual confirmation that man can do such a thing. This response carries with it the further recognition that the world will never be the same. Science in this way fulfills a part of man's innate curiosity. Almost all of astronomy and astrophysics, most of the earth sciences, and biomedical sciences in particular, contribute to fulfilling the innate desire of man to know, to understand, to comprehend the universe and himself. Scientific discovery ultimately has a cultural impact upon the philosophy of thought and upon the vitality of ideas. Science stresses that the individual, community, and the universe itself, is always in *a process of becoming*, and that none of this *transaction* can be made to stand still. There is a certain anti-inertial force provided society through scientific enterprise. Science debunks authority; it emphasizes the intrinsically creative aspect of man's own life and his capacity to create increasing freedom within the total domain of organic and inorganic evolution. In many ways, science provides useful implements for cultural development.

Science is not a body of dogma: It is a way of life. The requirements of the creative process impose self-discipline and intellectual integrity. In the pursuit of science only that which can be communicated and sustained by others is retained and dignified as part of the organized knowledge of science. The process of creating new concepts requires maximum freedom. Progressively less freedom is needed for the exploitation of available concepts, hence, for development and technology. The need for freedom, freedom in thinking, freedom in discussion, freedom to demonstrate the true nature of man and his society, freedom of publication, all required by science, is a further contribution to the strength of freedom throughout society. Acceptance of the spirit and methodology of science by society's leaders assists society in adapting to new situations without the kinds of fear which have attended drastic changes in the past.

Culture is affected by the challenge of the adventure of science, of the frontiers to be surpassed, of the beckoning effect of the unknown. Science provides concepts of enormous intellectual satisfaction, enrichment and entertainment. Science contributes to the discipline of a culti-

vated society and to the inspiration of its youth. Thorstein Veblen wrote in 1906, "In myth-making, folklore, and occult symbolism many of the lower barbarians have achieved things beyond what the latter-day priests and poets know how to propose. In political finesse, as well as in unreasoning, brute loyalty, more than one of the ancient peoples gives evidence of a capacity to which no modern civilized nation may aspire. To modern civilized men, especially in their intervals of sober reflection, all these things that distinguish the barbarian civilizations seem of dubious value—futile in comparison with the achievements of science. They dwindle in men's esteem as time passes. This is the one secure holding-ground of latter-day convictions, that 'the increase and diffusion of knowledge among men' is indefeasibly right and good. When seen in such perspective as will clear it of the trivial perplexities of work day life, this proposition is not questioned within the horizon of Western culture, and no other cultural ideal holds a similar unquestioned place in the convictions of civilized mankind."

Toward an Ideal Destiny

Cultural Differences Affecting Science in Relation to Society. A research enterprise that depends upon the patronage of a democratic society depends upon a relatively broad understanding throughout that society of the values of science and of the conditions under which science can flourish or will languish. An obstacle to such understanding is that society is made up of many different cultural groups, each of which has its own set of values and conception of the conditions necessary to its own kind of enterprise. Examples of such groups are schoolboys, preachers, artists, salesmen, teachers, thieves, sailors, playwrights, physicians, policemen, pilots, businessmen, factory workers, television sponsors, miners, research scientists, bankers, and soldiers. In general, there exist only limited cross-group familiarities, although schoolboys, as a group, study teachers, and vice versa; thieves study policemen, etc. An individual in one group is likely to judge the actions of members of any other group according to his own code: indeed, he may know no other. The simplest translations are between groups whose values, actions, and conditions of work are known to each other through continuing interaction. Yet difficult translations may be required between groups whose superficial familiarity with each other may blind them to fundamental underlying differences.

> The predominant system of values and conception of working conditions in our society (at this time) relates to the marketplace. By and large, the leaders of our society understand the values and conditions relating to successful business and political enterprise. This is the code which is also most often publicly interpreted by the communications industry. This code is therefore the commonplace and primary cultural reference by which actions are interpreted. For these reasons it is quite understandable, although regrettable, that a research enterprise is likely to be evaluated according to marketplace standards.
>
> A further general feature of the action interface among different cultural groups is that *the predominant group not only evaluates the actions of other groups in the light of its own system of values, but that it actively exerts pressures to compel conformity of action in accordance with that same system.* Anything else would seem "alien" and "illogical," if not "improper," according to the code of the predominant group. This tendency is entirely natural, and is equally unreasoned. As an example: pressures are exerted, directly and indirectly, by the predominant group of righthanded persons to disguise or eliminate lefthandedness; at the very least, to require lefthanded persons to adapt themselves to the way in which hands are to be shaken, tables set, doors opened, faucets turned, writing desks arranged, etc.

These two general facts of cultural interaction, compelling judgment and conformity to a foreign code, have a powerful influence upon the action interface existing between scientists engaged in research and the patrons of science. Business and politics are fields of competition, as is science. Yet, the basis for the competition is fundamentally different. Business and politics are mainly for the purposes of social service, social power and social control. To a large degree the marketplace and public opinion determine what is correct,

what measures success, and what standards of conduct must be met. Most trustees of universities and managers of business as well as most members of the Congress and Federal Executives, are men with extensive experience in the professions of business, law and politics, but little or none in the direct pursuit of science. They are, therefore, in general, prepared to predict the social usefulness of a product, to estimate the popularity of a public policy, and to evaluate the risks and costs of an economic venture. They are culturally bound, perforce of their own code and previous experience, to evaluate a scientific enterprise in accordance with such terms. Only very rarely are such individuals experienced in judging creative scientific endeavor, scientific concepts, or the conditions essential to professional excellence in science. Moreover, *because technology requires conditions that are easier to appreciate according to marketplace standards, and because science and technology are often lumped together, mistaken judgments arising out of a confusion of these two activities are unfortunately often reinforced in an individual's experience, to the obvious detriment of science.*

Attracting the Ablest Scientists to an Organization. There is no substitue for setting the highest standards, and for providing the greatest attraction possible, for key scientific personnel. A relatively few top-quality individual scientists can provide an aura of excellence for the entire organization that will conclusively ensure future recruitment and retention. Such individuals will illustrate the creative process, the internal self-discipline, the professional competence, and the intellectual devotion required by science. They will live out the satisfactions which derive from intellectual pursuits and set the intellectual and experimental pace for the scientific community, according to their own lights. They will ensure the establishment of traditions most suitable for individual professional development and achievement. Other scientists, whether beginners or established investigators, will draw pride from association with these individuals and their professional accomplishments.

It is clear that without substantial evidence of encouraging creative accomplishment and of providing creative individuals positions where they can accomplish their maximum, an organization is bound to languish as a scientific institution. Without substantial *professional recognition* both within and outside of the organization, no other recognition is meaningful. It might be urged that since only a relatively few individuals within an organization are likely to be highly creative, it is not necessary to indulge in developing a truly creative environment: this is a ruinous misconception. If an institution is unable, for whatever reasons, to attract and to keep the indispensable (even though small) fraction of highly creative scientists upon whom its professional reputation depends, it can lose nothing not already lost.

When even a few highly creative scientists find that a given institution is best from the point of view of their individual professional development and accomplishment, then there are few obstacles to the administration of that organization:

(1) Recruitment and retention of top-level scientists is made easy.

(2) A scientist who must leave the organization because a position can no longer be made available to him, leaves with a sense of pride in his professional experience and association with the organization; on the outside, he is a knowledgeable advocate for its scientific program and its professional support.

(3) Internal professional ideals to seek greater freedom, dignity and responsibility for the individual scientist, tend in a self-controlling, group-correcting way to elevate the standards of excellence of scientific performance entirely in the absence of administrative intervention.

(4) The stature and license of administration as representative of this respected community of scholars become automatically enhanced.

(5) Advocacy for support of the organization's program becomes more objectively scientific and less political in character; this, in turn, has a strong and favorable effect back upon the professional reputation of the organization.

(6) The value system of individual scientists becomes more closely identified with the professional excellence of the organization and less concerned with emoluments; yet

at the same time improved emoluments become even more evidently deserved and easier to justify and to acquire.

The ablest scientists seek an environment where limitations to their accomplishing important intellectual work will be mostly *internal*, where few limitations can be assigned to the environment. They seek a setting where they can have the greatest scope and freedom for both the prelogical and logical steps in their scientific work. These issues seem intangible, but their implications reach into every aspect of daily life: does the organization buy the scientist's time and then give it back to him to employ according to his own conception of time's most fruitful utilization? What are the time-demands which distract from the main goals? Is there a tendency to short-cut significant research in favor of more tangible or "practical" results? Are salaries, promotions, and both the tangible and intangible supports of research provided according to the highest professional standards, and no others? Does legitimate professional activity need to be justified on the basis of nonscientific criteria?

It is clear beyond peradventure of doubt that these issues need to be settled by scientists according to professional standards of aspiration and discipline which they take responsibility for setting: no one else is suitably qualified; no one else has a higher stake in the continuing exercise of those practices which will yield the highest standards of professional excellence for the organization. These issues are similar whether the organization is under the aegis of a university, an industrial concern, or the Government. The essential value judgments in job selection are made on the basis of the professional identifications and the history of professional accomplishments within that organization. New organizations are judged on the basis of their initial program leaders, and on evidence that individual scientists can contribute in a responsible way toward an ideal destiny for an institution that has not been bound down by slovenly traditions, nor by the even heavier yoke of mediocrity.

The ultimate level of accomplishment and performance in any scientific organization depends not only upon the techniques evolved for helping it live up to the noble ideals for which it was established, and the degree of aspiration and respect of its sponsors, but it depends in large measure upon the degree of aspiration and self-discipline exercised by its scientist members. If professional judgments hold sway (and this is essential), and if, in the aggregate, they are such as to lead to continuing internal improvement in the professional standards of the organization, that organization can withstand the impact of raids on its scientists, hence on its life blood, by competing enterprises; it will enjoy high morale and both internal and external respect. Responsibility for high professional standards must be borne by the profession, borne with steadfast and depersonalized objectivity, aiming always toward the highest realizable levels of scientific achievement.

The Extension of Professional Responsibility. Since World War II, the impact of science (as realized through technology) has outstripped most other forces influencing business, law and politics. In present circumstances, the leaders of society and patrons of scientific enterprise are bound to be dependent, in all of the complex and confusing decisions relating to science, upon an adventitious knowledge and judgment in these affairs which is contributed by scientists, consultants and committees of scientists. So much *developmental* action is possible, urgent, or even mandatory, in response to social, political and military needs now expressed and for which technology can supply partial answers provided the necessary developmental monies can be put forward, that the combined Federal and non-Federal budgets are insufficient to support them all.

How can these competing interests for technology be resolved? How can resources be safeguarded to ensure a broad range of scientific activities, upon which the future of technology depends? It is upon this jousting-ground of technical and scientific discriminations that some very large social responsibilities are being transposed to scientific and technical consultants and committees from their traditional location in the hands of business and political leaders. This may be inevitable; yet, it can be dangerous insofar as we may misperceive the extent of the transposition of responsibility, or blind ourselves to the fact that wherever responsibility be

transposed it must continue to be responsibly borne. Now, it is the scientist who finds himself in less familiar terrain. If he acts without regard to such responsibilities, or denies accepting general social responsibilities for scientific and technical decisions he is forwarding, he becomes guilty of failure to assume his full and proper *professional responsibility*. Any denial of his bearing the full implications of such responsibility is as pale as the statement of an advertising man that he bears no responsibility for the tides of public taste nor for the creation of new marketplace demands.

The displacement or transposition of responsibility in affairs relating to science and technology is taking place more and more rapidly. There is no going backward. It may be that business, legal and political leaders can learn to differentiate science from technology, and from business enterprise, and to translate objectively from one code to another. To the degree that this is achieved such leaders deserve to continue to bear in full the social and political responsibilities and opportunities yielded by science and technology. On the other hand, it may be that scientists can learn to accept and be accepted for a fuller share of social and political responsibilities relating to science and technology. This will require the scientists to bear a more difficult and baffling set of responsibilities than they have usually borne heretofore. To some degree both metamorphoses are taking place simultaneously, but as yet they have taken place to only a minor degree in comparison with the full scope of the problem. The general pattern is one of oversimplification and of artful dodging of responsibilities on both sides. *A solution lies first in recognizing the facts of the convergence of several different cultural patterns into an obligatory working relationship required to solve important social problems, and second a deliberate placing of responsibility where it can be most fittingly borne.* Any attempt to *eliminate* the cultural differences in either direction only destroys natural intracultural safeguards and interferes with the development of full professional integrity and responsibility.

One reason why science may appear to be so effective in the Soviet Union is that the Russians consider themselves to have a scientific society. Therefore they encounter no conflict between social, political and marketplace criteria and the system of values and conditions essential to science. This tends to affect general features of their educational system as well as scientific and technological enterprise. In other countries one can identify further differences in respect to cultural interactions affecting science, as compared with either the Soviet system or our own. The observable differences are not suitable to advocate as worthy for use by our society, but they do reinforce the fact that *every cultural interface pattern is an evolved pattern. Conscious and objective application to the problems we face and to the opportunities before us will undoubtedly identify a better destiny to set up for ourselves. Because of our role in the world, this discrimination and the social determinations it yields will be important for mankind as a whole.*

The Ideal of Professionalism. Our primary concern as scientists relates to growth in intellectual and creative power, in ourselves and our colleagues, to improvement in our mental grasp and understanding, particularly of the more general and comprehensive theories of science. Our business is thinking. Our products are conceptual, intellectual. The serious pursuit of new knowledge provides a special kind of discipline, different in important respects from that of any other intellectual calling. What scientists seek is the development of improved concepts that will possess pragmatic intellectual value and will stand testing against all valid sense experiences. The intellectual value concerns whether the concept provides a greater generalization or simplification of ideas, or whether it accounts more explicitly for the facts it relates. Although in biomedical science we can point to tangible evidences of progress in terms of new enzymes, new germs, and new therapeutic agents, these are by-products yielded through the pursuit of less tangible goals. A new level of understanding can be brought about either by beginning with an investigation

of explicit problems (a particular disease entity, or a given sick person) or through the investigation of general problems (the mode of physical-chemical action of enzymes, or how information may be genetically transmitted). In either case, an important clue to the scope of the ultimate scientific accomplishment is found in the admonition: "There is no harm in studying a special subject: the harm is in doing any kind of work with a narrow aim and a narrow mind" (J. Hughlings Jackson, 1877).

There is no such thing as a logic of creativity: the creative process is really pre-logical. The expository order of explanation as to what we have accomplished in science, and how we have tested our ideas, conceals the actual order of discovery. Logical features of science are essential for the logic-tight testing of new concepts against sense experiences. Yet the logical features of new concepts cannot be cleared up until a solution to the problem is evident. Not only is it necessary, in order to be creative, to be rid of interfering pre-committed opinions and prior assumptions, but the experience of creative work makes it increasingly difficult thereafter to hold provincial views. Scientific knowledge, and with it the truly professional scientist, can weather the shattering of its own fictions. Its power is such that it will defeat systems of thought that rest upon suasion, coercion or the exclusion of experience. The pursuit of new knowledge has therefore a liberating as well as a sternly disciplining influence on the individual and on his community. Pessimism and insecurity, and a lack of confidence in being able to do something worthwhile in the arable field of science because of externally imposed limitations, bring a decay of intellectual and moral fiber. Professionalism declines along with the decaying of intellectual standards.

The pursuit of professional excellence, like the pursuit of scientific knowledge is ennobling and liberating as well as disciplining. It is also everlasting. Changes in the character of the scientific frontier, changes in the socio-economic and socio-political context in which the enterprise is conducted, changes in the personnel of scientists, administrators and sponsors, all contribute to a dynamic *transaction* in which failure to be reflective and self-critical, individually and collectively, can lead to the establishment of practices (by direction or by default) that are likely to have devastating consequences for both the individual and the organization.

What seems to be required is a restitution of an ideal of professionalism as practiced, for example, in the medieval guilds. By this is meant an increased sense of personal dedication, a greater sense of individual responsibility to one's own work and to work of one's colleagues, an enlarged sense of identification through individual and group accomplishment, a greater thrust of pride in the mastery and exercise of professional skill. This attitude is clearly to be distinguished from the despairing concept of an impuissant, helpless cog, the contemporary dirge of one's being an "organization man." The degree to which a scientist's professional code of action, experience and responsibility is disallowed obstructed or diverted by his surroundings, and the degree to which a scientist permits this to take place, measures certain inevitable losses of professional power and professional integrity. *The only real safeguards of excellence in the conduct of research depend directly upon the professional characteristics of initiative, self-discipline, and what might be called "creative temperament."* These characteristics cannot be supplied from the outside. They can be, and often are, however, eroded and given away. It is not the hazard of avalanche that ruins scientific enterprise; it is the slow bit by bit loss of professional ideals. Ideals, by embodying themselves in institutions, become enduring historic forces. "An ideal is a picture of the place you will never quite, but always strive to reach. Its attainment happens in little pieces of the striving . . . in any one small piece of honest intellectual exchange, with my neighbor, with my book . . . a new beginning toward the unattainable is forever right at hand" (Robert Redfield, 1955).

Reprise

Biological and social evolution has gradually achieved greater degrees of obligate cooperation and interdependence within the individual, family, organization and society. Although each step of this progression has been at the expense of certain arbitrary modes of selfish behavior on the part of

the individual, family, organization and society, this petty constriction has been counterbalanced by substantial gains in freedom and self-determination at each of these levels. The progressive gains can be appreciated readily through an examination of comparative physiology and behavior, and through an examination of the facts of history over a time scale of centuries. The resultant achievements have meant substantially greater degrees of freedom and self-determination within a widening framework of cooperation, faith and mutual interdependence. This finds its expression in many phenomena: increasing urbanization, increasing dependence upon federated activities and increasing mutual interdependence on the international scale. Nonetheless, developments are not always and uniformly in the direction of enlarging spheres of interdependence: in the field of international travel and exchange, there has been a half century of deterioration associated with increasing formalities which are not entirely offset in practice by technological improvements.

It is patently true, in the aggregate, that never before in the history of man have there been so many individuals, families, organizations and societies so inextricably interdependent. The generalization is illustrated everywhere one looks: tool manufacturing, heavy industry, labor unions, the communications and transportations industries, the interdependence of universities with the Federal Government, and many other larger and lesser examples. The assumption of interdependent relations, whether contracted by individuals, members of a family, organizations, a given society, or assemblies of nations has often been urged and been identified on the basis of narrow motives and as a fulfillment of narrow and provincial interests. The extent to which this narrow view obtains is regrettable; it represents an unwholesome and incomplete recognition of larger relationships.

The situation can be improved through the encouragement and development of healthier and wiser motives and aims, on the part of the individual, family, organization, society and international confederation. This demands the assumption of more extensive responsibilities at each level, the attempt to be more rational about more complex equations, the continuing search for and endeavor to achieve a higher level of integration. Far from this being contrary to nature, it is in fact an extension and more adequate representation of vital principles that are active throughout all of life. These principles operate to allow increasing degrees of freedom and self-determination. These principles are susceptible to a vast acceleration of their effect through conscious acceptance of them as they apply to social existence. After all this is only the centennial of man's achieving a working conception of biological evolution.

Does man have freedom to manipulate the channels between his ideals and his actions? Can he choose his purposes? Research in the mental and neurological fields now supports the surest evidence in this regard that has even been put before mankind. *Opportunities of consciousness and the degrees of freedom of will are now known to be greater than we had any reason hitherto to believe. Freedom of choice and opportunity are ours. We need to utilize these not simply out of anxiety for individual or societal security, but for far more positive reasons—reasons that take into their scope the whole of mankind.* Freedom of the individual, family, society and species has been increasing over the centuries, and now, with the advantages of new insights, with incentives sharpened by prospects of tragic and debasing alternatives, this freedom can be greatly extended by the individual, organization, society and species. This requires only a wider recognition and release of our vital biological heritage which has brought us to the present level of evolution and understanding. *It needs only the wider recognition and release of natural tendencies toward altruism, faith and mutual trust.*

To be free is to be determined by one's own nature. This refers equally to the individual, family, organization, society, or mankind as a whole. A wise and resolute exercise and increase in man's freedom through this means is the most direct way to liberate ourselves from the undesirable exercise of our own baser tendencies. Such an effort can effect changes in our lives that will immeasurably forward us in "life, liberty, and the pursuit of happiness." Are we too blasé to take seriously the high-hearted language and aspirations of our forebears? If we take such goals

seriously, do we think that there is some easier or more direct way for their realization?

Long-Range Intramural Program Developments

The Assembly of Scientists, NIMH–NINDB. The idea that there should be some kind of "faculty" organization at the National Institutes of Health is probably as old as the idea for establishment of the Institutes. Such an organization would serve as a general forum for improving communication among scientists, as a means for formulation and expression of opinion by scientists, and as a mechanism for rendering advice and taking action to promote professional excellence and scientific achievements. As Dr. Seymour S. Kety pointed out, a "faculty" organization would provide the scientists as a whole with the same freedom of conscience and freedom of expression of opinion that has already long been afforded individuals. It would encourage an increase in the sense of participation and responsibility within the profession of scientists at the NIH in the same way that this has been cultivated by great universities.

Clearly, long-range goals of the Institutes are inextricably bound up with the worthiness of its scientists; and the professional careers of the scientists are inextricably bound up with the reputation of the Institutes. For two years, the Laboratory chiefs of the Basic Research Program, NIMH–NINDB, devoted their twice-monthly meetings to a discussion of the relative values of such a "faculty" organization and of ways in which the idea might be democratically put into effect. During this period, Dr. Kety provided a further notable contribution by recommending the examination of a model faculty organization, the highly effective Faculty Senate of the University of Pennsylvania, established in 1952.

In the spring of 1958, following thorough discussion with the Branch chiefs of the Clinical Investigations Programs of NIMH and NINDB, and after discussion in executive staff meetings within the two Institutes, the matter was brought before the Scientific Directors for consideration as an all-NIH "faculty" organization. The Scientific Directors suggested that a trial of the idea should be made among the scientists of the two initiating Institutes; if, after a period of experience, the results seemed to be worthwhile, the plan could be considered for wider adoption within the NIH. The conception of an "Assembly of Scientists" was thereafter presented before an open meeting to which were invited all scientists of the two Institutes. Yet, it was another full year, until the Spring of 1959, before the Assembly was finally launched. At a second open meeting of scientists of the two Institutes, Officers *pro-tem*, Dr. Hal Rosvold as chairman and Dr. Karl Frank as secretary, were elected by secret ballot following open nominations from the floor. There was unanimous expression of interest, in principle, in the establishment of an Assembly of Scientists. The chairman *pro-tem* was instructed to appoint a committee to draft a constitution which would then be presented to the scientists. In due course a Provisional Constitution was adopted, and in the fall of 1959 a revised constitution was formally adopted by a mail vote of 230 members of the scientist staff of the two Institutes. They also expressed their wish voluntarily to participate in future activities of the Assembly.

As far as is known, such a "faculty" organization within the Government is without precedent; yet this is not surprising in view of the fact that the National Institutes of Health are themselves without parallel in mission or spirit of organization within the Government. The potential value of this Assembly to the ultimate stature of the Institutes, and to the level of professional regard in which the scientists themselves will be held, is limited only by the vision, determination, and willingness of members of the Assembly to assume individual and collective responsibility of a constructive nature. Through the Assembly they have a unique opportunity to create an example for scientists elsewhere at the NIH and more generally throughout the Federal Government.

The Principle of "Tenure." Although employment security in the Government is accorded employees in all categories after only one year of employment, one year is too brief a period in which adequately to develop or evaluate the skills of junior scientists. If tenure were to follow automatically whenever a scientist works for a full year at NIH, three disagreeable alternatives would be forced: (1) either the Institutes would have to

be expanded indefinitely, or (2) there would be inadequate space for essential research operations after only 2 or 3 years of such practice, or (3) there would be no opportunity to provide research training for aspirant scientists. Since the most effective scientists are often asked to fill attractive research and teaching posts elsewhere, there is a continuing risk of losing the best research talent from the Institutes, the very leaders with whom aspirant scientists seek to study, the ones who chiefly account for the professional reputation of the Institutes. It is therefore imperative for safeguarding the professional stature of the Institutes, the opportunity for scientists to pursue research effectively, and for junior scientists to receive an adequate foundation in research training, that all of the scientists participate in a definite plan whereby the younger scientists can be provided training and experience in research for definite but limited periods of time, e.g. for from 2 to 3 years. Although this period might be extended for an additional year in exceptional cases, that would ordinarily be the final limit. The only possible execptions would be vacancies resulting from the retirement or departure of senior scientists. "Tenure" is therefore to be effected by establishing a firm understanding, prior to the employment of a new junior scientist, of the time-limited nature of his appointment. The practice of a policy of "tenure" is so standard throughout the academic world that it is widely understood and respected by scientists. Such pre-vision precludes the embarrassment and misunderstanding which otherwise may arise among scientists, each of whom is devoting his utmost energies to research, and for whom, inevitably, there can be only limited local research resources.

The Principle of "Sabbatical" Leave. Creative scientific endeavor demands mastery of subject matter and exercise of initiative, self-discipline, and personal devotion at a level that cannot be sustained indefinitely without intellectual refreshment and revivification. Anyone attempting highly creative scientific work, with the intense preoccupation and internal involvement that this entails, tends to "go stale" without periodic relief in the form of opportunities to renew his mastery of the field, to learn new technical and conceptual skills, and to obtain a new perspective on scientific values relating to his work. To some degree this kind of "change in pace" is effected by the individual investigator within his normal working pattern; nonetheless, over a span of years, he is likely to become even less aware of the conceptual strictures which may impoverish his accomplishing more effective and creative endeavors. Universities of high standing have long recognized as prominent among the essential requirements for sustained high-quality creative scholarship the need for senior faculty members to be given extended periods of time away from regular duties, usually at 7-year intervals.

The Laboratory chiefs of the Basic Research Program initiated discussions on this subject and were encouraged by the Director of NIMH, Dr. Robert H. Felix, to draft plans for a "Sabbatical" Leave Program. Under the chairmanship of Dr. David Shakow, an NIMH committee established the essential administrative considerations for such a program which was then endorsed, in principle, by Dr. Felix, Dr. Pearce Bailey, Director of NINDB, and by the Scientific Directors. Authority for this practice is found in existing regulations providing for the *Work Assignment* and for the *Training* of Institute scientists. The "Sabbatical" Leave Program will provide the senior scientists belonging to the permanent staff, upon whom the Institutes stake their research mission and reputation, an opportunity at 7-year intervals to engage in sabbatical activity of their own choice. Dr. Harris Isbell was the first scientist to be sent on this new leave program; others in the two Institutes are already on leave or are proceding with plans for participation in this augmented opportunity for personal intellectual growth and career development in preparation for further creative work at the National Institutes of Health.

Educational Programs Relating to the NIH. "Scratch a scientist and you will find a teacher." An important aspect of professional activity is helping others to acquire intellectual and technical skills and to have experience in the extension of these skills to new frontiers. The most characteristic form of such "profession" takes place between scientist-preceptors and their junior colleagues. Nearly every collaborative research undertaking involves similar vital intellectual

556044—60——11

exchange, a function intrinsic to the life of an investigator.

Early in the history of the National Institutes of Health there was an expression of need for more formal and organized opportunities for participation in both directions in the educational process. The NIH established a branch of the U.S. Department of Agriculture Graduate School which has grown steadily in attendance and autonomy. The Research Associates Program, established 3 years ago, has added to the preceptor-apprentice relationship a complementary means for a broad-based education in bio-medical research, through the provision of course work and seminars extending into fields other than the Associate's primary specialization. This last year, members of the NIH Scientific Advisory Committee established a non-profit corporation, The Foundation for Advancement of Education in the Sciences, Inc., which will facilitate a further extension of educational opportunities at the NIH. Dr. Daniel Steinberg is chairman of the Board of Directors of the new Foundation. The Board also includes other representative leaders in the field of science education outside the NIH. The Foundation, like the Graduate School Branch it takes over, will be largely self-sustaining from tuition. The increase of intellectual experience which the Foundation can provide will undoubtedly prove beneficial to the recruitment and sustained intellectual vigor of scientists who derive insight and satisfaction from participating in professional educational activities.

The Construction of a Greenhouse Facility. For several years scientists in the Laboratory of Cellular Pharmacology have expressed their need for a facility for direct investigation of alkaloid synthesis in plants. During the year a neat little greenhouse was constructed and put into operation under the direction of Dr. Harvey Mudd. Several alkaloids, particularly those related to groups of tranquillizers, psychotomimetic drugs, narcotic agents, depressives, and cerebral stimulants can now be studied in relation to their metabolic precursors and the ways in which they are handled and inactivated by plants. It will also be possible, by this means, to label complex compounds by feeding plants with radioactive building blocks, in many cases saving difficult laboratory synthetic procedures.

The first procedures undertaken by Dr. Mudd and his colleagues have established certain common features of metabolic pathways which are common to higher mammals, single celled organisms, and higher plants. This revelation confirms that it will be practicable to examine a number of complex metabolic pathways first in plants where the growth and harvesting of large quantities of particular metabolic steps will facilitate solution of a number of important problems. With certain key steps established in plants and with knowledge of the essential substrates, enzymes, cofactors, etc., it will be possible to confirm and extend these findings much more quickly in higher mammals. Members of the laboratory will now be able to work back and forth between plant and animal biochemistry and to look for variants and consistencies over a very broad biological field of endeavor.

General Commentary

This year has witnessed a continuing harvest of outstanding research papers from the Basic Research Program. The entire enterprise can be readily justified on the basis of a few of the really creative ideas brought forth. The status of the program is also measured by the large number of invitations which come to its scientists to provide papers for national and international meetings, and to lecture before or to join the faculties of outstanding universities. Judging by the increasing qualifications of scientists seeking positions here, it is evident that the program is gaining in reputation as an intellectual and experimental resource for effective scientific training and experience at all levels. Nearly every major university in this country and some seventeen foreign universities are represented by one or more scientists employed in the program this year.

Dr. Mortimer Mishkin was sent to work for 3 months at the Nencki Institute in Warsaw, Poland, and Dr. Stefan Brutkowski of that Institute has been sent by the Polish Academy of Science to work with Dr. Mishkin and Dr. Hal Rosvold for about a year as a guest worker.

Altogether, 16 of our scientists were sent abroad for periods of work and intellectual exchange during the year. There were nine scientists in the program who attended international meetings outside this country. Distinguished scientists from more than 20 different countries visited the Basic Research Program this year.

One of the traditional ways of improving the creative power of an organization—through the use of expert consultants—has been actively exploited this year as in the past. The 12 members of the Boards of Scientific Counselors of the 2 Institutes, NIMH and NINDB, have continued to give encouragement, intellectual stimulation, and to provide programmatic as well as scientific advice. Some 37 other expert consultants participated and advised in relation to special aspects of the program. Professor Torsten Teorell of the University of Uppsala, Sweden, and Professor Ulrich Franck of the Max Planck Institute in Darmstadt, West Germany, came to work for a period of time with Drs. Ichiji Tasaki and Constantine Spyropoulos at the Woods Hole Marine Biological Laboratory this summer. Similarly, Drs. Sydney Brenner and Francis H. C. Crick from Cambridge, England, paid working visits to Dr. Bernhard's Section on Physical Chemistry. Altogether, about a dozen foreign scientists spent working periods in the program. Through a chance encounter with Dr. Emanuel Piore, Director of Research for the International Business Machines Corporation, an exceptionally exciting research collaboration has been arranged. Dr. Sidney Bernhard introduced a group of engineers and mathematicians of the IBM Research Division, visiting Bethesda at Dr. Piore's suggestion, to the conceptual problem of "breaking the code" for the nucleic-acid sequencing of amino acids in genetic transmission, and more generally in all protein synthesis. Dr. Bernhard and Dr. Dan Bradley had conceived of a way in which the bulk of the presently tedious chemical identifications of one after another of the amino acids in serial order could be short-cut by utilizing advanced electronic computers in elaborate logical analyses. Dr. William Duda of IBM has since then been devoting full time to the difficult mathematical end of this investigation. Hs has been able to put to work the best IBM computer programmers and to commit the most modern computer equipment for this purpose. In September Dr. Duda accompanied Dr. Bernhard to Copenhagen to participate in discussions on this subject at the International Symposium on the Genetic Control of Protein Synthesis. Dr. Bernhard had been invited by the Symposium Program Committee to give an address at the Symposium in replacement of Professor Linderstrøm-Lang, an internationally famous Danish scholar noted for his work in this field, who died unexpectedly last spring.

The project to "break the code" of amino-acid sequencing by this means is still in early stages of development. Each step thus far has proceeded favorably, but it is a "long shot" as to whether the concepts and techniques may prove successful: nonetheless, both IBM and the Basic Research Program are confident that whatever is learned along this important line of investigation will be worthwhile. Whereas it now takes years of conspicuously conscientious and compulsive chemical work to determine the sequence of amino acids in even relatively small proteins, the new method shows promise of reducing this time to a matter of a few weeks. If this turns out favorably it will vastly accelerate the analysis of differences in the almost countless proteins of importance to biology and medicine, and it will make practicable the identification of the sites of defect of genetically determined developmental and metabolic errors.

During the year Professor Leo Szilard published the theory of aging which he developed while serving as a consultant to the program. He now has in preparation two new and equally challenging papers on the theory of antibody formation. After long deliberation, Dr. Szilard declined employment in this program in favor of accepting a long-term NIH extramural grant which would allow him to retain his post at the Enrico Fermi Institute in the University of Chicago and still to collaborate with our staff for extended periods as a guest worker. Unfortunately, the same week that Dr. Szilard was informed of favorable action on the NIH grant, he also learned that he has a highly malignant tumor which is effectively inoperable.

A Personal Note

Prior to appointment as Director of Basic Research for the two Institutes, I discussed with everyone concerned a deliberate limitation on the length of time I felt it was reasonable to commit to such heavy administrative responsibilities. This year, shortly before the completion of 3 years in office, I asked permission of the two Institute Directors to be relieved from this work "in the near future." My reasons for adhering to such a time limit are threefold: First, I believe it desirable for a scientific program to have a change in leadership from time to time. Dr. Kety has already set a precedent for this. Any leader of a research group has conceptual limitations which are likely to become an increasing interference to the program as his time in office is stretched out beyond about 3 to 5 years. Second, as Ian Stevenson puts it, "The possession of the power to make decisions can eventually persuade anyone that he also has the proper knowledge to do so." My third reason for wishing to adhere to such a time limit derives from a very personal desire to continue full-time research.

BASIC RESEARCH—(NIMH–NINDB)

Laboratory of Biophysics

The central objective of the Laboratory of Biophysics is to understand the nature and the implications of the ion movements fundamental to the initiation and propagation of a nerve impulse. The staff—John W. Moore, Richard Fitz-Hugh, Robert E. Taylor, William J. Adelman, W. Knox Chandler, John H. Gebhart, and Ernest R. Whitcomb—have continued to progress towards this objective, in part with the collaboration of the Naval Medical Research Institute, and the Computation Laboratory, National Bureau of Standards.

The characteristics of individual ionic movements as first determined from measurements of the squid-axon-membrane current and potential under controlled electrical, geometrical, and ionic conditions have led to far-reaching conclusions. But subsequent work has made it necessary to undertake an examination of the extent to which the measured membrane currents depend upon the adequacy of these controls. The records and analyses generally confirm the 1958 preliminary conclusions that the membrane potential of strong axons could and had usually been reasonably well-controlled and that the qualitative characteristics of these axons were similar to those of less-difficult axons. The conditions for an adequate control have been found to be more stringent and the understanding of failures to be more difficult than had been anticipated. The confusing effects of instability can be reduced by restricting the membrane current measurements to the region of best potential control but an estimate for the accuracy of control must await further investigations of the resistance between the control electrodes and the capacity of the membrane.

The sodium potential of Hodgkin and Huxley measured by voltage clamp may be assumed to be as good a measure for sodium-ion concentration changes inside the axon as it has been found to be for those outside. Thus measured, the net sodium inflow of the surviving axon at rest and the increase by stimulation are in general agreement with isotope measurements. The net flow of sodium is reversed by voltage pulses above the sodium potential as predicted by the sodium theory. By the same procedure, it was found that ten precent of normal external divalent ion concentration increased the resting net sodium inflow to about three times the normal value.

The phenomena of finite and infinite trains of impulses, anodal break excitation, refractoriness, and accommodation depend upon certain mathematical properties shared by a large class of systems, electronic and chemical, as well as physiological. The well-known Van der Pol relaxation oscillator equations have been generalized to include these phenomena and the Hodgkin-Huxley squid-axon equations have been reduced to a qualitatively similar form. The generalized equations can be represented on a plane with regions corresponding to the physiological states described by classical neurophysiology. Thus, a basis for deeper and more useful understanding of well-known phenomena is emerging.

Digital computations for a medullated axon with a modified Hodgkin-Huxley membrane at the nodes give an impulse velocity of 11.9 m/sec for

various super threshold stimuli but the long latency at threshold requires much more expensive computer runs than have yet been undertaken. Analog computations have shown that with reasonable modifications, the Hodgkin-Huxley squid-axon equations produce action potentials agreeing closely with those obtained by Dr. John Dalton from a lobster axon in normal and excess potassium media.

In a joint project, with Dr. Seymour L. Friess, NMRI, on the action of synthetic acetylcholinesterase inhibitors, pure optical and geometrical isomers in the ethylenediamine series and in the 1,2-aminocyclohexane family have been compared by *in vitro* enzyme inhibition and nerve blockage. Although both lines of investigation are interesting and promising, the results are so increasingly divergent as to further decrease the probability of a casual relationship between the two processes. A shift of emphasis from desheathed whole nerve to single node preparations is giving the results a clarity not possible when the drugs diffuse to many sites of action.

Laboratory of Neuroanatomical Sciences

The Laboratory of Neuroanatomical Sciences is organized into five sections, one a field station in Puerto Rico. Each section chief has composed a summary of the section's research activities during 1959. The research of each section is sufficiently unique to make an overall summary of activities less meaningful than presentation of separate accounts.

SECTION ON DEVELOPMENT AND REGENERATION

Research activities of the Section on Development and Regeneration fall into categories of (a) neurogenesis, (b) regenerative potentialities of central and peripheral neurons, and (c) experimentally induced structural alterations in the central nervous system.

(a) Drs. Sidman, Miale, and Feder incorporated tritium-labeled thymidine into deoxyribonucleic acid (DNA) of cells preparing for division. Autoradiography with tritium-labeled thymidine (thymidine–H^3) provided a method of marking such cells in the relatively inaccessible mammalian embryo. Pregnant mice were injected intravenously with thymidine–H^3 and killed at various intervals. Autoradiograms were prepared of sections through the embryonic brains. Eleven-day embryos, fixed 1 hour after exposure to thymidine–H^3, showed heavy labeling of most cell nuclei in the external half of the primitive ependymal layer in the wall of the cerebral vesicle, and almost no labeling in the inner half. Thus the external half of the primitive ependymal layer was a site of DNA synthesis. Six hours after exposure to thymidine–H^3, the labeled nuclei occupied the inner (ventricular) half of the primitive ependymal layer, and most mitotic figures at the ventricular surface contained labeled chromosomes. Forty-eight hours after injection, labeled nuclei had migrated laterally; some had entered the developing mantle layer, but many remained in the primitive ependyma and had repeated the cycle of DNA synthesis, migration, and division. Development of the primitive ependyma was similar throughout the embryonic nervous system. The cells of the primitive ependymal layer behaved synchronously. The primitive ependymal layer is a pseudostratified columnar epithelium within which nuclei of undifferentiated cells migrate to and fro in relation to the mitotic cycle.

It was found that the primordium of the cerebellum made its appearance after 11 days of gestation. At this time 3 zones comprising the primordium were recognizable. Cells of the ependymal region migrated into the intermediate zone to participate in formation of intracerebellar nuclei. Development of the various components of the cerebellum of mice was traced on different days of gestation. The cells destined to become Purkinje neurons migrated from the primitive ependymal region to their definitive positions in the future cerebellar cortex and rapidly differentiated there.

(b) Drs. L. Guth and C. J. Bailey evaluated the relative roles of the sympathetic and parasympathetic pupillary nerve fibers to determine whether autonomic neurons can maintain the function of autonomic effector organs other than those that they normally innervate. The atropinized pupil dilated significantly in darkness and a significant portion of this dilation was abolished by sympathectomy. Contrary to current opinion the sympathetic nervous system plays an active role, rather than merely a tonic one, in maintaining pupillary

dilation in darkness. They found that pupillary size was partially restored under the influence of "foreign" nerve roots, although these roots did not mediate a "darkness reflex." This was accomplished by transecting the sympathetic rami of T 1 to T 3 to allow collateral sprouts from rami of T 4 to T 7 to innervate the pupillary ganglion cells of the superior cervical sympathetic ganglion in cats. The nerve fibers apparently retained their original specificity, inasmuch as the heterogenously reinnervated pupil dilated in response to decreased environmental temperatures.

Dr. Guth, with Drs. James Campbell of Columbia University and Lamar Soutter of Boston University, are testing in monkeys whether or not diaphragmatic function can be maintained by the recurrent laryngeal nerve. The proximal recurrent laryngeal and distal phrenic nerve segments were anastomosed. Regeneration has been in progress for nearly 1 year. Dr. Karl Frank of the Laboratory of Neurophysiology will cooperate with Dr. Guth early in November to determine electrophysiologically the state of the diaphragmatic reinnervation in these animals. Dr. Guth previously conducted a similar study in rats and demonstrated that vagophrenic anastomosis did, indeed, restore function to the denervated hemidiaphragm. If the experiment in the monkey succeeds, it will be possible to consider application of the operative procedure to those human disorders characterized by pathology of the phrenic nerve.

The chief of the section and Dr. E. R. Feringa, collaborating with Drs. J. B. Campbell, A Bassett, and C. Thuline of Columbia University, have continued to study regeneration in the mammalian spinal cord. A study of transected spinal cord of 16 monkeys with gaps of several millimeters was carried out. In a report of this work presented at the April meeting of the American Academy of Neurology, postulates for evaluating regeneration in the central nervous system were laid down. These are: There must be proof that the spinal cord was severed; clinically observable signs of restitution of motor and/or sensory functions; proof of physiological conduction across the healed lesions, including electrical recordings from the spinal cord following stimulation above and/or below; trans-synaptic potentials must be demonstrated; finally, there must be unquestionable verification of reestablishment of histological continuity. All of these are needed in one and the same animal. To date, no single experiment in this or any other laboratory has fulfilled all these postulates in mammals. Current studies in cats and monkeys are in progress combining the techniques of surrounding the lesion with Millipore to prevent encroachment of connective tissue, and treatment of the animals with Piromen. Multiple short segments of frozen-dry, peripheral-nerve homografts, have been implanted in some. A preliminary operation to fuse the skeletal elements of the spine prior to spinal-cord transection has led to improved histological and physiological results in cats. By combining several procedures, each of which has some demonstrated merit, it is hoped to achieve fulfillment of our postulates.

Drs. Feringa, Campbell, Bassett, and Thuline have explored methods of grafting segments of large peripheral nerves, using the goat as the principal laboratory animal. Homologous nerve segments, banked by freezing, freezing and irradiation, and freezing and drying, were implanted in gaps produced surgically in the sciatic nerve of these animals. Preliminary studies in cats, with smaller nerves, demonstrated swift functional and anatomical regeneration across gaps of 1 cm., bridged with frozen-dry segments of nerve wrapped in Millipore sheaths. It is hoped that the studies in goats will provide information adequate to permit the method to be extended to human subjects for the repair of large peripheral nerve gaps.

(c) Studies on alteration of the structure of the central nervous system resulting from asphyxia neonatorum and nitrogen asphyxiation of newborn and young monkeys have been carried out in collaboration with members of the field station, Section on Perinatal Physiology, in San Juan, Puerto Rico.

Asphyxia neonatorum was induced in monkeys near term by detaching the placenta at hysterotomy under local anesthesia, keeping the fetal membranes intact. Eleven to sixteen minutes later the fetuses were delivered from their membranes and resuscitated by pulmonary insufflation with oxygen. The infant monkeys showed neurological deficits during life. Five were killed by perfusion-fixation at 2 to 9 days of age. Brains of these and of two which were not asphyxiated were studied. A common pattern of structural al-

teration was encountered in the nervous system of the asphyxiated monkeys. Nuclei were symmetrically affected; those most consistently and severely damaged were the nucleus of the inferior colliculus, gracile and medial cuneate nuclei, roof nuclei of cerebellum, ventral posterior group of thalamic nuclei, globus pallidus, putamen, and vestibular nuclei. The cerebral cortex was severely damaged in only one monkey. Lesions began with primary nerve cell and, less frequently, neuroglia cell lysis and loss. Secondary damage of myelin sheaths, and reactions of astrocytes, endothelial cells, vascular adventitial cells, and phagocytes were noted. A relation of lesions to vascular distribution was not apparent. Hemorrhages were not encountered.

Six newborn monkeys and four juveniles were asphyxiated in N_2, resuscitated similarly (cardiac arrest in one), and killed 10 days later. Appropriate controls were provided. Nitrogen asphyxiation failed to produce the pattern of brain damage seen after fetal asphyxia. No focal changes were observed 10 days after cardiac arrest. Asphyxia neonatorum and N_2 asphyxiation have quite different effects on the central nervous system of *Macaca mulatta*.

Guinea pig fetuses were asphyxiated at term by clamping the uterine blood vessels during laparotomy and resuscitated by insufflation of their lungs. Observations of degree of asphyxia and degree of resulting neurological deficit were made. Asphyxia produced neurological deficits at least transiently. Asphyxiated animals and their control litter-mates were tested in an alternation maze during the first week of life or during the 12th week. Some of these were tested also in a water maze at about 18 months of age. Asphyxiated animals made more errors than the controls in learning, although relearning tests did not show that they forgot more rapidly than controls. There was no correlation of learning performance and degree of asphyxia. Histological examination of the brains revealed damage similar to, though less severe than, that reported previously (1942). Hemorrhages were seen only in acute stages. Chromatolytic changes in neurons, loss of neurons, and neuroglial reactions appeared in the brain stem and thalamus. Circumscribed bilaterally symmetrical loci of damage were encountered in the thalami of long-term animals. The signs of damage were less marked in animals living for a year or more than in those dying during the first few days or weeks. The neuroglia cell changes subsided with time. Long-term survival animals exhibited minimal brain damage. This experiment yielded further evidence that asphyxial episodes at birth produce neurological deficits, learning defects, and brain damage. It casts doubt on the thesis that the short asphyxial episodes seen clinically do not cause some undesirable sequelae.

SECTION ON NEUROCYTOLOGY

As the Section has developed, its interests and problems investigated fall into two categories: morphological and chemical.

In the first group our investigations this year have been directed toward learning more about the relationship between the neuron and its supporting structures, the glia in the central nervous system and the capsular cells in the sensory ganglia.

In the peripheral ganglia each neuron is enclosed within a capsule consisting of Schwann cells. In the dorsal root ganglia, the capsule characteristically is closely apposed to the surface of the neuron except in certain spots where the two surfaces diverge, forming bays or lakes into which the capsular cell sends protruding ridges and microvilli. These spots are associated with intracellular vesicles in the capsule. It is possible that these formations are related to ionic movements during the activation of the cell surface which is part of impulse propagation. In the ganglia of the eighth nerve, myelin coats many of the neurons. We have studied these in the goldfish. The myelin occurs with various degrees of compactness. Nodes of Ranvier are absent. The presence of myelin completely surrounding a neuron raises the question of transport of nutrients and exchange of metabolites between the neuron and the extracellular space. Such cells are perhaps more amenable to physiologic studies than those of the central nervous system where extracellular space is also limited by the close packing of all elements.

Dr. Brightman and Dr. Albers have completed a histochemical study of species differences in the distribution of cholinesterase activity in the

central nervous system. Pseudocholinesterase occurs in the vascular endothelium in the rat, goldfish, and toad, but in the glia of the cat and rooster. The significance of this distribution is not clear at present.

The chemical studies of this section have been carried out under the direction of Dr. Albers. His investigations have been concerned with the role of γ-aminobutyric acid in the metabolic reactions of the brain. This study is based upon the observation that γ-aminobutyric acid and its direct precursor, glutamic acid, occur in large quantities in the brain. The enzyme which catalyzes the conversion of glutamate into GABA is localized in gray matter, possibly in the neurons, and is not found in other tissues of the body.

The section has been host this year to Dr. John Hills, NINDB trainee, and Dr. Mary Grillo, research fellow, both of whom will be studying certain aspects of the peripheral nervous system. Dr. L. Embree has joined with Dr. Albers as a Research Associate.

Dr. Palay gave a Phillips lecture at Haverford College in Haverford, Pennsylvania, and also lectured at the Rockefeller Institute, New York; the University of Washington, Seattle; Columbia University, New York; George Washington University Medical School, Washington, D.C.; Annual Symposium of the Gastroenterological Research Group, Atlantic City; and in the Linnean Society, London. Dr. Albers participated in a national conference on "Inhibition in the Central Nervous System and γ-aminobutyric acid."

SECTION ON EXPERIMENTAL NEUROPATHOLOGY

Several series of studies were completed as part of a long-range plan to establish extra- and intraspinal factors concerned with maintaining normal spinal-cord structure and function.

A comparative miscroscopical study of the vascular system revealed the widespread distribution of intervascular connective-tissue fibers in 13 animal species, including monkey, dog, raccoon, cat, rabbit, several rodents, opossum, and pigeon. The significance of establishing such a fibrillary system in animals is (a) normally vessels tend to undergo involuntary changes which should not be interpreted as due to experimentation, disease, or aging, (b) such a fibrillary system contributes to the stability of normal vasculature, (c) such intervascular connections may have a deleterious effect on blood circulation in cerebral edema when blood vessels are stretched and their lumens distorted, and (d) differences in regional susceptibility may be ascribed to variations in number and size.

Another study, aimed at the oligodendrocytes along the vessels, disclosed that in all animals these cells are arranged in rows, small groups, or larger clusters near perivascularly arranged neurons or near points of arborization of vessels. None is arranged in a manner to support older concepts that these cells are concerned with the function of neuronal perikarya or processes. On the other hand the particular arrangement of these cells suggested that they are concerned with intrinsic control of blood flow to neurons. The complex arrangements of oligodendrocytes and vessels were more striking in the spinal cord; this observation may point to the need for a more intricate control mechanism in this organ than the brain to permit utmost economical distribution of blood between activated neurons. These results, presented at the Conference on Microcirculation in Physiology and Pathology May 4, 1959, at the NIH, have opened new avenues for anatomical, histochemical, physiological, and pathological investigations. They have initiated further research both to determine the exact distribution in a tridimensional model of the central nervous tissue and to assess the reaction of these cells under abnormal conditions.

As part of an investigation on the response of the tissue to abnormal capillary blood flow, a dog material injected with oil was studied and reported jointly with Dr. Roy L. Swank, University of Oregon, Medical School, Portland, Oreg. The tissue changes, which varied greatly in size, were always the result of capillary obstruction. The tendency of perivascular conversion of fibrinogen to fibrin was less striking in dog than in man. Two other observations were: a striking diffusion of iron as indicative of disturbed iron metabolism and an aggregation of oligodendrocyte nuclei in small foci of necrosis where neurons had disappeared indicating different resistance of oligodendrocytes and neurons to ischemia.

The significance of abnormal staining of neurons was reviewed. An extensive study of histological material and of previous publications formed the basis of a brief report given before the American Association of Neuropathologists (Annual Meeting, Atlantic City, June 1959) and of a larger review to be published in *Ergebnisse der Anatomie*. Conclusive evidence of the artifactual nature of dark neurons was given. As a consequence, contemporary views on the role of so-called dark neurons during normal activity and disease reaction could not be confirmed. Only material free of dark neurons should be used for cytological and neuropathological studies. It was gratifying that principles of fixation and autopsy were established which prevented normal neurons from undergoing artifactual changes.

In order to understand how to control the factors involved in histological preparation a series of measurements was performed. With the aid of karyometric methods it was possible to demonstrate that the size of neuroglial nuclei was greatly influenced by fixatives. The shape and staining of nuclei, in particular of astrocyte nuclei, varied with the technique used. With some of the procedures the astrocyte nuclei were rounded whereas with the best procedure available they exhibited an extreme degree of pleomorphism. This pleomorphism occurred in most regions and animal species except several of the small rodents. For investigations concerned with identification of astrocyte and oligodendrocyte nuclei only such species should be selected where astrocyte nuclei are easily identifiable because of their pleomorphic shape, as in the chinchilla.

As a consequence of these studies all histological material is now prepared according to a standard procedure: (a) use of a modified Heidenhain's Susa solution as perfusate for fixation; (b) delay the autopsy 4 hours after the perfusion; (c) immediate transfer of blocks to alcohol; and (d) staining of the microscopical sections by the periodic acid-Schiff procedure and then immersion in gallocyanin-chrom alum, pH. 1.7, at room temperature for 24 hours.

Two series of animals were studied by Dr. Helen D. Ramsey to establish the distribution of extradural fat; one concerned the conditions in the cat from newborn to adult and the other a comparative study in the rabbit and monkey. This is the first systematic study of the intricate manner in which fat is deposited in several animal species. The extradural fat permits the complex movements of the vertebral column without tearing the spinal cord and rootlets.

The Section has been host to Dr. Mignon Malm (University of Stockholm, Sweden) during 1959. Dr. Helen D. Ramsey left the Section during the current year.

SECTION ON FUNCTIONAL NEUROANATOMY

Professional personnel of the Section of Functional Neuroanatomy are: Richard Gacek and Grant L. Rasmussen, Chief. This section concerns itself primarily with nervous pathways and connections of the brain and spinal cord.

In order to better understand the neural mechanism of hearing, studies of the auditory afferent system, so long neglected, have received particular attention. Point-to-point interneuronal relationships existing between the organ of Corti and the cochlear nucleus, and the manner of projection from the latter to higher auditory nuclear groups, have been restudied in more detail than heretofore by the experimental anatomical approach. The study dealing with the projection of cochlear nerve fibers on the cochlear nucleus has been completed for both the cat and chinchilla. The special arrangement of the nerve terminals and manner of distribution to the different subnuclear groups of the cochlear nucleus have been determined and correlated with the sites of lesions. This information has been brought together in the form of a plastic model of the cochlear nucleus which permits one to gain a three-dimensional view of the course and distribution of cochlear nerve fibers that transmit nerve impulses from the different tonotopic regions of the organ of Corti.

The efferent or recurrent connections of the cochlear nucleus have been studied extensively in the cat. The most important finding is the discovery of two numerically important bundles of efferent fibers originating from higher auditory levels. One has its cells of origin in the nuclei of the lateral lemniscus, its fibers descend in the stria acoustic dorsalis to the dorsal cochlear nucleus of the opposite side. The other arises from the superior olivary nucleus and terminates about cells of

556044—60——12

the ventral cochlear nucleus of the same side. These efferents, plus the one previously described (1958), together constitute a rich feedback innervation from higher auditory centers. The fact that recent physiological experiments of several workers have shown that neural activity of the cochlear nucleus can be suppressed by stimulation of higher auditory regions leads one to assume that this phenomenon is effected through the descending nervous connection described above. Dr. Gacek has played an important part in these investigations particularly in performance of the operative procedures involving placement of the lesions visually.

Two new features of the olivocochlear bundle have been revealed that the neurophysiologist must take into account. One is the presence of a homolateral component of the well-known crossed olivocochlear bundle; this arises from the S-shaped olivary nucleus and joins the crossed efferent cochlear bundle before leaving the brain. The other finding is a connection of the olivocochlear bundle with cells of the anterior ventral cochlear nucleus.

The study of efferent and afferent connection of higher auditory levels is being continued.

Robert Boord, a student of the University of Maryland working here under a PHS predoctoral fellowship, completed his study concerning the question of possible presence of an efferent cochlear bundle in submammalian animals possessing a poorly differentiated hearing apparatus. The experimental results demonstrate for the first time an efferent component of the cochlear nerve in the pigeon and alligator which is homologous to that found in mammals. The efferents have been traced as far as the receptor epithelium of the primitive organ of Corti and of the lagena, a structure of unknown function and not present in mammals. Knowledge of the presence of the efferents in animals having simpler organs of Corti than those of mammals is important relative to the determination of the question of whether or not the receptor cells have a dual innervation. It should be much easier to settle the question of ultimate termination of the efferents in an animal having a simply constructed receptor organ. This work is being used as a thesis for the degree of doctorate of philosophy.

Dr. Gacek has pursued further the problem concerning the ultimate termination of the efferent vestibular fibers. His attempts to solve this question utilizing various well-known experimental neuroanatomical techniques have proved fruitless. He has traced the efferents up to but not beyond the basement membrane of the receptor epithelium.

Dr. G. Dohlman, internationally known for fundamental contributions on the anatomy and physiology of the vestibular receptors, joined the section in May and pursued histochemical studies on these organs. He plans to return from Europe near the beginning of next year to resume studies which concern determination of efferent innervation of the receptors by histochemical method (cholinesterase activity) and the mechanism of stimulation of hair cells of the vestibular receptor areas.

Mr. Kent Morest, a senior medical student of Yale, rejoined the Section under the COSTEP program for 2 months and completed the study begun last year on fiber connections of the area postrema. He will return July 1, 1960, as full-time investigator.

SECTION ON PERINATAL PHYSIOLOGY

During the past year the activity of this Section has been experimental investigation and extensive data collecting on adverse factors in the perinatal period of rhesus monkeys resulting in neurological and psychological deficits in the offspring. The first adverse factor tested was asphyxia neonatorum.

Data on menstruation in rhesus monkeys under standard conditions continue to be collected. Menstrual cycles of individual monkeys are subject to wide variation. There has been no systematic relation between regularity of cycles and fertility. Matings result in conceptions throughout the year.

Data are being collected on maturation of rhesus monkeys. A nursery, patterned in many ways after those in hospital use, is maintained for the care of infant monkeys. Records are kept of daily weights, food intake, temperature, and respiratory rate. Heart rate is recorded electrically, grasp reflex is routinely measured; and the maturation of the ability to self-feed is assessed. Normal ranges of values have been established. This knowledge has been applied to the care of infants damaged by asphyxia.

Neurological deficits of experimentally induced asphyxia have been investigated. Monkeys of known mating dates were delivered by caesarean section near full term. Fetuses were asphyxiated by removing the uterine contents intact and waiting until intra-amniotic respiratory efforts ceased or were about to cease before freeing the infant from the fetal membranes. Asphyxiation times were varied; some infants were able to breathe spontaneously, while others had to be resuscitated. Resuscitation was accomplished by intermittently inflating the lungs through an endotracheal tube. Activity, respiratory effort, and heart rate were recorded.

Some newborn monkeys were asphyxiated by placing them in a glass jar through which nitrogen was flowing. Other monkeys were delivered at once by caesarean section to serve as controls. Asphyxiated and control infants were raised in the laboratory and required the same constant nursing care as healthy and sick newborn human infants. Motion pictures were taken during the experiments and at intervals thereafter; neurological examinations were performed regularly; electroencephalographic tracings were taken at intervals, and a great variety of physiological data were recorded for later study, review, and comparison. Infants which seemed unlikely to survive, as well as some healthy ones, were killed by perfusion-fixation for histological studies.

Technically satisfactory asphyxiation-resuscitation was occomplished in fetuses and newborns. The differences in the responses of fetal and newborn monkeys to asphyxiation observed during these studies fell into three groups: (a) the fetuses were quiescent except for respiratory efforts, whereas the newborns struggled, defecated, and salivated; (b) the heart rate fell slowly in the fetuses, while in the newborns the heart rate fell abruptly and then rose slowly; (c) the fetuses could be resuscitated 5½ minutes after their last gasps, whereas the newborns could not be resuscitated if asphyxiated for 1½ minutes past their last gasps. These findings complement those of Dr. Dawes and his associates, working in this laboratory, who have shown in acute experiments differences in blood pressure, cardiac glycogen reserve, and blood sugar between fetuses and newborns asphyxiated past their last gasps.

Standardized neurological examinations have been performed periodically from the time of birth. Motion picture records of the examinations are made at established periods, as are electroencephalographic recordings. Photic stimuli are used to activate the EEG. The neurological and electroencephalographic studies are carried out on both the asphyxiated and control animals. Neurological deficits of considerable degree and persistent nature have been observed in some of the asphyxiated monkeys. Within these, there has been little tendency for lateralized motor deficits. The general pattern has been one of choreoathetoid movements and a marked lag in development of isolated movement, such as pawing, reaching, and picking up small objects. The EEG's of most of the asphyxiated animals did not differ greatly from the normal. The most marked finding has been a depression of the electrical activity during the first 5 or 6 post-asphyxial days.

Miss Saxon has been studying the psychological effects produced by asphyxia neonatorum. Using both asphyxiates and normals in a test battery she has found no significant differences in learning ability between the groups so far tested (5 asphyxiates; 5 normals). Also, no significant correlation was found to exist between the length of asphyxiation and object discrimination learning. However, normal controls have been found to be significantly more emotional than the asphyxiated subjects. Monkeys with severe neurological defects could not be subjected to these psychological tests.

A year ago plans were initiated for a collaborative study between members of the field station Section on Perinatal Physiology and a group from the Nuffield Institute for Medical Research, Oxford, England. Dr. Geoffrey Dawes and three members of his staff from Oxford received a grant from United Cerebral Palsy Research and Educational Foundation to permit travel to Puerto Rico for this work. They arrived in early August and conducted experiments on fetal physiology of the primate during August, September, and early October. Twenty monkeys were mated for this project. Eight infants were born spontaneously and 12 by cesarean section. Nine of the twelve mothers that were subjected to section survived. Several other newborn and young monkeys were

made available for the study. Physiological and biochemical data were collected and are being readied for publication. Plans are being made to continue the research in Puerto Rico in another 9 months. The most substantial findings are summarized in the following paragraphs:

Observations have been made on 12 fetal monkeys *in utero* under pentobarbitone anaesthesia, ranging in gestation age from 115–158 days (term is about 168 days). A leg was delivered through a small uterine incision and a catheter was inserted into the femoral artery for recording blood pressure and to obtain blood samples. In 5 fetal monkeys, an arm was also delivered through a separate incision and the brachial artery was catheterized so that samples could be withdrawn from tributaries of the ascending and the descending aorta simultaneously. In the three youngest monkeys (126 days gestation or less) a uterine incision was made over the neck of the fetus and a catheter was introduced into the left carotid artery.

The mean O_2 saturation of the femoral arterial blood was 58 percent (range 51–63). Simultaneous samples taken from a brachial artery were, on the average, 9 percent higher; values as high as 77 and 79 percent were observed. The blood-lactate concentration was of the same order as that seen in six monkeys, 8–12 days after birth (7–17 mg/100 ml). In three fetal monkeys, femoral arterial blood samples withdrawn at the peak of a uterine contraction (producing a rise of intrauterine pressure of up to 35 mm Hg) contained less O_2 (1.5–9 percent saturation) than did samples withdrawn during a quiescent period between contractions. On delivery of the fetus, the uterus contracted strongly and the arterial O_2 saturation fell to low values, comparable with those commonly observed in human-cord blood samples, even though the placenta was not yet separated. It is therefore suggested that cord blood samples, taken after delivery, may give a misleading impression of the conditions of intrauterine life in the primate. And it is interesting that the same difference between the oxygen content of the blood in the ascending and the descending aorta exists as has been shown previously in ungulates.

Dr. Combs, Dr. Dennery, and Miss Saxon have been carrying out electroanatomical studies on connections between the cerebellum, diencephalon, and cerebral cortex. These experiments were all carried out initially in cats, but similiar studies will be made on asphyxiated and control monkeys in order to seek neurophysiological evidence of damage through asphyxiation to this important motor pathway. With electrical stimulation of the cerebellum, cortical responses were readily obtained from the contralateral anterior sigmoid (motor) gyrus. Smaller potentials were obtained from the contralateral posterior sigmoid gyrus. Ipsilateral responses, when present, were small, inconsistent, and only found in the anterior gyrus. The active cerebellar zone formed a longitudinal strip consisting of the lateral one-half of culmen, the medial two-thirds of crus I, the medial one-half of crus II, and the paramedian lobule. The largest response resulted from stimulating the anterior two-thirds of the medial one-third of crus I. The results were as readily obtained in Nembutalized as in curarized preparations.

In a related study, methodic stimulation throughout the diencephalon while recording from the ipsilateral sigmoid gyri has shown that the active diencephalic areas include essentially the sensory pathway and the course of the brachium conjunctivum.

In 17 animals with prior mesencephalic destruction of the medial lemniscus and the brachium conjunctivum, the active areas were the same with the exception of the anterior part of the red nucleus, the fields of Forel, the zona incerta, and the mesencephalic tegmentum. It has also been established that single-shock stimulation of the cerebellar hemispheres will induce in Nembutalized cats a bilateral multiple alpha-rhythm response in the cerebral cortex. This is most pronounced in the contralateral ectosylvian (auditory) cortex.

An observational study of behavior and social organization of rhesus monkeys in the free-range colony on Cayo Santiago (now numbering 300) was begun in June 1956, by Mr. Altmann. Dr. Koford from the University of California is continuing these investigations. When the Laboratory assumed control of the Santiago colony, it consisted of about 150 monkeys. Since then, they have increased to nearly twice that number; there are now about 280. The rate of mortality is low, about 5 percent per year. Five females have lived more than 20 years on the island. The ratio of

mature males (5 or more years of age) to mature females (4 or more years of age) is 1 to 5. Mating activity commences late in July. Nearly all young are born during the period of 4 months commencing in February. Approximately 65 percent of the mature females have infants born this year. At least since mid-1956, the monkey population has been divided into two principal social groups, one approximately twice as large as the other. Except for an occasional subadult male, members do not shift from one group to the other. The groups normally occupy separate parts of the island, though they share much common ground. Near the center of each group are two or three of the largest males, which are clearly highest in dominance rank. Nearby are females with their young, up to 3 years old. Subadult males, low in dominance, are usually at the periphery of the groups.

Mr. Chandler, under the direction of Dr. Gavan at the Medical College of South Carolina, is carrying out anthropometric studies on the Cayo Santiago colony. They are conducting a longitudinal growth study with main emphasis on normal, development morphology. They hope to identify the normal rate, duration, and course of growth and to isolate some of the factors which may modify this pattern.

OTHER ACTIVITIES

The scientists of the Laboratory of Neuroanatomical Sciences have been called upon to participate in activities not directly related to conducting experiments. Several serve on committees and advisory panels.

Dr. Palay transferred from the Anatomy and Physiology Fellowship Panel to the Cell Biology Study Section, DRG. He is also Secretary of the Assembly of Scientists of NIMH–NINDB.

Dr. Lloyd Guth is a member of the Anatomy and Physiology Fellowship Panel, DRG. Dr. Milton Brightman is Vice President of the Washington Society of Electron Microscopy. Dr. C. J. Bailey recently left this laboratory to serve as Executive Secretary of the Mental Health Study Section, DRG.

Dr. Rasmussen serves on the Committee on Hearing and Bio-acoustics as a representative of NIH.

Dr. C. M. Combs holds a courtesy appointment as Associate Professor of Anatomy, and Dr. H. N. Jacobson, as Associate in Obstetrics, at the University of Puerto Rico School of Medicine. Neither one of these appointments carries teaching duties but both provide valuable contacts with other scientists.

The chief of the Laboratory serves on the following committees: Foreign Fellowship Committee, DRG; Anatomical Sciences Training Committee, DGMS; Committee on Primates, National Academy of Sciences-National Research Council; Executive Committee, American Association of Anatomists; Membership Committee, American Academy of Neurology; Committee on International Collaboration, American Academy of Neurology; Research Advisory Board, United Cerebral Palsy Research and Education Foundation.

Editorial tasks have engaged some of the investigators' time during the year. The chief of the Laboratory is editor of *Experimental Neurology;* Dr. Palay is on the editorial board of the same. Dr. Rasmussen is editor of a monograph entitled *Neural Mechanisms of the Auditory and Vestibular Systems* which is the sixth in a series of "Symposia in the Neuroanatomical Sciences," edited by the chief of the Laboratory. Dr. Guth is translator and editor of Ramón y Cajal's book on neurogenesis.

Laboratory of Neurochemistry

Until more space becomes available to the Laboratory, it will not be feasible to establish one or two desirable additional sections and to give more adequate working space to the two present Sections. Until such a time, we have had to abandon our search for a Laboratory chief. During the year. we submitted plans which reflect our space and budgetary needs. We are hopeful that a new building will be constructed here to accommodate some of the growing needs of the Basic Research Program and that such construction will be available for occupancy sometime during 1964. The Laboratory of Neurochemistry will at that time be due for major expansion. It is our plan in the meantime, after construction appropriations have been committed, to recruit and assemble the necessary personnel to make this

a most effective and resourceful establishment for the investigation of neurochemical problems.

Not a moment is to be lost in this enterprise because advances in biochemistry and related achievements in other complementary mental and neurological disciplines are ripe for imaginative exploitation by a strong team of neurochemists. Although the Laboratory then will be much expanded and as yet only two elements of the total breadth of interest in this field are represented, it is clear from what we already have within the Laboratory and within the program that this expansion of neurochemistry will represent growth from strength within this essential discipline.

SECTION ON PHYSICAL CHEMISTRY

Dr. David Davies has been carrying on further investigations of the formations and structures of complexes of macromolecules related to nucleic acids. Although the interaction of the synthetic polynucleotides, polyadenylate and polyuridylate, can lead to a complex which has the double helical configuration and paired complementary structure of native DNA, X-ray diffraction studies of the strongly aggregating system polycytidylate-polyinosinate show no DNA-type structure. Centrifugal studies of this latter complex indicate strong aggregation, and spectral studies, moreover, show gross changes in both the rotational and vibrational frequencies associated with the purine and pyrimidine ring substituents of these molecules. The diffraction pattern of this simpler complex appears to be closely related to that obtained for the as yet undetermined structure of native DNA.

Dr. Dan F. Bradley, with Dr. M. Kenneth Wolf of the Laboratory of Neuroanatomical Sciences, NINDB, Dr. Gary Felsenfeld of the University of Pittsburgh, and Dr. Audrey L. Stone, a visiting scientist in the Section, has been carrying out extensive studies on the aggregation of dyes bound to polyanions. There exist a large number of cationic dyes which exhibit striking metachromatic color changes when used to stain polyanions such as desoxyribonucleic acid, ribonucleic acid, heparin, and hyaluronic acid in tissue sections. Perhaps the single most important discovery is that the structure of a polyanion determines in part the strength of dye-dye interaction so that the color of bound dye can tell us about the polyanion to which it is bound. We have collected a considerable body of data which supports the generalization that the more rigid and well-ordered the polyanion, the weaker the dye-dye interaction. For example, all samples of native, well-ordered desoxyribonucleic acid (DNA) examined show the same low value of this interaction, but, when they are disordered by heat denaturation, the strength of the interaction increases. This observation has been developed to the point where the color of bound dye can be used to determine the degree of nativeness of DNA specimens. Applying this general principle to structural changes in synthetic nucleic acids it has been possible to confirm certain hypothesized transitions. A quantitative method of analysis in the microgram range for nucleic acids, mucopoly saccharides, and synthetic polyanions has been developed.

Dr. David Davies and Dr. Sidney Bernhard, in collaboration with Dr. T. Viswanatha of the Laboratory of Pharmacology, NIAMD, and Dr. John C. Kendrew, of the MRC Unit in Molecular Biology, Cambridge, England, have been attempting to establish the detailed molecular configuration of the atoms of an enzymatic site and it has been found possible thus far to crystallize some small enzymes and enzyme fragments. Some of these have been crystallized with specific substrates and/or heavy atoms. X-ray diffraction patterns of one heavy-atom labeled enzyme have been obtained.

A new project this year is the study of enzyme models carried out by Dr. Sidney Bernhard with Drs. Ephraim Katchalski and Arieh Berger of the Weizmann Institute. Since the variety of enzyme-catalyzed reactions is enormous, it is necessary in any finite study to limit the models to either a particular enzyme or particular class of enzymes. In this investigation we have limited ourselves to the largest-known class of enzymes with a common *catalytic* amino-acid sequence. The sequence -glycyl-aspartyl-seryl-glycl- is catalytically inert. Derivatives of this sequence with the hydroxyl of serine esterified (a proposed enzyme-substrate intermediate) show no unusual, reactive, chemical properties. Derivatives of this sequence in which the "beta" carboxyl group of aspartic acid is converted to an ester or an amide have striking properties. Thus, for example, the

beta-benzyl-ester derivative of this sequence is hydrolyzed (debenzoylated) at a rate one million times faster than normal benzyl esters. The reaction has a "turnover rate" at neutral pH comparable with enzymatic reactions. Molecular model constructions have resulted in a theoretical proposal of the three dimensional conformation of this molecule in the course of reaction. This "intermediate" conformation has been proposed as the catalytically active form of the enzyme, the configuration being stabilized by the protein superstructure.

One of the most important concepts in molecular biology is that the function of a protein is determined by the sequence in which the component amino acids are linked together. The first step in the theoretical approach to the problem of sequence determination in proteins was to develop a logical system for processing bits of information about individual peptides to determine the unique sequence in the protein. Drs. Sidney Bernhard and Dan Bradley have developed such a system in collaboration with Dr. W. L. Duda at the IBM Company. We have provisionally named this system the Logical Unique-Sequence Tracer and have used it to reconstruct long sequences (100 amino acids) from what seemed to be a hopeless confusion of data. However, the system cannot be used to its maximum efficiency by an individual because it involves a very large number of logical decisions so that it is being programed for a high-speed digital computer. With this program in operation, sequencing can be done with a minimum of experimental data. It is planned to carry out *Gedanken* fission experiments to find out on a statistical basis for real proteins how much information value is contained in various-sized peptides, the optimum size of peptides, value of determining only partial sequences on peptides, etc. A preliminary result of great interest is that a method of "indifferent" fission in which a relatively large number of peptide bonds are broken with about equal velocities, *e.g.*, by acid hydrolysis, is suggested as a method which might provide the optimum distribution of breaks in the protein to achieve maximum information. We hope to test this suggestion experimentally in conjunction with scientists in England who are working on the sequence of myoglobin.

SECTION ON LIPID CHEMISTRY

Dr. Roscoe Brady has continued his investigations on the pathways of synthesis of complex lipids. The report from this section in 1958 that malonyl coenzyme A is the key intermediate in the biosynthesis of long-chain fatty acids has been confirmed by two other groups of investigators. Work during the past year has dealt mainly with purification of the requisite enzyme system and the preparation of specifically labeled intermediary compounds. A 600-fold purified enzyme system from rat liver tissue has been used to investigate the detailed mechanism of the condensing and reducing reactions required for fatty-acid synthesis. When the appropriate intermediary compounds are incubated with the enzyme, the only cofactor required for fatty-acid formation is a source of hydrogen atoms. Triphosphopyridine nucleotide (TPNH) is the preferred material although diphosphopyridine nucleotide is about two-thirds as effective as TPNH.

Conversion of acetyl coenzyme A to malonyl coenzyme A was also discovered in this section in 1958, and a report of this reaction was published early this year. This finding has also received confirmation in laboratories in the U.S. as well as Germany and Japan. The enzyme system has been purified, and the reaction exhibits the following dependence: There is an absolute requirement for adenosine triphosphate and the vitamin biotin. The presence of divalent metal ions is necessary, and the respective efficacy is $Mg^{++}>Mn^{++}>Co^{++}$. A detailed study of the mechanism of this carboxylation reaction is being carried out with a purified enzyme preparation.

Dr. Eberhard Trams has worked on the formation of complex sphingolipids which has resulted in the discovery that splenic tissue obtained from patients with Gaucher's disease catalyzes the formation of the accumulated offending cerebrosides *in situ*. With the use of various precursors of the cerebroside molecule, it has become apparent that the entire molecule may be synthesized *de novo*. These observations tend to render unlikely the suggestion that the etiology of Gaucher's disease is an excessive accumulation of catabolic materials from red-blood-cell destruction and is consistent with the failure of other investigators to demonstrate an increased level of plasma cerebroside in Gaucher's disease.

We have prepared labeled psychosine (sphingosine-O-galactoside) for investigating the biosynthesis of gangliosides, the polyhexose sphingolipids present in ganglion cells of the cortex. Preliminary experiments indicate that the enzymatic synthesis of these compounds has been successfully demonstrated for the first time in cell-free preparations of brain tissue. We have also devised an ultrasensitive analytical method for the quantitative determination of gangliosides. The availability of this procedure is required for studying the metabolism of gangliosides. We expect to undertake investigations of this nature in cortical biopsy specimens obtained from patients with Tay-Sachs disease. This condition is characterized by the accumulation of abnormally large quantities of gangliosides in ganglion cells.

A method has been devised by Drs. Eberhard Trams, Roscoe Brady, and Eugen Hecht for the quantitative determination of free sphingosine in plasma. The procedure was perfected in cooperation with Dr. Charles Sweeley of the National Heart Institute, and initial determinations indicate a level of 1 to 14 micrograms of sphingosine per milliliter of plasma. We expect to use this technique to determine the normal level of sphingosine in the cerebrospinal fluid and in samples obtained from patients with demyelinating diseases. It has also been observed that free sphingosine can act as a prothrombin conversion factor. The significance of this finding on the mechanism of blood coagulation is under investigation.

Within the past year, confirmation has appeared for the demonstration in this laboratory by Drs. Bernard Agranoff and Roscoe Brady of a new class of intermediary compounds called *liponucleotides*. These highly reactive compounds are formed by the enzymatic reaction between phosphatidic acids (the monophosphate ester of a diglyceride) and cytidine nucleotides. Cytidine diphosphate diglyceride is the required intermediate for inositol phosphatide formation. Inositol phosphatides exhibit very rapid metabolic turnover in brain and are markedly affected by physiological and pharmacological agents such as acetylcholine and chlorpromazine. Inositol phosphatides have been implicated in trans-membrane secretory processes, and the role of these materials in these reactions is under investigation. In the course of these studies, the nature of the metabolic antagonism between inositol and choline has been demonstrated. These substances compete with each other for the syntheses of essential phospholipids in growing animals.

Dr. Bernard Agranoff spent the past year working at the Max Planck Institut für Zellchemie in Munich where he participated in studies which dealt with several important steps in the pathway of the biosynthesis of terpenes. Specifically, he demonstrated the enzymatic isomerization of the 5-carbon intermediate isopentenyl pyrophosphate to dimethylallyl pyrophosphate. The latter compound is required for the condensation of two 5-carbon fragments to form the 10-carbon geranyl pyrophosphate and subsequently the 15-carbon farnesol pyrophosphate. He participated in experiments which demonstrated the conversion of these larger molecules to squalene, the immediate precursor of cholesterol and other terpenoid materials such as the steroid hormones and vitamins A, D, K, and E.

Laboratory of Neurophysiology

The Spinal Cord Section, under the leadership of Dr. Karl Krank, is proceeding with analysis of fundamental work on generation of impulses in nerve cells. The work is immediately aimed at determining what parts of the neuron produce the A and B spikes and analysis of the role of the dendritic processes. Clamping technics have been applied and the results are consistent with the previous hypothesis that the A spike originates in the axon and that the B spike originates from membrane at least partly outside the soma. Work is going forward to plot the potential field around a single cell activated by antidromic stimulation. This project is dependent on application of some method of improving the signal-to-noise ratio.

Practical methods for information-retrieving are under development in joint projects of this laboratory and the Laboratory of Clinical Sciences. A modification of a method devised by Mr. Robert Cox will be used by Dr. Frank in the first phase of this project. This is an important and pertinent area. Means must be found to raise signal-to-noise ratio for microelectrode work as well as for other purposes.

Work is moving in the direction of analysis of integrating mechanisms of the neuron.

It is generally assumed that CNS membrane potentials are sensitive to anoxia, and a recent publication purported to prove that assumption using intracellular recording technics. In a series of excellently designed experiments, Dr. Phillip Nelson, Dr. Frank, and Mrs. Mary Becker have shown this is not true. The stability of the membrane in hypoxia also has important consequences for certain aspects of spreading cortical depression. Previous work has shown that, with macroelectrodes in the spinal grey, hypoxia produces a quick negative swing. The present work indicates this quick negative swing is essentially an artifact, probably due to abnormal sensitivity to hypoxia of the neurons injured by the electrode.

The Section on Special Senses, under the leadership of Dr. Ichiji Tasaki, has several developments. Perhaps the most important one is serious application of adequate theoretical treatment to tracer studies. This work was done in collaboration with Drs. Torsten Teorell from Uppsala, Ulrich Franck from Darnstadt, Germany, and Leslie Nims from Brookhaven. It has been recognized for some time that tracer data often contain inadequate information. Recently, rigorous theoretical treatment of these problems has been accomplished by Dr. Nims and others. Dr. Tasaki and his collaborators have extended these theoretical developments and have made practical application of these concepts on both living and nonliving membranes, to get accurate information on ion movements.

An extensive study of the movement of radioactive tracers across the squid axon membrane was carried out by Drs. Constantine Spyropoulos, Tasaki, and Teorell. The tracers examined include Na^{24}, K^{42}, Ca^{45}, tritiated water, Cl^{36}, labelled phosphate and sulfate. The data obtained were treated on the basis of the new concept developed by these investigators.

The Section on Limbic Integration and Behavior, under the leadership of Dr. Paul MacLean, has proceeded with a broad program in the general area of brain and behavior and the limbic system in particular. This program involves a comprehensive employment of behavioral observations, conditioning and learning studies, electrical examination of the CNS, biochemical lesions, and neuroanatomical work.

In the past there has been a notable lack of information about the localization of genital function in the brain. The Section on Limbic Integration and Behavior is employing the squirrel monkey in a systematic exploration of the brain to identify structures involved in penile erection. Thus far positive locus has been discovered within a major subdivision of the limbic system.

Three other studies of a complemental nature are also in progress. One pertains to the function of the mammallary bodies. A second, conducted by Drs. John Gergen and MacLean, is aimed at analyses of the interaction between the highly excitable hippocampal system and other parts of the brain, as well as the interaction between the hippocampal system and associated neuro-endocrine mechanisms. The third pertains to a study of the sexual and social behavior of the squirrel monkey. Dr. Detlev Ploog is conducting, on a small group of squirrel monkeys, a valuable naturalistic study, which is remarkable not only because of the nature of the information obtained, but also as an illustration of what can be done with a small group of animals in a crowded laboratory setting. Dr. MacLean's introduction of the squirrel monkey into this laboratory has been in itself a valuable contribution, and it may have been partly instrumental in introducing the squirrel monkey to the National Aeronautics and Space Administration. An anatomical atlas is being prepared to aid in the laboratory use of the squirrel monkey. The variety of work done by this section is unique, and is also a unique example of good basic science with closer than usual relation to clinical research.

The Section on General Neurophysiology has been proceeding with several projects. One of these is an intensive program conducted by Drs. Eric Kandel, Alden Spencer, and Floyd Brinley, involving unitary extracellular and intracellular recording from the pyramidal cells of the hippocampus of the cat. This work deals with the fundamental excitation processes of the neuron. A great deal of new information has been obtained. Similarities with and differences from data derived from the spinal motoneuron have been demonstrated. The discovery of prominent depolarizing after-potentials in hippocampal cells is particularly significant in view of the relatively

high excitability of the hippocampus. It is interesting to note that the electronmicroscope pictures show a smaller than usual extracellular space for the hippocampal neurons. This supplies inferential support for the hypothesis that accumulation of K ion in extracellular space is the cause of the depolarizing after-potentials. There are also important inferences in this argument for mechanisms of spreading cortical depression. This program is gaining some knowledge of actual integrative mechanisms of which the firing of the cell axon is the end point.

A valuable project, using tracers to examine K ion release from the cortex by various chemical agents and spreading cortical depression, was accomplished.

Dr. Barbara Renkin is proceeding with an interesting method of conducting analysis of sensory discrimination at cortical and thalamic levels.

Dr. Felix Strumwasser has made progress in the preliminary instrumentation of telemetering techniques to be used in studying manmmalian hibernation. He has made microelectrode studies of cells in brain and dorsal-root ganglion of the frog. Incidental to these studies a useful method was developed for extracellular stimulation of single cells permitting excitability studies to be made of units normally at rest. Dr. Strumwasser is applying this technique to a study of excitability changes during some processes involved in learning. Technical advances in recording from the dorsal-root ganglion of the frog have permitted a study of the relative roles of the different parts of the membranes of these cells.

Dr. Freygang, working this past year with Dr. Richard Adrian and Mr. Andrew Huxley at the Physiological Laboratory, Cambridge, England, has been investigating the electrical characteristics of the membrane of muscle fiber with a special interest in properties that are peculiar to this kind of cell. Such properties need to be understood in order to relate the electrical events to the contractile properties of muscles.

Laboratory of Cellular Pharmacology

When the Laboratory of Cellular Pharmacology was originated in 1954 a broad program of research was devised centering around three main lines of investigation: (1) biological methylation, (2) amino acid metabolism, and (3) comparative biochemistry. Although large overlaps have made subdivision of the various research activities among these different areas somewhat artificial, the device has nevertheless been useful in classifying our activities.

While these areas continue to hold the interest of the laboratory, in the latter part of 1958 and more clearly 1959, some reorientation of the Laboratory's efforts took place, and the major areas of investigation can now be grouped more profitably around four topics: (a) mechanisms and pathways of protein biosynthesis, (b) biological methylation, (c) biological oxygenation and (d) alkaloid biosynthesis. These new areas stem more or less directly from the three original ones: for instance, alkaloid biosynthesis is conceptually related to our lasting interest in comparative biochemistry and obviously studies on protein biosynthesis represent an extension of earlier efforts in the field of amino-acid metabolism.

From an administrative standpoint, the most notable advance has been the establishment of a new Section on Alkaloid Biosynthesis and Plant Metabolism, and the inauguration of a new research program in this area. The research greenhouse facility has been in operation under Dr. S. H. Mudd since early spring and is now fully functional and adequately, if not yet optimally, staffed. More significant is the fact that the research program which had been originally formulated for this project and had formed the basis for its justification and realization is already beginning to bear fruit, precisely along the lines we had anticipated.

Our findings in this area illustrate once again the value of studying fundamental biochemical mechanisms in whatever biological material is most convenient with the assurance that the facts in a given form may well apply to widely divergent forms.

Specifically, it has been established that, in the biosynthesis of the alkaloids, N-methyltyramine, hordenine, and gramine, by cell-free extracts of barley or millet, the methyl group of these compounds is donated by S-adenosylmethionine. Moreover, it was shown that barley can indeed synthesize S-adenosylmethionine which is identical to that made by vertebrates even to the extent of having the same stereochemical configuration about the asymmetric sulfur and α=carbon atoms.

Together, these facts very strongly suggest that the predominant pathway of plant transmethylation lies through S-adenosylmethionine just as it does in vertebrates and microorganisms.

A matter which requires further exploration is suggested by the structural resemblance of two of the particular alkaloids studied to the adrenal hormones and of the third to serotonin: If the role, as yet unknown, of these compounds in plant metabolism can be elucidated, perhaps we shall get a hint about the role of these neurohormones or related hallucinogenic materials.

Another area of interest in regard to this system points up the particular advantages of plants as complex, biological forms, containing many specialized types of tissues and elaborate anatomical and hormonal systems of intraindividual communication, and yet much simpler than the vertebrate and vastly more responsive to environmental manipulation and experimental control. Thus, the formation of the alkaloids now being studied is known to be under not only genetic control but under other controls as well, so that the formation occurs in a dramatic outburst at a specified stage of ontogenesis and in restricted types of tissues. It seems not unlikely that a study of the interplay of the control mechanisms at work here will give some insight into the important question of how enzyme formation and activity are governed in higher organisms. The genetic, environmental, tissue-specific and hormonal factors cooperating in this system are undoubtedly complex but it is hoped that the great advantage offered by the relative malleability of the plant to experimental control will aid in elucidating these questions.

With the realization of our plans in the alkaloid biosynthesis area it became desirable to divide the laboratory at least administratively into three sections under the leadership of Dr. Seymour Kaufman, Dr. Mudd, and myself.

Dr. Kaufman's section continued to center its interest in the area of biological oxygenation. The two different enzymatic hydroxylation reactions being studied by Drs. Kaufman and Ephraim Levin are yielding results complementary to each other in many ways. In both the phenylalanine and the DOPamine hydroxylating systems, new roles have been found for well-known vitamins—folic acid and ascorbic acid respectively. Specifically, it has been established that these cofactors play the role of electron donors in the reactions in which they respectively participate. In both systems, a substrate-dependent (i.e., phenylalanine or DOPamine) oxidation of the cofactors takes place resulting in the oxidation of tetrahydrofolic and to dihydrofolic acid in the phenylalanine system, and of ascorbic acid to dehydroascorbic acid in the DOPamine system. While the ultimate electron acceptor in both reactions is oxygen, it is possible that an enzyme-bound metal or even the substrate molecule may serve as the immediate electron acceptor.

These studies, in addition to their intrinsic scientific value, contribute significantly to the area of basic research in the field of neurology and mental health for they are of obvious and direct significance to the problems of synthesis and function of the catecholamines, and to the pathogenesis of phenylketonuria oligophrenica. Indeed, the basic facts discovered by Dr. Kaufman in these studies are being utilized by Dr. Kaufman and Dr. William P. Weiss, of the Laboratory of Clinical Science, in a clinical study of phenylketonuric children in the hope of discovering and devising some way to alleviate morbidity in this area. Although phenylketonuria contributes only a small fraction of the patients in the area of mental retardation it would be highly gratifying to be able to contribute in some measure to the solution of this medical problem.

The work of the Section on Proteins centered primarily around two problems of fundamental interest. The protein-synthesis project, which is occupying the main interest of the laboratory, is a multipronged attack on what may be considered one of the central problems of biochemistry. At the present time, it appears most profitable to concentrate our efforts on a study of the chemistry, molecular configuration, and biological properties of S–RNA, a polynucleotide of relatively small molecular weight which appears to function as an acceptor and donor of activated amino acids for protein synthesis. S–RNA has been studied with respect to its physicochemical properties, as revealed by electrophoresis ultracentrifugation and viscosity measurements; its chemical nature, as demonstrated by base composition; and its enzymatic and biochemical characteristics. A significant feature of the biological activity of S–RNA

is its specificity for different amino acids but the basis for this specificity is yet unknown. Work from this laboratory has established that molecular weight plays no role as a specificity determinant and furthermore that the different S–RNAs which are specific for the different amino acids all have similar or identical molecular weight. This suggests that the basis of the biological specificity must reside in the nucleotide composition or sequence, and by a study of this problem it is hoped to obtain some clue as to principles of biological coding mechanisms. In these objectives the Laboratory has enjoyed the collaboration of Dr. Maxine Singer of the Laboratory of Biochemistry in NIAMD.

The second problem (which has progressed favorably in the last year) has been the study of the properties of thetin homocysteine methylpherase. It has been known for some time now as the result of the work of this laboratory that this protein, which represents approximately 1 percent of the total liver protein, is capable of undergoing an interesting and rather unique reversible polymerization reaction. The biological significance of this reaction is not yet fully understood; the possibility is under investigation that it may be related to the complex changes occurring during cell mytosis or even more generally that this protein may fulfill some cyto architectural role in the cell.

During 1959, the Laboratory enjoyed and benefited from the association of three visiting scientists:

Dr. Olga Greengard, formerly of Middlesex Medical School, London, England;

Dr. Othmar Gabriel, formerly of Vienna University and now at Columbia University;

Dr. Claude Blanc, formerly of Marseille University and now in the Laboratory of Cellular Pharmacology.

Section on Technical Development

During the calendar year 1959, the Section on Technical Development continued its role as a supporting organization. About 250 projects of various kinds were completed, serving the needs of virtually every section in each laboratory of the research program. Projects of representative types will be mentioned further on in this report.

The Section has continued to be a source of material and components for investigators or their representatives. Procurement is carried out with the needs of the entire basic research program in mind. Obsolete equipment and components have been shunted to Surplus Property in order to better utilize the available space for an up-to-date stock. The blanket purchase-order arrangement with Capitol Radio Wholesalers has proven itself so well, due to complete inventory and fine cooperation, that it is now possible to maintain adequate stock in the Section on Technical Development at the expense of only a half rather than a whole module of space. This provides one additional work area and makes practical the recruitment of another technician.

Addition of Mr. Paul Byrne, a general laboratory mechanic, to the staff during 1959 resulted in a very significant decrease in the waiting time of the researchers who have a purely mechanical problem, and permitted detaching one man for thirty days' duty at the Marine Biological Laboratory, Woods Hole, Mass.

Additional power supplies, waveform generators, and other universally used instruments have been made available, on loan, for short-term experiments or as interim equipment during purchasing delays.

The Section's fund of technical information and new product listings is being improved continually. As new equipment and components are developed, literature is requested and filed. A technical library is slowly and carefully being built, with some 60 volumes of text on physics, electronics, semiconductors, optics, and biological techniques now on hand.

The Section's time is divided among several functions: counsel, construction, development, maintenance, and repair. Each category is a part of both internal operation and services to the designated Laboratories. Time permitting, ideas originating within the Section are implemented, where they will be of value in the future, although not yet the object of a specific request. The majority of time is spent in the construction of new instruments and devices to specifications presented by investigators. Equipment previously used is adapted or modified for another purpose. Repairs are made on both commercial equipment and the specialized units that have been developed at the NINDB or NIMH.

Working space and direct assistance are still provided to the scientist who wishes to work on a problem himself. This continues to be a much used and much appreciated function of the Section. It also seems to fit in well with projects underway by members of the Section.

The goals of the Section on Technical Development are unchanged. Foremost is the development of a better tool for the scientist to use, in an effort to create the means for the realization of an idea. As thinking extends a tool to its limits, the justification and need arise for another and better tool. Secondly is the concept of direct assistance whenever possible—assistance meaning another pair of hands in the scientist's own laboratory, the loan of equipment and components, and the exchange of ideas.

In regard to projects completed, a résumé of several representative types will be given.

An auxiliary cathode-ray tube display system was designed and built. This "slave scope" works with the Tektronix Type 502, a dual-channel oscilloscope. Waveforms may be viewed on either channel of the "slave" simultaneously with the same waveform on the corresponding beam of the master. Adjustments are provided to equalize such variables as intensity, focus, sensitivity etc. It is used in conjunction with a camera, enabling permanent records to be made. Another slave system, using the Tektronix Type 535 Oscilloscope was built. It is similar to previous models using the Type 535, the variation being one of control circuitry.

It seemed advisable to develop a power supply capable of powering a Type 122 pre-amplifier, which normally uses batteries as a power source. Due to the expense of batteries and their inopportune failures, a supply was designed and built, using plug-in stages. Later, due to an increase in cost of these units, it was built without the plug-in units. A third model now on the design boards will make use of the regulating capabilities of zener diodes. This model should be useful as a battery replacement for the more demanding d.c. amplifiers.

Design and construction of a working model of an electrode holder and advance mechanism are now complete. With this unit, dual concentric micropipettes can be accurately positioned and the inner controlled with respect to the outer. Not only is this a critical mechanical assembly, but provisions had to be made for electrical connections and for mounting to the amplifier in such a way that removal can be rapid. Negotiations are completed for the production of a small number of these mechanisms by a private concern.

A device for the control and programing of light stimuli has been developed for experimentation with rats. By means of programing the light patterns, apparent movement can be simulated. This apparent movement can be regulated with respect to time. The effect of the moving stimuli upon the animals is then observed and recorded.

Several high-input impedance probes have been constructed with miniaturization, shielding, and ease of operation as considerations. The input electron tube is mounted in especially machined aluminum shields, turned down to a few mils in thickness to reduce size and weight. Work is now progressing towards design of a probe unit to be compatible with the electrode holder and advance mechanism mentioned previously.

An intercom has been designed for use between an observer and two subjects, all located in separate rooms. Signals, as well as verbal communication, are possible with this system to be used in dream studies in conjunction with an EEG recorder modified for this purpose. Design and construction of amplifiers and signal oscillators are complete. Further EEG modifications are pending.

Additional enumeration probably serves no purpose since a complete list cannot be presented in a report of reasonable length. The projects are widely varied, including repair and modification as well as other new equipment. The coming year will show further progress along the lines of more and better service to the research program. This progress will be due to the increasing proficiency of the present staff and, hopefully, to the addition of another capable electronics person.

CLINICAL INVESTIGATIONS—NIMH

INTRODUCTION*

The inception of the clinical investigations program seven years ago afforded an unusual opportunity for the development of a mental-health research unit. Eighty beds were made available, together with ample nursing, social service, and physical and recreational therapy support. Since the beds were in a Clinical Center rather than in a hospital, there were no service obligations, no teaching responsibilities. In fact, the beds did not even have to be devoted to patient care, but could be used for a variety of studies of normal individuals. There was similar freedom with respect to recruitment of investigators. At a time when research foundations were beginning to consider the merits of block versus project research grants, and when the idea of lifetime appointments for a limited number of research professors was just being broached, we were able to select several lifetime laboratory chiefs, a number of section chiefs, and to provide each of them with block support for long-range studies of broad scope.

In these circumstances it seems appropriate to scrutinize carefully the established psychiatric research organizations. Did the freedom from conventional teaching and service responsibilities and reasonable assurance of long-term support afford us an opportunity to study new problems or old problems in new ways? Should we endeavor to utilize these resources to establish a more or less traditional setting hopefully free, perhaps, from some of the common practical and material burdens which restrict freedom for research? Or should we to the best of our abilities try to develop a different type of setting and organization which might facilitate the production of data which could augment and extend the significance of the important studies being carried out in many excellent service and teaching hospitals?

It was felt that among the more important but least amply supported (by systematic observation) theories in psychiatry were those related to developmental and psychosocial determinants of personality formation and behavior. The great concentration of biological scientists at the National Institutes of Health promised powerful support for investigations of the organic bases of normal and abnormal behavior. At the same time the location of our facilities in a structure which was architecturally designed and administratively geared for an organic approach to chronic disease dictated against the establishment of a community health facility with in- and out-patient, consultative, and home-visit services, which might also have provided a unique setting for significant mental-health studies. It was decided, then, to make the study of behavior rather than psychiatric treatment our central theme, and to study normal behavior as well as a selected variety of behavior disorders. This did not mean that treatment would be neglected; on the contrary, it was hoped to establish a highly effective therapeutic setting. The openness and freedom of expression which occur in successful therapy provide information about thoughts and feelings which cannot be gained by other means. The study of therapy brings to light some of the important forces which operate to change behavior patterns. This has obvious implications for education, and for any culture which would hope to bring about a fuller and more creative life. Similarly, to the extent that mental illness can be looked upon as an experiment of nature, the study of the social, psychological, genetic, and biological variables which may appear in consistent patterns with different types of personalities and disorders would provide the basis for a more powerful theory of behavior than is now available. The establishment of longitudinal studies of child development would provide an ideal instrument for verification of hypotheses derived from cross-sectional approaches, and would in turn feed back suggestions for scrutiny by the groups studying other phases of behavior. Finally, the opportunity of setting up a clinical neuropharmacology center in collaboration with Saint Elizabeths Hospital would significantly extend the scope of the experimental studies in which we could engage.

In keeping with this broad goal we did not limit our recruitment to the clinical therapeutic disciplines, but sought anthropologists, sociologists, and psychologists on the one hand; pharmacologists, physiologists, and biochemists on the other.

*Prepared by Robert A. Cohen, M.D., Ph. D., Director of Clinical Investigations, NIMH.

The range of disciplines and the areas of study are roughly indicated in the table below:

	Pathological behavior			Normal behavior			Animal behavior
	"Acting out" disorder	Disorders of thought and affect	Psychosomatic disorders	Adult	Aged	Child	
Anthropology							
Sociology							
Psychology							
Psychiatry							
Pharmacology							
Physiology							
Biochemistry							

Not all these areas are being intensively studied, nor is each discipline represented in each study. In their individual reports, the laboratory and branch chiefs have given substantive reviews of the work in progress. Before proceeding to them, I wish to point out some of the issues we have encountered in our efforts to give substance to this idea for a mental-health research program.

The first problem faced was that of staff recruitment. For such a goal as has been outlined, it would have been desirable to have brought together laboratory chiefs representing at least several of the important disciplines, to have given them time to get acquainted with each other's concepts, to outline several investigative problems of mutual interest in addition to others they would pursue individually, and then to assemble the supporting staff. Even in this demiparadise, however, time was a precious commodity. A commitment had already been given to open the first ward, 4½ months after the Director of Clinical Investigations was appointed, and by the end of the year a 50-bed unit was supposed to be in operation. Although this time schedule was not strictly enforced, it is not necessary to stretch one's imagination to picture some of the difficulties encountered. Both the investigative groups and the hospital organization were built up from scratch. Every appointment had to be made through the U.S. Public Health Service Commissioned Corps or through the Civil Service system. And when finally a staff was assembled, each member bringing with him the point of view and way of operating which were traditional at his previous assignment, we had to forge a new set of procedures which would be suitable for our goals, for our staff as a whole, and for our new setting.

Since it was not possible to recruit the laboratory chiefs and bring them together to plan our joint program before operations actually began, the directors of each of the major divisions were appointed as soon as the interest of a suitable individual could be enlisted. Each laboratory chief joined in the consideration of those who were appointed subsequently, which has made possible good personal as well as potentially good intellectual and working relationships. This, in my opinion, is relevant to the effectiveness of interdisciplinary research since I believe that some conceptualizations may require more than one mind, more than one way of looking at behavioral events. However, the result of this manner of program growth has been the development by each laboratory and branch chief first of his own individual program. It took 5 years to bring the group together, and now after 7 years, I believe that it would be fair to say that each laboratory has defined its major research goals; in each of them creditable investigations are well under way, and some significant studies have already been completed. In individual projects there has been considerable collaboration between laboratories, and in some of the laboratories several disciplines are strongly represented. In addition, there is a degree of overlapping interest between laboratories in several important research areas, even though each group varies its approach and focus. All this could serve to support a coalescence of interest in one or two problems of major theoretical importance which might constitute a limited portion of each laboratory program. While many factors favor the gradual formulation of several such broad research studies, it remains to be seen whether they are all that is necessary for truly creative interdisciplinary work.

I believe the first phase of the development of our research program is over. We are no longer laboring just to secure a firm footing, but can turn more of our attention to the circumstances under which we work. There are two sets of conditions—one external, the other internal—which affect our creativity, our sense of satisfaction and fulfillment in our work, and, consequently, our productivity

The National Institutes of Health developed from the old U.S. Public Health Service Hygienic Laboratory, and the list of our forebears includes such distinguished names as Rosenau, Reid Hunt, Voegtlin, Stiles, Mansfield Clark, Goldberger, Francis, and, more recently Kornberg. Despite this excellent record, every senior investigator we have approached in our recruitment program has had serious questions about freedom of research in a Government institution. Where public money is spent, controls of some sort are invariably instituted, questions asked at frequent intervals, certain regulations observed. Research workers traditionally believe that regulation should come from within themselves, and not imposed by external authority. Personal contact is important to them, and they are concerned about working in an organization whose very size makes it necessary that some decisions affecting their work be made at levels several steps removed from them. Those of us who look upon our land as the cradle of liberty, must feel some concern at the fairly widespread belief among scientists that, as liberty has become institutionalized, and scientific freedom restricted, a Government appointment should not be seriously considered.

On the other hand, however, there are many who believe the Government has a responsibility to conduct basic research, and are interested in becoming personally involved in the development of what they believe can become an excellent research institution. In my opinion we have made notable progress toward that goal. At the bench level, research problems can be selected by the investigators who will work on them. Unless new and additional space, equipment, and/or personnel are sought, the investigators have a high degree of control over the resources which will be employed in the attack upon the problem. As I see it, the most serious and difficult question which confronts us is that of program evaluation. This regularly comes up when promotions are considered and when proposals for expansion are presented. It becomes an issue when any request is made calling for a break in routine and established procedures. It is generally accepted as desirable to have strong representation of related scientific disciplines in each of our major program areas. It is obvious to everyone that in the large population recruited as a result of this decision, there must be variations in the competence and creativity of the research staff—some may approach genius, others of us may never be more than pedestrian. It is equally obvious that as the program expands and as the numbers of scientists increase, decision making can no longer be confined to the relatively small group who had the vision and who have borne the responsibility for the development of the National Institutes of Health as they stand today.

The Government operates as a line organization however, and line organizations make no provision for delegation of decision-making responsibility. The man on top remains responsible. As program grows and numbers increase, he has less and less contact with the individual members of his staff. Administrative routines are established to deal with regularly recurring and commonly accepted procedures. But the break in routine calls for individual consideration and makes a demand upon the chief's limited time. He no longer is able to maintain his earlier personal involvement in each phase of the developing program. Therefore he sets up a number of intermediate persons or groups to review requests and to advise him concerning the appropriate decision. In fact, if the program grows large enough, he may even call upon outside groups to assure him that the proposed plan has been carefully considered and merits support. In this process what begins as a highly personalized research issue or problem or request becomes progressively depersonalized as it passes from level to level on its way through the decision-making machinery. Such circumstances are obviously not conducive to creative effort, particularly when—as sometimes but not always happens—the effort may constitute an assault upon cherished concepts. It is here that the problem of individual creativity enters the picture. Most of us would grant an Einstein, a Fermi, or a Darwin any condition he deserves, but is equal freedom to be granted to the mine-run investigator? My answer to this question would be emphatically "Yes!" There is considerable evidence to support the belief that creativity is fostered by assigning the decision-making responsibility to the level at which all the immediately relevant facts are available. The development of the Assembly of Scientists is a step in this direction; the more the scientists as a group can share in the decision-making process, the more

responsive will it be to their needs as productive investigators.

But the most important answers to the question of what is needed to do thoughtful research lie not in our stars but within ourselves. It has, perhaps, been more stressful than we realize to come to this new type of institution and to settle down immediately to the pursuit of new knowledge. If a group from Walla-Walla should be first to settle on the moon, those of us who arrived a year later might expect to find a number of institutions closely resembling those we know exist in Walla-Walla. In fact, the very names of New Orleans, New York, New Haven, and New London suggest that for ages past the thoughts of explorers turn toward the scenes of their childhood. Although it is true that research was fostered first by the learned societies, it soon found its home in the universities where, until recent years, it largely remained. Although universities were supported by lordly patrons, research could hardly be said to have lived a life of luxury. Traditionally, it was conducted in a garret, with simple, home-made equipment, at odd hours snatched from teaching and family responsibilities, and often drew its inspiration from the pressure of conflicting forces with which the teacher or doctor was wrestling in the course of his daily life. In some instances the researcher did not even have the blessing of a university position—Einstein worked in a patent office, Beaumont at an isolated Army post. Probably such events as the successful development of the atomic bomb and Ehrlich's discovery of the effectiveness of 606 gave support to some of the thinking which led to the building of the Clinical Center. After all, if Einstein developed the theory of relativity in a patent office, what greater ideas might he have had if he had worked from the beginning in a large research institute? If Ehrlich had tested the therapeutic effectiveness of all 606 of his preparations simultaneously, might not millions of people have been spared the ravages of syphilis? If Shakespeare composed his poetry for a group of ragged players, what beautiful images might he not have brought forth if he had written from the comfort of an endowed chair?

Most of us came from universities and hospitals where we had to teach and had to treat patients. We had longed for an easing of these burdens, for time to pursue our ideas in leisurely and thoughtful fashion. But few of us ever dreamed that we would one day be given an institution not only with freedom from but an injunction against teaching and service, and told simply "create!" Research ideas come in unplanned-for places and at unplanned-for times, and not necessarily most often when one's professional life depends upon their appearance. It took an environment such as NIH which, despite its limitations, has, perhaps, the greatest degree of academic freedom to be found and makes us realize how truly protective teaching and service may be. When one earns his bread by these means, his research becomes a delightful avocation which can only serve to increase his self-esteem. Under such circumstances one dares to be unconventional, to let fancy roam—and perhaps it is no accident that basic research has flourished in universities more than it has in industrial laboratories, the occasional notable exception only serving to prove the rule. The fact is that NIH does resemble a foundation for industrial research in that our success is measured by a product alone, and not only by teaching and service as might be true in a university. Even though the product is basic research itself and not a better way to make lipstick, the concentration upon it and exclusive dedication to it might conceivably impede progress under certain circumstances. The atomic bomb and Ehrlich's final discovery of 606 were engineering feats. A basic-research institute cannot be organized for that type of activity.

Whatever the correct interpretation may be, I have seen the new staff member, after his first breath of freedom, begin to look around him for the chains he laid aside. Invitations to lecture are eagerly accepted. Some of us feel that we get our best ideas while teaching a group of eager, dedicated graduate students; others, while we are wrestling with the responsibilities of treating desperately ill patients. We find ourselves longing for some of the university structure and atmosphere. Still others find themselves at times wanting more colleagues to work with them; somehow, in order to seem truly worth while, the program in which they engage should cover every conceivable facet of the research problem in question. I do not mean by this remark to cast doubt on the value of exhaustive attacks upon important

problems, but simply to point out that on occasion we may have an impulse to assuage unwitting anxiety about the merit of a more-or-less limited problem by expanding its limits as if we were thereby increasing both its importance and its merit.

These, as I see them, are some of the important problems which face us in our effort to develop to its fullest the remarkable opportunity afforded at NIH. For this new type of institution we shall have to build, as the universities did over a longer period of time, structures which foster the highest degree of creative thought on the part of bench scientists and administrative leaders alike—structures not necessarily modeled precisely after those which have proved successful in universities, but which are fashioned out of the unique attributes of our own situation.

Report of the Chief of Clinical Care*

During the past year we consolidated our clinical care activities into two services. The Adult Psychiatry Branch service has three nursing units providing the settings for the study of family relations in schizophrenia, for the study of first-year college students who develop a psychiatric illness requiring hospitalization, and for psychosomatic studies with a group of normal control subjects. The Clinical Science Laboratory service, with two nursing units, uses one for a group of male chronic schizophrenic patients in the study of biological factors in schizophrenia, and the other for a group of normal subjects who serve as controls. Each unit is administered with considerable autonomy, integrated with the total program of the respective clinical service involved, and the services coordinated within the framework of policy and practice for care of patients within the Clinical Center.

The Psychiatric Nursing Service has been organized along the same lines. Interchange of nursing staff personnel between the nursing units of a service has facilitated development of greater understanding of the total research activities of the service, resulting in more interest and effectiveness in carrying out the work. The enlightened and devoted interest and support of Miss Agnes Middleton, Chief of the Psychiatric Nursing Service, and her staff, in meeting the many problems in connection with the nursing care of the patients, has contributed immeasurably to the development of our Clinical Investigations program.

The clinical service of the Child Research Branch was discontinued June 30, 1959, with the termination of the clinical studies of that Branch. The clinical facilities used by the Child Research Branch were transferred: Nursing Unit 4–E to the Adult Psychiatry Branch, and Building T–4 to the Biosocial Growth Center.

The Biosocial Growth Center, administratively a unit of the Office of the Director of Clinical Investigations, NIMH, began operations July 1, 1959, under the direction of Dr. Wells Goodrich. This new program is designed to investigate the earliest influences on normal growth, through concurrent studies of parental attitudes in prospective parents with a group of newly married couples, parents of newborn, and parents of healthy 2-year-old boys. At the same time, studies of neonates are being carried out in collaborating hospitals, and of normal 2-year-old boys in a nursery school setting in Building T–4. The research in Building T–4 is carried out as an out-patient operation through the Admission and Follow-Up Department of the Clinical Center.

In the past we have obtained the recommendation of the Medical Board and the approval of the Director of NIH for some of our psychological studies with normal subjects on an ad hoc basis. Recently, greater recognition was given to the NIMH need to carry out certain psychological and sociological studies in which the usually required medical work-up is not indicated or is contraindicated. The Director, NIH, has approved the Medical Board recommendation for an amendment to the Organization and By-Laws of the Medical Staff of the Clinical Center, regarding psychological and sociological studies in Building T–4. This is a step in the direction of facilitating approval procedures for psychosocial studies of normal subjects.

On several of our units we have patients and normal subjects who remain here for long periods. It is therapeutically desirable to provide work opportunities for these people, consistent with their clinical status and the treatment goals for them. We have had some success in finding work for

*Prepared by William C. Jenkins, M.D., Chief of Clinical Care.

some of them but a wider range of such activity is needed. We are continuing our efforts to find a more favorable solution to this problem.

Adult Psychiatry Branch*

The Adult Psychiatry Branch has undergone major changes in the past 2 years. During 1958 there was extensive recruiting of new staff members, reorganization of existing projects, remodeling of space, obtaining of equipment, and general tooling up for the planned new research. In 1959, data collection got underway in several new lines of inquiry. A tangible sign of change during the year was the opening of two new research units in the hospital (3-East and 4-East). In January we began admitting as patients to 3-East college freshmen from a variety of eastern schools who encountered serious difficulty on going away to college, and in June we admitted to 4-East our first group of healthy young volunteers for psychophysiologic research. This latter unit makes possible a degree of close observation rarely possible in psychosomatic studies. In addition, it permits utilization of environmental conditions for experimental purposes, and brings our various psychophysiologic workers—necessarily an interdisciplinary group—into close contact with each other.

In our **Psychosomatic Section** we are attempting to determine the extent to which the day-to-day interactions of the human organism with its environment are reflected in endocrine function. Research in recent years has shown the extent of CNS regulation of endocrine function and, consequently, a wide range of visceral functions. Our research is attempting to determine whether the emotional fluctuations of everyday living are associated with concomitant fluctuations in concentration of hormones that have widespread physiological significance. In this connection, we would like to quote from a chapter by our close collaborator, Dr. John Mason, in the 1959 *Annual Review of Physiology:*

> * * * It appears in general that the importance of central nervous system influences upon visceral functions, particularly endocrine regulation, has not been fully appreciated, probably for a number of reasons.
>
> In the first place, there has been the rather well-established general impression, supported by experimental data, that visceral functions are largely autonomous and responsive primarily to metabolic needs. The existence of these plausible concepts of self-regulation has possibly created resistance to the idea than an additional set of regulatory influences of considerable practical importance might be exerted upon visceral functions by the central nervous system.
>
> Probably a more important reason, however, has been the crucial matter of experimental approaches and methods. It is clear, first of all, that a comprehensive experimental approach to neural regulation of visceral function can only be achieved by the pooling of skills and viewpoints of a broad range of scientific disciplines. These combined approaches must permit, on the one hand, an analysis of central nervous system mechanisms (by the techniques of neuroanatomy, neurophysiology, neuropharmacology, neurochemistry, experimental and clinical psychology, and psychiatry) and, on the other hand, the analysis of specific visceral functions (by the techniques of chemistry, physiology, pathology, immunology, and internal medicine). Impetus for collaboration between various combinations of disciplines within these two general groups has come from both directions. Many behavioral scientists have come to view hopefully the measurements of visceral function, which are relatively objective and quantitative, as a major approach to the experimental analysis of emotional states, which have been extraordinarily difficult to evaluate by psychological techniques alone. Similarly, physiologists and internists have become increasingly aware of the necessity for a greater understanding of the impact of emotional disturbances upon bodily function and of the possible participation of emotional factors in the development of disease.
>
> Within the past 5 to 10 years substantial new methodological developments have emerged in many fields and have made possible a new order of directness and accuracy in psychophysiological research.

A typical problem in research of this sort is the difficulty in utilizing the best available chemical methods for hormone measurement. The active participation of Dr. Mason and his colleagues provided microanalytic techniques that permit reliable detection of small differences.

Another typical problem in this kind of research is the difficulty of obtaining reliably observable emotional responses for experimental purposes. This is sometimes done by observing naturally occurring stressful situations and sometimes by experimentally devised situations. These approaches tend to complement each other and we are attempting to use both.

Research on human behavior and adrenocortical function in recent years has established the following points: (1) Plasma and urinary hydro-

*Prepared by the Chief, David A. Hamburg, M.D.

cortisone elevations occur in circumstances of distress; this has been shown in several laboratories utilizing in aggregate several hundred human subjects. (2) There is a linear relation between the intensity of anxiety, anger, or depression and plasma hydrocortisone levels. (3) Particularly high hydrocortisone levels occur in the presence of disintegrative anxiety. This finding has recently been followed and confirmed in an ingenious experiment by Dr. Sheldon Korchin and associates. Dr. Korchin joined the Adult Psychiatry Branch as visiting scientist during the year. (4) Remarkably consistent hydrocortisone elevations occur in "first experience" situations—those characterized by a high degree of novelty and ambiguity.

We have been pursuing some of these findings during the past year—partly by way of additional checking, partly searching out further implications, partly inquiring whether epinephrine and norepinephrine are budged under these same circumstances.

In current studies, we are attempting to relate fluctuations in emotional state to fluctuations in plasma and urinary levels of hydrocortisone, epinephrine, and norepinephrine. We are utilizing three situations to observe changes in emotional state: (1) adaptation to new environment, (2) spontaneously occurring events in the life of each subject, (3) standard commercial films that effectively present common human problems. In each situation, modest but clearly detectable shifts in emotional state can be observed, and evidence is rapidly accumulating that there is concomitant variation in levels of hydrocortisone, epinephrine, and norepinephrine. The fluctuations in emotional states are determined in several ways: (1) personal interviews, (2) ward observations, (3) self-rating questionnaires, (4) projective tests. In this work we have benefited from the collaboration of Dr. Joseph Handlon of the Laboratory of Psychology.

Adaptation to a new environment has proved to be a potent stimulus for adrenal activity. We have found substantial hormone elevations in normal controls during the first few days following admission to the Clinical Center. This is a high-tension experience for most of these individuals, since they typically have only a vague image of the experiences they are about to undergo and are concerned about possible risks. Under these circumstances we have observed not only significant elevations in plasma and urinary hydrocortisone but also in the excretion of epinephrine and norepinephrine. The norepinephrine elevations are more consistent than the epinephrine elevations. This is in keeping with earlier findings suggesting that hydrocortisone and norepinephrine move closely together, while epinephrine shows a somewhat different pattern of response to environmental stresses.

Another point of interest has to do with differences in the diurnal pattern of hydrocortisone levels during the first few days after admission. Almost all individuals show significant early morning elevations in comparison with their basal levels. However, some individuals maintain relatively high levels throughout the 24-hour period, while others show a sharp decline, sometimes having no detectable plasma steroids in afternoon and evening. The 24-hour urine, which is so extensively used in medical research, only reflects these differences in diurnal patterns to a modest degree. Thus, if one were relying on 24-hour urines alone, a good deal of this difference in regulatory pattern would be obscured. This may have methodological implications for other fields of medical research. At present, we are searching for possible behavioral correlates of the different neuroendocrine regulatory patterns.

Our data this year have shown that individuals are quite consistent in their adrenocortical responses. Some individuals consistently respond to the stresses of ordinary living with substantial steroid output; others show relatively slight response under the same circumstances. These differences tend to emerge more clearly under stress than under basal conditions. Thus, we observe patterns of adrenocortical function that are characteristic of individuals, and we are trying to correlate these with enduring patterns of behavior that are also characteristic of the individual. In the near future we hope to put our working hypotheses on this problem to a predictive test.

We are exploring various techniques for evoking emotional responses experimentally and measuring concomitant hormonal changes. Our main effort in this direction during 1959 has been the use of movies. In human stress research, it is essential to keep ethical considerations in mind when

formulating experimental plans for evoking reliably observable emotional responses. Movies have the advantage of being clearly justifiable on ethical grounds. In addition, they permit generalization to the common experiences of everyday living; they do not represent an extreme or unusual psychological stress.

Our findings with the movie technique so far may be briefly summarized as follows: (1) moderate prefilm hydrocortisone elevations occur consistently, just as pre-experimental elevations have occurred in other contexts; these elevations are associated with a background of uncertainty and tension about the experiment; (2) against the background of this tension and moderate steroid elevation, sharply contrasting film effects have been demonstrated: pleasant, absorbing films consistently produce a drop in steroid levels, whereas powerful films tend to produce further elevation. In connection with the latter films, an important methodological point is the "fit" of a given film with the attitudes of a particular audience. A film that constitutes a powerful stimulus for one type of audience may be a weak stimulus for a different sort of audience.

Evidence has accumulated over the past few years that the pituitary-adrenal system is a sensitive reflector of the degree of behavioral arousal of the organism and presumably of tonic states of excitation in the CNS. If this is correct, we might expect to find evidence of such organismic arousal under conditions of elevated corticoid levels by studying what the brain does when it is not immediately interacting with the environment, as during sleep. By seeking such relationship we hope to further elucidate the psychophysiological adaptation of the organisms to stress, to explore individual differences in reaction to stress, and possibly to further understanding of those maladaptations to stress which contribute to mental and psychosomatic illness. Thus, we are seeking a possible relationship between adrenocortical function and the quality of sleep and dreaming, employing the latter as behavioral indices of tonic states of organismic arousal.

Electroencephalographic and electroculographic records are obtained of sleeping subjects, concurrently with the study of their blood and urine 17-hydroxycorticosterone levels. Both types of procedures are carried out under circumstances when the corticoid levels are elevated, and when they are at stable baseline levels for the subjects under study. Under these contrasting conditions, the electrophysiological measures are assessed in terms of sleep depth and the number and duration of dream periods, while the manifest dream content is rated on a dimension of "threat" expression. Thus far, the subjects have been normal volunteer controls. Data collection under the conditions described has now begun. The first phase of the study has been concerned with studying techniques of assessing the depth of sleep, the occurrence of dreaming, and the collection and rating of dream content.

In our **Personality Development Section,** we have selected for study one of the commonly occurring experiences which may stimulate personal growth for many people, but may also lead to breakdown for others: the transition from high school to college. Our aim is to determine some of the sources of problem-solving effectiveness in adolescence. We hope to learn about the kinds of factors in our subjects' life experience, family, personality, and current environment which seem to be related to their finding effective ways of dealing with stress during the period under study.

Under the leadership of Dr. Earle Silber, we selected a group of students on the basis of the following minimal criteria of mental health: (1) evidence of competence in school work, (2) evidence of ability to make and maintain interpersonal closeness with at least one person outside the immediate family. All the students in our sample were exposed to the demands of a college preparatory course in a good public high school; most of them had a similar socio-economic background. The sample was drawn from the senior class at the Bethesda-Chevy Chase High School, which numbered 530 students, about 53 percent of whom were girls. There are 20 students in the sample we chose for intensive study.

In selecting the 20 students the following steps were carried out: all of the students were in the senior class, were exposed to a short talk telling about the project and asking for their cooperation. Then a letter was sent to all senior-class students, and the students were asked to sign this letter if they wished to volunteer. Parental signatures were also required. The letters were then returned to the school, and also gave consent for us to have

copies of the students' transcript records. We picked students in the top half of the class who would be going to colleges not too distant, so that we could visit them if we desired, and also students who seemed to reveal some concentrated interest as reflected in their extra-curricular activities; and also those who seemed to have made a favorable impression upon their teachers as evidenced by personality ratings, which was part of the transcript record. From a volunteer group of about 100, we selected 39 as probably meeting our criteria, and carried out a screening interview with them. The 20 intensive cases were chosen from these 39 and were seen for a series of weekly interviews.

A special projective test was constructed by Dr. George Coelho and Dr. Silber, patterned after the Thematic Apperception Test, consisting of 11 pictures designed to elicit characteristic modes of dealing with certain potentially stressful situations. There were two sets of pictures—one for boys and one for girls—including scenes such as posting of final grades and couples sitting close in a car.

Content of the subsequent interviews included investigation of the following areas: academic work, peer group relations, values, constructive experiences during high school, dating, plans for marriage, the family situation, areas of responsibility within the family, description of the parents, decisions about going to college, anticipation of college life, plans for living at college, academic preparation, and attitudes about college organizations.

During the summer months we continued our interviewing to obtain information about the student's general history, medical history, and personal development, as well as to keep in touch with some of the events in his current experience. To understand more of the dominant values of the high school and its formal and informal organizations, and the background against which our individual subjects operated (that is, the current high school environment), a series of four group interviews—along with the individual interviews—was held with four students designated as group leaders.

We have also gotten acquainted with the parents of our students and during the summer months, all of the parents (with a few exceptions) were seen in a joint interview. We plan to continue interviews with the parents. We have, wherever possible, seen our students just before departure for college, to see what changes may be present on the eve of actually leaving home.

Essentially then, we have had an opportunity to get acquainted with a group of 20 college-bound students while they were still in high school. By beginning with each student while he was still in high school, we had an unusual opportunity to study the techniques of personal problem-solving in the high school years, as well as the anticipatory phase of the transition to college. We have also had an opportunity to get acquainted with the student's past history and to have some direct experience with his parents. We have now begun interviewing our students after they have moved into the freshman year at college. We made a field visit to each of the colleges to see the student after he had been away at college 6 to 8 weeks. We interviewed some of the students when they were home for Thanksgiving, all of them during Christmas vacation, and plan to see each one again during Easter vacation. At the end of the freshman year, we plan another intensive series of interviews to review the entire year. This field study has benefited from our collaboration with Drs. Morris Rosenberg and Leonard Pearlin of the Laboratory of Socio-Environmental Studies.

Our interest has been in clarifying the behavior of competent adolescents, and particularly in learning how they cope with the stresses of this transitional period. Further, we are attempting to make systematic comparisons of these students with those who break down during the freshman year and are hospitalized in the Clinical Center.

In order to study sources of interference and conflict in the developmental process, we have hospitalized a group of adolescents in whom there has been a significant area of developmental failure, serious enough to make adaptation to the demands of a first-year college experience temporarily impossible. We selected the situation of the freshman in college because it provided an opportunity to observe the impact of a new situation with new demands, both internal and environmental, upon the adolescent.

The 3-East project has been in operation since January 1959, under the leadership of Dr. Roger

Shapiro and Dr. Harold Greenberg. Since the opening of the ward we have hospitalized twelve college freshmen who were in sufficient emotional difficulty to make it advisable that they leave school. These patients have represented a wide range of diagnostic categories including acute schizophrenic reaction, paranoid personality disorder, other types of character disorders, and some symptom neuroses.

Our first efforts with this group involved organizing an effective program of therapy. This has included individual psychotherapy on a 3 or 4 hour a week schedule for all patients, meetings of the entire patient group twice a week, the development of an activities program, and therapeutic interviews with the patients' families.

Some of the research questions we hope to answer from data available from the therapeutic program. Other questions we are trying to get at in specially designed research interviews with the patients, their families, and their friends. Our questions up to now have been essentially in two areas. The first area includes questions about the nature of the new and presumably stressful situation (the experience of the college freshman), and about the psychological equipment the individual brings into the situation; what his resources are in coping with it, what directions his adaptive efforts take. The second area includes questions about factors that might determine such behavior in individuals, and have up to now been chiefly investigations into aspects of his previous life experience which might account for some of the idiosyncratic responses we observe. In addition to information along these lines available from the data of individual therapy, we are now in process of designing a standardized interview in which one of us will attempt to get at this material specifically with each patient.

Several other sources of data about the patients' adaptive efforts in the college situation are available. One is the observations of friends, teachers, counsellors, etc., close to the patient during his attempts to adjust in his first year of college. We have sent one of our staff to the college setting in which each patient has experienced difficulty, to interview a number of people who were close to the patient, and get a picture from them of the coping efforts made by the patient and the kinds of situations he had to cope with there. We have been particularly interested in the kinds of efforts the patient made to form friendships in the college setting and have addressed many questions to the patients' friends about the nature and quality of relationship that existed between them and the patient.

We have attempted to use observation of the patient's adaptation to the new experience of hospitalization and living in the ward situation as another source of data about the nature and quality of his coping efforts. Again we have been particularly interested in how he goes about forming new relationships and have asked nursing personnel to make an ongoing record of the developing relationship between themselves and the patient. We hope to learn how these patients relate to a variety of individuals, and if we can to clarify why for them formation of new relationships as a type of adaptive behavior is so difficult and when it occurs is often unsuccessful in reducing their anxiety and maintaining their self esteem.

With regard to the second area of questions, most of our efforts to understand the personality development of our patients have involved study of relationships within the families in which they have grown up. Interviews with parents and siblings have been conducted with particular interest in what could be learned about their conscious and unconscious attitudes toward the patient, their feelings about the patient's assets, their image of his liabilities, and their readiness to see him as a separate individual. We also want to obtain from the parents as thorough a picture as possible of their own experience as adolescents. As they recall their own adolescence, we hope to gain some knowledge of what sources of anxiety existed for them as adolescents and how they dealt with them. We then want to determine how much their child's adolescence has revived this anxiety in them, and how much they have responded with anxiety to their child's explorations in an area of anxiety in his own adolescence. This may tell us something about the developmental experiences which promote and which impair adaptive behavior in adolescence.

During 1959 the **Section on Family Studies,** under the leadership of Dr. Lyman Wynne, has deepened its investigations of family relations in schizophrenia. Broadly speaking, the work of the past year has strengthened previous impres-

sions that the familial patterns of interpersonal relationship of schizophrenic patients differ from those of nonschizophrenic psychiatric patients and that the schizophrenic offspring occupies a different family role and has had a different familial experience from nonschizophrenic siblings.

Two independent approaches have been useful in producing new ideas and data which bear upon these problems: family psychotherapy, and psychological testing of entire families. All 25 families which have been studied intensively in this project during the past 2 years have been seen in "family therapy" in which both parents, hospitalized patient, and siblings are seen together in exploratory, psychoanalytically oriented, psychotherapy. In effect, the communication and interpersonal patterns of the family system are not reconstructed in family therapy but are observed directly. The very recent addition to the facilities of one-way observation windows to the interview rooms has expanded the research opportunities in studying the family therapy sessions.

The family-therapy approach has led to the conclusion that the traditional use of individual interview descriptions of family interaction is of very dubious reliability. Repeatedly, when family members describe disturbing events that have just happened within a therapy session, major aspects of the events are distorted or omitted by *all* the family members. However, direct observation illuminates behavior that could not be reported by the family members because it has occurred outside their awareness.

Although this project is primarily oriented toward untangling the relation of family patterns and schizophrenia, the use of family therapy as an exploratory research tool has led to a significant byproduct: a contribution to the development of the theory and technique of family therapy, an approach which has recently achieved considerable recognition as a new and potentially valuable addition to the therapeutic repertory of psychiatrists. Observations and ideas within our group about the advantages and limitations of family therapy, including the relation of family therapy to concomitant individual psychotherapy, have been evolving into a conceptual framework during the past year.

The criteria for selection of families compared in this project have minimized all presenting differences except one: the presence of a schizophrenic (or psychiatrically ill and hospitalized), versus a nonschizophrenic young adult offspring. In other respects, the families can all be characterized as consisting of two parents and at least one sibling of the presenting patient, as having predominantly American middle-class customs and values, and as sharing a willingness to explore jointly the difficulties involved in the psychiatric illness.

During the past year procedures for evaluating both the diagnostic and the sociocultural background characteristics of the families selected have been systematized and sharpened. Beginning with background interviews by the psychiatric social workers of the project, a procedure for recording historical material as it cumulatively unfolds has been developed.

In comparing the families of schizophrenic and of nonschizophrenic psychiatric patients, the work in family therapy has been useful in suggesting a number of hypotheses of significant differences. For example, the familial response to "deviant" behavior of a schizophrenic family member (that is, behavior outside the code or subculture of the particular family) generally has the effect of fragmenting the behavior into concrete aspects; for example, by ignoring the most obvious intentions of the deviant family member and giving only a very limited, literal interpretation to a trivial aspect of the behavior, such family responses reduce and confuse the central meaning and intent of the behavior into incoherence and uncertainty about the validity and authenticity of one's own perceptions and intentions. In contrast, the familial response to "deviant" behavior in nonschizophrenic families typically involves, for example, relatively straightforward accusations and the production of guilt in the deviant person, but without reducing his capacity to trust his own senses about what he intended.

Such hypotheses, as they become progressively refined, seem to lend themselves to systematic comparison studies relevant to basic features of schizophrenic experience. Procedures are now being developed for making systematic use of family therapy transcripts in such comparison studies and plans are under way to compare these and other families, using standardized procedures

focused on particular aspects of these hypotheses.

In addition to family and individual interviews, three other sources of data have expanded in their contribution to the family studies program during the past year: (a) nursing observations, both the traditional observation of individual patients and the observation of patients interacting with their families, who are encouraged to visit on the nursing unit; (b) home visits, with the entire family present, have become a regular part of the work with all families; naturalistic observations have been made for various lengths of stay by staff members having different roles—nurse, social worker, psychiatrist; and (c) art therapy, as a tool for diagnostic and therapeutic work with entire families, as well as individuals, has, in selected instances, been quite informative.

Parallel to the direct clinical work with families, a consultant to the project, Dr. Margaret Thaler Singer, has been studying "blind" the psychological test protocols obtained from entire families seen in the project. Given first the protocols of the family members but not of the patient, Dr. Singer predicts which families have a schizophrenic member and then, given the patient protocols, matches patient with family. Thus far, with eleven families, a remarkable accuracy has been achieved. Most interestingly, these results could not be obtained using traditional methods of interpreting test protocols by their content. However, when attention was paid to the form of the thinking used by the subjects—for example, in the way in which attention and meaning were manipulated—then accuracy of differentiation was possible. The convergence of these test findings with the clinical findings previously mentioned has been striking and will be intensively examined in further evaluations of the material already obtained and in future investigations of additional families.

Clinical Neuropharmacology Research Center*

The past year has seen an increasingly fruitful interaction between the CNRC and Saint Elizabeths Hospital, and has further defined areas of mutually complementary interest. This interaction has been manifest in a number of steps taken by the Hospital to assist the program during its formative period. Dr. N. Waldrop, formerly Chief of the William A. White Service, has been promoted to the newly created post of Associate Director of Research for Saint Elizabeths Hospital, and, in this capacity, has given valuable assistance in the implementation of the CNRC program within the total Hospital setting. A new Biometrics Branch has been created by the Hospital. This is to collaborate with appropriate personnel of the CNRC, and the Biometrics Branch of NIMH, in meeting the statistical needs of the program. The Chief of CNRC, and the Director of Training of the Hospital are actively exploring ways and means whereby the training potential of the CNRC could be used to optimum advantage, without unduly infringing on the time of CNRC personnel. Already, requests have been received from residents of the Hospital to work in the laboratories of CNRC over and above their normal duties, and a resident has been assigned to the CNRC by the Hospital to collaborate in an ongoing program of clinical investigation in the wards of the William A. White Service. The regular internal and guest seminars of CNRC have attracted wide participation from the Hospital. Taken together with joint staff conferences, regular teaching rounds within the William A. White Building, and the spontaneous formation of small groups centered around subjects of common interest, they have greatly strengthened the links (both personal and administrative) between CNRC and the Hospital. The Research Committee of the Hospital, of which the Chief, CNRC, is Chairman, now comprises four members of the CNRC staff (Dr. I. Whitfield, Dr. H. Weil-Malherbe, Dr. G. C. Salmoiraghi, and Dr. M. Hamilton). The work of this Committee (which meets at monthly intervals to consider research proposals, both from the CNRC and the Hospital) has gone some way towards defining standards, and devising procedures designed to further research within Saint Elizabeths Hospital.

Despite these hopeful trends, however, it would be idle to ignore some deep-seated difficulties which confront the CNRC and the Hospital in the pursuit of an advanced program of research and of

*Prepared by the Chief, Joel Elkes, M.D.

556044—60——13

training. Staff shortage, at professional and non-professional levels, takes first place, and is acutely felt in the William A. White Service, as well as in other parts of the Hospital. For similar reasons (and despite every effort to participate in a common research program) the Pathological Laboratory of the Hospital can only meet a very limited number of requests for studies arising out of the program; a priority system had to be strictly adhered to in the allocation of research projects requiring such laboratory studies. Furthermore, a number of medico-legal issues connected with the pursuit of clinical investigations within the confines of the Hospital remain unresolved, thus favoring the use of established forms of treatment, and holding in abeyance the introduction of procedures of a more novel and exploratory nature. Lastly, the lack of intermediate treatment facilities such as Out-Patient, Day Hospital, Night Hospital, and Club facilities is being increasingly felt by the CNRC, as the rehabilitation of the Center, and the Hospital, gathers momentum. The Hospital is fully aware of these needs, and, within the stringent financial limitations imposed upon it by present circumstances, is doing its best to bring these facilities into being.

There is one further aspect which has become clearly apparent during the past year of the Center's operation. The physical separation of CNRC from the Clinical Center at Bethesda has been increasingly felt by members of staff, and the hope has been steadily expressed that some aspects of the neuropharmacology program could, with advantage, be pursued at the Clinical Center. The physical representation of the subject of neuropharmacology, both at Saint Elizabeths Hospital and at the Clinical Center, would make for more ready interaction between the program of CNRC and related aspects of the programs of the Laboratories of Clinical Science, Adult Psychiatry, and Psychology of Clinical Investigations, NIMH. Furthermore, it would also encourage the circulation of personnel between the Clinical Center and the CNRC facility, and thus reduce the sense of isolation of CNRC personnel, as well as increase the contact between Saint Elizabeths Hospital and the Clinical Center. There is, in fact, every evidence that such a move would greatly enhance the mental climate within both the CNRC and the Saint Elizabeths campus, and, through closer liaison of the Hospital with the Clinical Center, contribute to the steady evolution of an academic setting and attitude within the Hospital.

For the purposes of the present report, the division of the Center into Sections of Psychiatry, Chemical Pharmacology, and Behavioral Sciences is adhered to. It has, however, become increasingly apparent that, while serving a very useful purpose during the early formative period of the Center, these terms no longer fully connote the principal themes to which the program of CNRC wishes to be committed. It is hoped that these themes may receive their formal recognition in an internal reorganization of the Center during the coming year. During the interim, Dr. G. C. Salmoiraghi has made a valuable contribution to the evolution of the Center by assuming responsibility for central laboratory services common to all Sections, by assisting the Chief, CNRC, in the coordination of the programs as a whole, and by representing him on appropriate occasions.

SECTION ON PSYCHIATRY

The slender resources of the William A. White Service of Saint Elizabeths Hospital have encouraged Drs. A. Hordern, of CNRC, and J. Lofft, of Saint Elizabeths Hospital, to initiate a comparative study of two phenothiazines within six wards of the Service. The broad aims of this study were essentially fourfold. In the first place, it was hoped to explore the limits of feasibility of a number of procedures, and to determine their most economical use. Secondly, it was hoped to define ways and means whereby the mere pursuit of such a study could contribute maximally to the training of staff, and thus to the care of patients not directly involved in the study. A third aim was to test various clinical research instruments (and more particularly rating scales) in terms of their usefulness and reliability in the day-to-day setting of a busy mental hospital ward. Finally, it was hoped to determine the effect of such a clinical research program on the attitudes and aspirations of the staff.

In this preparatory study, therefore, information regarding the relative merits of the two drugs used (Trifluoperazine and Prochlorperazine) was

regarded as incidental; the real yield lay in a contribution to the solution of some controversial methodological problems, of which seven received particular emphasis. These were (1) the role of structural milieu in ward management; (2) the effect of interpersonal milieu, and nursing care in the management of patients; (3) the mobilization and cultivation of latent research skills in the nursing staff; (4) the design of the type of trial best suited to meet the requirements of (1), (2), (3); (5) the assessment of patient behavior; (6) the assessment of attitudes towards a clinical research program in a hospital ward; and, finally (7) the most economical way of collecting, tabulating, and processing data. Although the study involved only 24 specially selected patients, the simultaneous pursuit of the program in 6 wards proved an interesting, and (from the point of view of staff training) helpful feature. The patients were divided into six groups of four, and were observed in special day rooms (decorated by the patients) in six wards of the Service. In each ward the group of four formed the nucleus of a larger group of ten patients, the six additional members consisting of chronic schizophrenics receiving their customary medication. The six groups were consistently nursed by selected day and evening nursing assistants, a carefully defined routine being followed in each case. Regular group discussions with personnel contributed to the training of personnel, and familiarized them with the regular instruments being used. Thirteen different rating scales, the details of which are given elsewhere, were employed. According to needs these were administered at daily, twice weekly, weekly, monthly, and three-monthly intervals.

The results of this study to date are distinctly encouraging. The methods have proved feasible, and economical of staff time. Personnel attitudes throughout the study have been monitored. A trial of this kind would appear to be an economical training device in group nursing and rehabilitation techniques. There appears little question of its positive effect on the mental climate of the various wards. At the initiative of the staff of Saint Elizabeths Hospital, the study is now to be extended to include a further 126 patients who are to be transferred especially to the William A. White Service for rehabilitation; it is hoped that parallel studies will be initiated in other services of the Hospital. A visual aid exhibit, prepared by the principal investigators, has been a useful demonstration and teaching device, and is to be shown in an expanded form at a forthcoming meeting of the American Psychiatric Association.

In an attempt to study social interaction within a chronic mental hospital ward in a quantitative way, Dr. S. Kellam is developing an objective method for the visual recording, and measurement of the relative association, or isolation, of the individual patients within the ward setting. Information regarding the number of contacts of a patient is noted by nurses and aides on a time-sample basis, and recorded on a specially constructed grid by means of mobile map tacks. The clustering of these tacks readily reflects social contacts, a ward log supplementing this visual device. The method is already giving useful objective indications of the group structure of the ward, and the prestige systems existing in the wards. It should be of particular interest in an examination of devices or procedures (including drugs) designed to render the patient more accessible to the milieu in which he functions.

In an attempt to study factors making for chronic hospitalization, Dr. D. Lipsitt has examined the role of so-called "dependency" in the psychiatric in-patient, and of the possible, though unintended, collusion between the needs of the over-dependent patient and an over-protective attitude in a ward milieu. The term "dependency" is in obvious need of clarification and measurement. Two hundred patients form the subject of this study. The instruments used include selected scales from the Minnesota Multiphasic Personality Inventory, a review and scoring of the patient's case record, a psychiatric interview, and behavior ratings; other instruments are being developed. Present trends indicate that long-term patients deny their dependency (showing a higher denial and a low dependency score), whereas patients admitted for the second time tend to accept, admit, and overtly display their need for dependency. It is hoped that, with growing experience in this area, dependency scores may be tested for predictive value and, perhaps, be put to use in selecting appropriate treatments for patients with varying dependency needs.

In a cognate vein, Dr. M. Geller is developing methods for study of the transition of the chronic

schizophrenic patient into the community, with special reference to the use of the pharmacotherapies and group therapeutic techniques to facilitate such transition. Two matched groups of eight patients are being used as a pilot sample in this study. Experience in the conduct of group sessions with chronic schizophrenics and in the recording and analysis of data is being gained at present, though this, of necessity, is a slow process. It is hoped that utilization of analytically oriented group psychotherapy may, in time, allow one to exploit the assets of the chronic patients during trial visits and during an initial period (of, say, 6 months) following discharge. It is also hoped that by providing regular group therapy during this stage of transition the patient may be successfully weaned from his undue dependence on the hospital and encouraged to make a successful adjustment in the community.

Drs. Harwood, Hordern, and Lofft, together with a number of other colleagues, are studying the effects of Imipramine on plasma 17-hydroxycorticoid levels in eight depressive patients, and are attempting to correlate these findings with clinical responses to the drug, as well as its effect on protein-bound iodine and plasma catecholamine levels. This study is in progress; no biochemical data is as yet at hand.

Dr. R. Gentry, CNRC, and Dr. N. Waldrop, of Saint Elizabeths Hospital, are gaining experience in the use of an optical transducer device for the study of changes in finger blood flow. This is to be used in a number of psychophysiological investigations, and may be found particularly helpful as an index of autonomic activity in word-association studies.

SECTION ON CHEMICAL PHARMACOLOGY

In continuation of previous work, Dr. Weil-Malherbe has attempted to refine his published method for estimating epinephrine and norepinephrine in plasma. It was found that the results obtained by the trihydroxyindole method were only slightly lower than those obtained by the ethylene diamine method. Studies on the chemical mechanisms of ethylene diamine condensation indicate that in the reaction with epinephrine a single compound is formed as a major product and that side reactions occur to an insignificant degree. In the reaction with norepinephrine, on the other hand, two or three major products are formed from catechol and dihydroxy-mandelic acid. The need for a reliable method for estimating catecholamines is an urgent and persistent problem, and it is hoped that Dr. Weil-Malherbe's critical reappraisal of existing methods (including his own) may be a useful contribution to the field.

In a cognate area, Dr. H. Weil-Malherbe and Dr. E. R. B. Smith are attempting to develop a method for estimating urinary metanephrine and normetanephrine by a modification of the method of Euler and Floding. The zinc ion was found to be an essential catalyst in the formation of fluorescent derivatives of these two compounds. Taken together with the methods now being developed in the Laboratory of Clinical Science, Clinical Investigations, NIMH, the availability of a quantitative method for the estimation of metanephrine and normetanephrine in urine should make it possible to account for the major metabolites of epinephrine and norepinephrine in man. It is intended to put these methods to use in appropriate pharmacological studies.

Dr. H. Weil-Malherbe and Dr. H. Posner are examining the effect of DOPA on the synthesis of catecholamines in the brain after depletion by reserpine. It had been shown in previous work that the intravenous injection of DOPA accelerated the reappearance of catecholamines in rabbit brain and, equally, that there was a significant redistribution of intracellular epinephrine, norepinephrine, and hydroxytryamine following administration of reserpine. The effect of Dopamine on these processes is being examined further.

In pursuit of previous studies, Dr. Szara is now developing quantitative methods for the estimation of the *6*-hydroxy derivatives of psychotomimetic tryptamine derivatives, with special reference to the estimation 6-hydroydimethyltryptamine and *6*-hydroxydiethyltryptamine. This has involved the organic synthesis of reference materials, and a comparison of these with material isolated from urine. A sensitive and specific spectrophotometric method, measuring 1/μg/ml of *6*Ohydroxydiethyltryptamine in urine had been developed. This has been found useful in following the *6*-hydroxylation pathway of diethyltryptamine, a compound shown by Dr. Szara to possess marked psychotomimetic properties.

In a promising juxtaposition of biochemical method with animal behavioral techniques, Dr. Szara, of this Section, and Dr. Eliot Hearst, of the Section of Behavioral Sciences, have attempted to study the relation of the rate of *6*Ohydroxylation of diethyltryptamine to the threshold required to elicit behavioral change in the individual rat. It was found that the rate of transformation of dimethyltryptamine to its *6*-hydroxy derivative differed from animal to animal, and that the rate correlated well with the thresholds needed to elicit such behavioral effects. The evidence thus pointed to *6*-hydroxylation as an important, and possibly essential, step in the production of behavioral change by tryptamine derivatives. It is also of interest that the *6*-hydroxylation of diethyltryptamine by a microsomal enzyme system provides one of the few instances of the production of a psychoactive agent *in vivo*. It will be of interest to examine the effects of ataratic and other agents on the rate of this *6*-hydroxylation process.

Dr. H. Posner has been following the metabolism of some phenothiazines in man, with special reference to possible excretion of phenolic metabolites in urine. By incubation of urine extracts with glucuronidase, he has obtained evidence suggesting that glucuronides constitute a considerably larger fraction of the excreted metabolites than sulfoxide derivatives. In an admittedly difficult study, Dr. Posner is attempting to develop methods of identification and analysis of the various metabolites of chlorpromazine and is hoping to use these in an intensive study, both of excretion patterns of phenothiazines in the individual patient, and in studies of the behavioral effects of a number of key metabolites in the experimental animal. As in the case of Dr. Szara, Dr. Posner is planning his biochemical studies in conjunction with Dr. Eliot Hearst of the Section of Behavioral Sciences; both studies are attempts to correlate individual biochemical variation in the metabolic handling of a drug with behavioral effects and thresholds for such behavioral effects.

Dr. Posner has also carried out an extensive survey of the urines of selected patients with the view to isolating suitable case material (such as phenylketonuria, alcaptonuria, tyrosinosis, prophyrinuria) for subsequent study. Although this survey of some 286 patients did not yield the material intended, it drew attention to certain anomalies in the Ehrlic indole reaction, which led Dr. Posner to examine the possible role of metabolites of chlorpromazine in this reaction. It is this incidental finding which led Dr. Posner to his present study of the disposition of the phenothiazine derivatives in man.

In conjunction with Dr. A. H. Stewart of Saint Elizabeths Hospital, Dr. T. Harwood has studied the effects on plasma 17-hydroxycorticoid levels in six patients being treated with the drug for symptoms of depression. In keeping with previous findings by others, the 17-hydroxycorticoid plasma levels were found elevated in these patients prior to treatment. There was a fall in these levels following iproniazid administration; though the degree of clinical response did not necessarily correlate with this alteration in 17-hydroxycorticoid concentration.

Dr. Cambosos has been engaged in the recalibration and development of the Chaney colorimetric procedure for the determination of protein bound iodine levels in serum. The modification has increased the sensitivity of the method about fivefold, making possible the determination of PBI levels in about 0.1 ml. of serum. This should thus prove useful in longitudinal studies (both clinical and experimental) where frequent blood sampling may be required.

SECTION ON BEHAVIORAL SCIENCES

The Section of Behavioral Sciences has continued its studies of the mechanisms subserving the coding of information along the auditory pathway, with special reference to an analysis of the role of inhibitory mechanisms in the cochlear nucleus. One such study, carried out by Miss P. Stopp, has centered on an analysis of unit response patterns of the avian auditory pathway, as followed by microelectrode, within the *nucleus magnocellularis* of the pigeon. It was found that, despite a lack of differentiation of the cochlear receptors in birds into inner and outer hair cells, the pattern obtained showed remarkable similarity to those observed in the much more highly organized auditory system of the cat.

In another study Dr. J. B. Fotheringham has attempted to influence the inhibition of unit activity normally seen in the inferior colliculus of the cat following appropriate two-tone stimulation.

A number of well-known centrally acting drugs were used. Significantly, none of these noticeably affected the discrete inhibitory process operating at this level. A similar lack of responsiveness was noted in the cochlear nucleus by Dr. A. S. Schwartz. The results thus tentatively suggest the existence of local (and possibly pericellular) barriers at high central level. In an attempt to test for these, pericellular microinjection will be attempted, though of necessity the experimental hazards of this procedure are considerable.

Dr. R. Gumnit has continued to study direct current changes in the auditory cortex of the cat in response to auditory stimulation. In view of the sensitivity of these changes to slight variations in experimental condition (such as degree of anaesthesia, moisture, and temperature of the cortex) rigorous control of these had to be assured, and optimum conditions defined. A cell suitable for direct current recording has been devised and tested. Results to date suggest the existence of direct current changes in the auditory cortex, and particularly a localization of these changes to Auditory Area I. The role of direct current changes in the handling of sensory information is still controversial, and any established facts in this area must be judged welcome.

Dr. R. P. Michael, a Guest Worker at the Center on a Rockefeller fellowship, has made a useful beginning in developing a cannulation and microinfusion technique, which, it is hoped, may make it possible to slowly inject small amounts of drugs directly into selected areas of the brain in the conscious monkey subjected to simultaneous behavioral study in an operant conditioning situation. So far, the method has been used in four Rhesus monkeys; approximate thresholds for chlorpromazine, injected directly into areas of the reticular formation, have been obtained. It is quite obvious, however, that a refinement of the technique is essential before any further study in this area can be undertaken. Time expended in developing this technique, however, may be well worth while, since (particularly if combined with biochemical and endocrinological studies) it may offer a reasonably direct approach to an analysis of the regional physiology and pharmacology of the brain stem. It is hoped that following Dr. Michael's return to England the work may be continued by other members of the group.

An independent area of investigation is being opened up at the Center by Dr. G. C. Salmoiraghi. In previous work, carried out with D. B. Burns, at McGill University, Montreal, Canada, Dr. Salmoiraghi, using extracellular microelectrodes, had attempted to determine the location and pattern of discharge of respiratory neurones in the brain stem, and to account for the rhythmic nature of respiration in terms of reciprocating inhibitory mechanisms. He is now hoping to extend these studies, and, more particularly, to elucidate further the mode of action of CO_2 and of other drugs on these mechanisms. The technical difficulties (particularly those connected with movement artefact) are very considerable; yet it is hoped that an elucidation of the mode of action of drugs on rhythmically discharging cells of the medulla may contribute to an understanding of the action of drugs on rhythmic processes elsewhere in the brain stem. Furthermore, the concepts developed by Dr. Salmoiraghi to account for the discharge of respiratory neurones may find their application in the medullary centers controlling cardiovascular phenomena. In view of the striking involvement of central autonomic centers in some phases of stress, and of mental disorder and the effect of drugs on such phenomena, a discrete analysis of the organization of these central steering mechanisms is deemed desirable, despite the long-term nature of such an undertaking.

Dr. Eliot Hearst, in conjunction with Dr. Murray Sidman of the Department of Experimental Psychology, Walter Reed Army Institute of Research, has continued a study on the aversive nature of a conflict producing stimulus in the rat. Animals were trained in situations which produced conflict, but which, equally, enabled the animal to escape into a neutral situation. In experimental situations allowing choice between positive (reward), negative (punishment), and neutral situations, neither positive nor negative situations alone activated escape into such a neutral situation. This escape, apparently, depends on an interaction between positive and negative elements.

As indicated earlier (under "Section of Chemical Pharmacology") Dr. Hearst and Dr. Szara (of the Section of Chemical Pharmacology) have joined in the study of individual differences in susceptibility of rats to diethyltryptamine. In-

teranimal variation in regard to responses to psychoactive agents is a relatively unexplored field. It is hoped to pursue these findings further using compounds other than the ones so far employed.

Child Research Branch*

SURVEY OF DEVELOPMENTS

1959 was a year of crisis and change for the Branch. In June 1958, Dr. Redl had announced his forthcoming departure. After a careful review of the studies nearing completion, and consideration of the developing needs of the total clinical investigations program, it was decided to terminate the clinical work of the Branch in July 1959. Suitable arrangements were made for the patients who had been under treatment, and a number of the research staff undertook the responsibility of staying on until June 1960, in order to complete the working up of some of the data previously gathered.

There were, at the time, two groups of patients under study. The one group, which had been in treatment for some 5 years, consisted of five teen-age boys who lived in the Children's Treatment Residence and will be hereinafter referred to as the cottage or residence group. The other patient coterie comprised eight latency-age children who had been admitted in September 1959, after a brief previous hospitalization for diagnostic study; they were housed on Ward 4E and will be referred to as the ward group. After the decision to terminate the program was made, all these patients were accordingly prepared for discharge and/or transfer to other institutions during the latter part of the spring. The residence patients, now faced with the prospect of a termination that they had known must come some day but which they had not anticipated quite so quickly, showed progressively severe breakdown in morale, controls, and overall adjustment. Behavioral upset became rife to the point of becoming overwhelming; staff morale followed suit; and it required a series of intensive staff discussions followed by confrontation of the patients, both individually and as a group, for everyone at the cottage to be able to reorient himself and face the future in an effective manner. In a sense the emotional upheavals in the staff paralleled those of the patients on every echelon; it was only after prolonged and intensive work on these issues that the various team members were able to regain their sense of proportion, see the salient parts of the reality before them, to master these, and communicate them to the patients.

Administratively, serious problems were faced in terms of future placement and followup planning for the cottage patients, and a number of conferences were held at various levels throughout the administrative structure, the upshot of which was a decision to provide financial backing for placement of three of the youngsters. After careful consideration of several residential treatment centers, it was decided to place three of the patients at the Berkshire Farms School in Canaan, New York. A contract was let for this purpose by the National Institutes of Health and the boys were transferred in July 1959. A fourth youngster was sent home temporarily pending our finding a place for him; he was finally admitted to the Spaulding Youth Center in New Hampshire. The fifth residence child was returned to his own home. An active followup program is being continued with a half-time social worker assigned to the project, to make monthly visits both to the parental homes and to the current placements of all the boys.

The ward program terminated somewhat less traumatically, since at the time of admission, the patients had been informed that they would be staying only until the coming summer. As summer approached, most of them were able to accept the fact of the termination without as severe a degree of disturbance. It was, nonetheless, a difficult episode for the staff who had been deeply involved in the project. Many of the staff left at the time the patients departed, some were transferred to other services, while 11 stayed on to work on the data that had been gathered over the preceding years and to prepare it for publication.

This work has proceeded and continues at present. The team structure is that of four full-time and one part-time senior researchers, three full-time and one part-time junior researchers, a half-time social worker, and two secretaries. Occasional meetings are held. Dr. Redl returns for

*Prepared by Joshua D. Noshpitz, M.D., Acting Chief.

regular consultations with each staff member. Some consultation takes place with former staff members, thus making it possible to amplify the recorded material in a useful way.

RESEARCH DEVELOPMENTS AND TENTATIVE FINDINGS

Projects Completed and Reported

Individual Therapy and Psychopathology. Two different styles of therapy were pursued in our two separate settings during the early part of the year. In the cottage, the five boys who had been in intensive, four-times-a-week individual therapy continued and terminated this therapy. On the ward, the ward group which had been in a situation-geared type of therapy showed increasing readiness to adapt to, to accept, and to respond to this approach—moreover, in some cases, they began to orient themselves toward the ward therapist in such a manner that they were in effect converting our original structure into a more formal type of individual psychotherapy, very largely by their own requests and attitudes. For example, they might insist on seeing the ward therapist only in his office, they wanted regular appointments, and they wanted him to avoid otherwise entering into their ward life. It was difficult to avoid the impression that the youngsters were able to make a remarkably rapid induction into a psychotherapeutic relationship in spite of very severe character problems. It will be important to reexamine and retest, in other settings, the possibility of utilizing such a method as an induction technique for the initiation of psychotherapy with hard-to-reach patients of this type. Little can be said about the possible long-range benefits of such an approach—the period of time the patients were in treatment was too brief. Our effort was in the nature of a pilot study.

Returning for the moment to the individual therapy with the cottage boys, the aspect of the interdigitation of this therapeutic process with the on-going ward life demanded the attention of a number of the investigators, including the cottage mother and two of the therapists. A paper by Kitchener, Sweet and Citrin, which was presented at the orthopsychiatric meeting in 1959, traced out in meticulous detail the simultaneous working of parallel themes with the therapist in the playroom and with significant ward personnel on the ward. Attention was addressed to the phenomenon of breaks which occurred in the behavioral and therapeutic relationships and the ensuing hostile flood which submerged the entire observing ego. The egodynamics were described with emphasis on the precariousness of primary-object relationships; the inability to endure tension because of the sense of unbearable helplessness and the certainty that no future gratification can be trusted; the sense of profound emptiness; the insatiable oral needs with the accompanying fear of their seduction, hunger, and destructiveness; the tendency to project the oral sadism and view the world as devouring; the intense castration anxiety along with the associated fear of the loss of inner substance. The resultant ego structure is one in which the acting out has the defensive function of denying the dependency and fear of disappointment and where it becomes imperative to maintain an illusion of omnipotence. Hence, projection, magical thinking, and conversion of passive to active are routine. Rapid shifting occurs in the level of ego functioning; the observing ego is not given much opportunity to develop or to play a role in personality actively. The capacity to learn from experience is thus markedly limited and thought plays but a small part in the patient's adjustment. Appropriate management tactics were described starting with the recognition of the necessary reliving and recapitulation of the most infantile layers of the patient's experience around incorporation, object loss and delay of impulse.

Two technical developments were presented the first of which concerns the management of aggression. In this area, techniques which permit the therapist to join in the support and defense help realine the child's collapsing ego and are more important than interpretation or prohibition. One way to achieve this is to turn the emerging aggression into a quasi-game. Another way is to feed the child's narcissism at a moment when he seems frightened. On the other hand, when the aggression is used in the form of manipulation, i.e. when the ego is pathologically strong rather than pathologically weak, it is important to employ interpretation or physical limit setting. The second development concerned choice in interpretation. It is essential that the interpretation be geared to the phase of treatment. Thus, when a

child is communicating primarily through motor behavior, interpretations must be accompanied by or made through concrete behavioral responses. When a child has improved to the point where he can utilize symbolic communication, then symbolic gestures of one sort or another should be the rule for the therapist too. Only later in treatment when verbalization becomes important to the child, can interpretive efforts of a verbal kind impinge effectively. Readiness to shift among these levels as the child shifts during any treatment hour is of the essence. Many similar devices need be used by ward personnel with a parallel respect for the shifting states and the precarious ego situation of the patients. The confusion that may in time take place between significant ward persons and therapists is considered an expression of the patient's finding uniform respect for and response to his treatment needs.

Problems of Technique. The approach to patients through the life-space interview as contrasted to individual therapy received its most thorough explication and documentation during the final months of the project. Many hours of life-space interviewing with the ward patients who were not in individual therapy served to demonstrate the readiness of some of these youngsters to move from concrete discussions of specific behavioral events and upsets into more family centered and conflict-oriented areas. Such conversations would sometimes progress into classical-seeming psychotherapeutic interviews. The cry: "Take me to the playroom" became a popular and frequent one to be directed toward the ward doctor. A major factor in this seemed to be the omnipresence of the ward doctor who was often there evenings and weekends so as to be continually available to pick up on moments of anxiety, loneliness, or simple need for some gratification as well as to handle the more intensive behavioral upsets. In addition, a mode of approach was employed in which ward personnel often joined the therapist in these interviews so that what might otherwise have been complex and tricky stories to get straight, now became sharply focussed, accurate accounts of what went on with the full details immediately available, and with the possibility of the interpersonal relationships that had been disturbed by the incident receiving effective and ready clarification. This opened an entirely new dimension to the patient's perception of staff people individually, and of the interacting adult world collectively, and ofttime seemed to have dramatic and influential effect. Aside from its therapeutic aspects, the training value of this procedure is very great; the changes in style and technique and the growth in professional maturity of the staff members were marked.

Problems in Ego Structure. Two areas that received major thought during the first half of 1959 were those of diagnosis and ego identity. The first involved the notion of making diagnosis a composite of several terms, i.e. each diagnosis would be a sentence composed of a number of clauses, rather than a single descriptive phrase of two or three words. Each part of the sentence was scheduled to be connected with a definite set of categories and the categories were in turn, the psychological past, the identity structure, the impulse orientation, and finally, the symptomatic behavior displayed by the particular patient. Various subcategories under each of these, such as, overstimulated, phantasy-oriented, erotic-aggressive, and so forth were defined, and various formulations were then possible to describe particular manifestations in specific children. This set of diagnostic categories was developed exclusively in connection with the hyperaggressive child; it is hoped that specialists in other areas of childhood disturbance might also develop appropriate category classes, so that the possibility would emerge to put a number of such category sets side by side, and winnow their essential similarities and differences with an eye towards developing a more significant set of diagnostic terms.

As a derivative from this essay, the categories applied to the psychological past were in turn viewed in their relationship to the development of antisocial behavior in adolescence. The psychological events ensuing at puberty were viewed as intruders on a scene that had been prepared for by a process of overstimulating, depriving, or overgratifying the child in the preschool period, with the inevitable consequences that ensued. It was anticipated that this type of approach might serve to complement the sociological approach to delinquency where it is possible to account for a delinquency-prone population, but more difficult to explain why only a small percentage of such a

556044—60——14

population actually becomes involved with the law. If necessary psychological factors which may have been present in the past of the particular child who becomes delinquent can be seen as additions to the sociologic vectors, both functioning as necessary, but neither as a sufficient cause, a more difinitive system emerges.

Similarly, the question of ego identity—what it meant, how it functioned, what its psychological and dynamic structure was, its relationship to the defensive aspects of personality, some of the forms it might take in latency children—was explored and will continue to be studied. In particular, the role of the choice for or against one's own chronological age was viewed as an essential polarity of identity-formation in childhood. Other such polarities included the choice of the reality-fantasy issue, i.e., was someone a real person or a make-believe person; and the choice between the covert and the overt, i.e., was the child "really" to be a concealed individual beneath quite a different outward appearance, or was he to be someone who was what he was on the surface. Finally, there seemed to be a choice between having any identity at all or no identity (which amounts, in effect, to the choice between nonpsychosis and psychosis). Some children were so vague, so amorphous, so lacking in outline, as to be labeled "diffuse" in our identity category.

Milieu Therapy and Life Space Interview. In attempting to define what milieu therapy and residential treatment needed to differentiate them from other types of housing of disturbed children, the simultaneous employment of ego support and ego analysis as technical modalities in creating a therapeutic milieu were described. The notion of how the milieu can come to grips with a symptom without allowing the child too easy gratification of his acting-out on the one hand, or attacking him and increasing his defensiveness on the other, were exemplified. The need for the milieu personnel to respect the particular ego configuration of the youngster and give strength to his defenses when they were weak and when anxiety was high, was studied at some length and the correlated approach of interpreting symptomatic acts as defenses when these were strong was equally stressed. These two techniques alternating back and forth with the same child were seen as essential methods of working with him in the environment. Thus, a theory of residential treatment built on ego analysis and ego support was developed.

A group of clinical studies on experiences with self mutilation in children was presented at a symposium on this subject at the orthopsychiatric meeting in 1959. Generally, these pointed up the interpersonal meanings as well as the masochistic and subjective aspects of this behavior. Self-mutilating behavior was observed to be: an attack on the person of the therapist in the case of a child prone to symbiotic fusion experiences; a symbolic act of control at a distance in youngsters who were not quite so primitive; a manipualtion of and a punishment for therapists; a coercive for achieving or forcing reactions of some kind out of the staff; and it might also have the value of a sort of masturbatory pleasure. Numerous case illustrations were cited.

A review of the thinking that had gone into the construction of the treatment cottage and of some of the problems that had developed around the use of space was also the subject of a paper. The notion of which elements in the spatial arrangements around a child's life can be combined and which need to be separated was examined at some length, and the impact of architecture on the treatment process was touched upon. In particular, the roles of staff residence space and child residence space in terms both of the need for nearness, the nature of distance, and the kind of distance required were observed both for their advantages and disadvantages.

An additional contribution with important technical implications was an account of the developments around informing the cottage patients that the program was about to end. When the youngsters learned of their impending separation and termination, a highly anxiety-charged situation developed which in turn generated a series of tactics for countercontrol. This was described in some detail: such elements as surprise, concerted adult action, group plus individual interviewing, intensive and protected programming during the crisis time, active limit setting, and, above all, recurrent interpretation, were blended into a combined approach with good success. This experience has many practical implications for the management of institutional crises.

Learning Disturbances. The modifications in school methods necessary for disturbed children were carefully studied. In one paper a five-step series of modifications from normal school procedure was described involving (a) shortening the small group school sessions to fit the task involvement span of the group, (b) adhering to the lowest common denominator of academic working level, (c) choosing materials which were as free as possible from the predominant problems of the children, (d) the design of procedures that were not ambiguous or dependent on a child's self control, and finally, (e) the attempt to demonstrate an understanding of the child's pathology while simultaneously discouraging the manifestations of it in the classroom.

In addition to these technical procedures, their counterpart—the impact of work of this sort on the teacher's personality—was examined by one of our teachers and the nature and kind of growth that emerged were explored. In particular, an account was given of the major alteration in philosophy that had taken place between the time that the first long-term group had been initiated into the classroom, and the time when the work with the second long-term group was begun. This change involved moving from a position in which the teacher attempted to follow the patient at the youngster's own level, wherever that might be, to a position in which the teacher defined a certain range of limits within which the youngster must operate with the understanding that if the youngster overthrew the traces or stepped out of these limits, he must leave the classroom until he could accept this configuration and return. This change had profound implications for the entire learning process, the milieu, and the adjustment of the teacher. To begin with, the child was confronted with a much more clear-cut learning task with which he could cope either by refusing or rejecting or fighting against it some way, or by accepting it—in each case he always knew exactly what was expected of him, and where he was. Secondly, the milieu knew that the school was going to be rather taxing and specific in its demands and that youngsters might come out of school tense and pent up from having had to control themselves in order to meet the school demands—this made necessary the planning of much activity after school. Third, the teachers came to view themselves more specifically as educators rather than as teacher-therapists and could work within a structure that permitted them to apply their teaching skills in a specific and articulate way. It will probably remain an area of contention in the field for years to come, whether in general the more structured school is better for a child within a residential setting, or whether the residential school should be primarily and essentially a flexible instrument which views education as part of the treatment process and hence bends as the child bends. In any case this was the evolution that took place here, and seemed to have beneficial results.

The fact that the "ideal" school conditions under which the teachers theoretically worked here did not free the school from the basic problems that every school must face was reported in one communication. These basic issues were: the need to cope with groups, the need to cope with the body of theory and techniques available to teachers, and the need of the school to live within a certain community and reflect the aims and attitudes of that community. Inevitably, intricacies in operation will arise on each of these levels, regardless of whether one is working in a school with tiny classes under the most carefully structured conditions, or whether one is working with huge classes and under the most diffuse conditions. The fact that the school is structured in a hospital and must thus come to grips with the attitudes, manner of life, values, and personnel structure that are normal to this type of social community places, on a hospital school, very special problems, different in degree but not in kind from those of the school in any other community.

The problems encountered in the transfer of the cottage patients from the in-hospital school to the public schools were studied in detail, and some generalizations, useful to any workers in the field who face the problems of entering institutionalized children into public schools were developed. In brief, three major issues were defined: (1) the decision as to *when* a child can begin to benefit from schooling outside the treatment institution, (2) the decisions that must be made around the planning for and selecting an appropriate school, and (3) the decisions about the quality and quantity of power and role distribution between school and institution to make it possible for the

child to utilize the school experience. In connection with the first of these decisions, it was noted that the direction in which the child was moving was more important than his current situation at any given moment, i.e., readiness for new experiences is often seen in potential ways by the amount of development the child has been able to make and the tendencies discernible in his overall adjustment rather than by how well he may do on any particular day within the institution. The obvious clinical issues, of course, must be considered, such as how he behaves, what he does in therapy, and how he handles community activities. In particular, the school record prior to admission must be analyzed for behavior crises and issues that occurred there. Attitudes toward the teacher are subjects for evaluation, as is the child's capacity to adjust to a new pupil group. Routines need to be weighed and the youngster's ability to adapt to them as well as the use he makes of ordinary school methods, all must be measured in sum. With this in mind, a simultaneous evaluation of school and child was undertaken and a number of criteria were developed. Some of these were: the attitude of the staff of the school under consideration toward psychiatric treatment within that school; the nature of staff personalities who would be involved with the student; the physical plant of the school; its geographical location in terms of how hard it is to bring the child there or for the cottage staff to get there in a hurry if necessary; the amount of crowding and nature of the session pattern at school; the relationship of that school to the child's family with special weight on previous contacts with parents or with siblings; the nature of the social structure of the school as compared to the social group of the child; the potentials for social groups within that school to become pathological; the age grouping of the children who are in the patient's grade; and the complexity of the school structure, e.g. junior high vs. grade school, to which the youngster must return. The attiude of the principal was of prime importance, and some observation of the teaching methods and classroom manner of all the potential teachers in advance of allowing a child to contact them was considered a sine qua non for any possible success.

All the above factors are of the essence in the resolution of the third major issue, i.e., the arrangements between school and treatment institution. A very carefully tailored program was arranged for each child at school, with the appropriate courses worked out well in advance. One parent surrogate from the institution represented each child at the school. In addition an educationally sophisticated representative of the treatment institution kept in regular contact with the school in order to consult with teachers and principals. Opportunities were afforded for continued discussion of all issues; there were regularly scheduled institution-school conferences designed to discuss homework and community expectations. Special tutoring was also a feature of the work. Important contacts were made with student counselors in all schools and with the student disciplinarians in junior high school. Reports from school were obtained for inclusion in the institutional records, and planning conferences for grade placement the following year were initiated early in the year in order to facilitate the patient's promotion with a minimum amount of stress and strain.

Behavoiral Measurement and Assessment of Change. A continuing group of studies had been devoted to surveying the social interactions of the cottage boys and many codeable observations were made on how they related themselves to others and how others related to them. In an earlier study, the interpersonal behavior had been observed at two periods a year and a half apart and the changes over these periods were examined. Two studies on this data were completed in 1959. In one the influence of various social settings on the interpersonal behaviors was explored, and it was established that knowledge of the social setting increased the variations in the types of behaviors observed on the part of the boys. What was not anticipated, however, was that the interactive effect between the child and the settings revealed much more information about the youngsters' behavior that did the sum of all the independent components of behavior that were observed separately. It was concluded that the behavior evoked by a particular setting was related more to the personality of the particular child than to any other single factor. As they grew older and continued in treatment, the children's ability to make differentiations and variations in social behavior

increased, this being dependent upon the nature of the setting. One of the important implications of this for further research is that it seems likely that observers would differ somewhat in their estimate of improvement depending on their locales of observation. In order for such an entity as improvement to be studied adequately, there would have to be a representative sampling of a variety of situations in which the behaviors took place.

The second major study compared the social behavior of the patients at the two phases at which they were observed with that of two groups of well-adjusted children who acted as controls. These control normals lived for brief periods in the same hospital ward as the patients and were observed in identical situations. Thus, with age and situation controlled, an attempt was made to decide whether the changes that occurred in the patients were due to the effectiveness of the treatment program or rather to maturation. It was found that a certain increase in appropriateness of social response observed in the disturbed children's peer relations did seem to be related to treatment rather than to age changes. In terms of their behavior toward adults, the children tended more and more to approach that of the normal controls. These favorable changes were also judged attributable to treatment. On the other hand, the disturbed children who had shown less overt dependent behavior toward adults when they were younger, came to exhibit increasing dependency toward adults as they matured. Since normal youngsters tended to become more independent as they matured, this too was viewed as a direct effect of treatment. It was concluded that the forces for change in social behavior are derived from adults rather than from other sources. Normal children tended to differentiate among social settings more than did the disturbed children, and their behavior was more predictable.

In general, both the normal and the disturbed children showed the same behavioral tendencies toward specific settings whether it was mealtime or play. The relationships between the behavior evoked by a particular setting and the personality of a particular child was a constant for the normals as it was for the disturbed children. This in a sense was the concluding work in this whole skein of observations on the interactions of children and adults within the treatment settings. The use of the normal controls enabled the investigators to establish more definitively both methods of observations as well as an evolution of the meanings of improvement for these youngsters.

A derivative study that emerged from the observations made on the control normals had implications for a psychodynamic behavior theory. Normal ego growth was seen as something more than an absence of pathology but rather as a series of positive achievements meriting descriptive formulation, and, in time, genetic investigation. Such elements as the development of time prespective, the sense of trust in the future, the sense of objectivity (i.e. detachment from narcissistic equation of self and object) the ability to delay, the tolerance for frustration, the realistic perception of cause and effect, the capacity for initiative and taking responsibility, the search for mastery, and the sense of self-esteem were categorized as areas for observation and measurement. A number of achievements of the normal latency child were observed and comments were made on the reflections of ego growth in the use of speech, in the development of peer relations and ego controls, and in the techniques employed by such boys to control their own impulses and to advance support to each other in the face of tempting and exciting situations.

Studies of improvement were also undertaken. One investigator recorded the comments and observations of various staff members about the state of each of the residence patients at 6 months prior to the move from ward to cottage, and then again 6 months after the move. In tabulating this information, five areas of improvement emerged. The first lay in the extension of ego horizons of the patients, so that totally new areas of functioning came into view. The second was the use of withdrawal as a healthy coping device in contrast to the acting-out-impulses behavior of the past—it was an interesting phenomenon that what in normals might have been considered pathological behavior, with these patients was used in the service of increasing mental health. Third, there were shifts in balance between individual- and group-centeredness; instead of the youngsters banding together and operating like a gang who are being oppressed and must resist the adults, each one began to individualize, differentiate and seek his own goals, and to express his own needs quite apart from those of the others. Fourth, there were

changes in sensitivity to danger potentials; the youngsters became aware of problems earlier, and recognized and responded to each other's recognition of them more quickly and subtly. And, finally, there was a development of a concept of self in such areas as sense of responsibility, pride, identity, humor, objectivity and sense of time which marked the progress of each of the youngsters.

A second study on improvement sought to evaluate the meaning of the term, to measure the impact on the staff of the phenomena of improvement, and to record some of the resistances and anxieties as well as the downright opposition that developed to the changes taking place in the patients. This latter was called the phenomenon of "improvement panic." It occurred when a staff which had been geared to coping with and responding to the most seriously disturbed behavior began to find that they were in fact encountering less disorganized and explosive situations than previously. As a result, such normal staff activities as recording and discussion showed up markedly; there seemed to be nothing to write and nothing to say about the less pathological patients. There was an increase in covert interdisciplinary tension; much argument about whether the patients were really getting better or whether this was just a naive, over-optimistic statement; and violent rejection of any attempt to observe the possible improvement at all closely. This was carried to the point of outright anger at the entire project together with a nostalgia for the "good old days," when things had been "different" and very difficult. The narcissistic investment in the child's pathology, the feeling of being needed by him, the awareness of one's own errors that a study of his improvement might reveal, were among the factors that appeared to be operative. Even the term improvement itself had many meanings. It might mean a change in overall mental health; it might mean alteration in the individual functioning of the person as a human being; it might mean an alteration in the ease with which one could live with a patient; it might simply mean an abstract function with no particular connection with behavior. In any case, the impact on the staff was painful, and sometimes more painful on the more sophisticated than on the less thoroughly trained members of the team. Of particular concern was the question of whether or not a given "bit" of improvement was "real" or whether it was merely a defense against treatment. Moreover, even if present in any one field, was it ready for transfer to other areas? Again, the question was raised as to whether improvement meant that the child would not break down again or whether the bases on which the progress rested were "genuine."

Some of the clinical changes that occur in patients when they start to improve were reviewed as well as some of the additional problems they might have to face; e.g., in coping with newer and more difficult situations, there would be greater anxiety about failure and about loss of control. The many problems the youngsters encounter with their peers, once they begin to improve, and their sensitivity to adult reactions, with the consequent use of their newfound abilities as bargaining and manipulative tactics also alter the acceptability of and the staff response to their improvement. To improve means to have more choices than before, which is in itself a source of turmoil—particularly if the adults now begin to leave the youngsters on their own more, and thus implicitly demand more of them. Inevitably the patients make some wrong decisions and must face the consequences of these. The clinical staff must cope constantly with the temptation to overexpect and overexploit the improvement and to make quick deals, so to speak, with a child whose improvement is really more of a mask and a defense than it is a genuine growth. The youngsters should not be made to feel that the response to their improvement is a reward for having been "good" or a privilege they have been granted. Without being indifferent to their growth, one tries to communicate to them that the improvement is something that is accepted just as growing taller would be. It is readiness rather than privilege that is the criterion for providing new opportunities.

On their side, staff members have difficulty repressing their own desire to gloat over success. Rivalry may develop among the various "fields" or disciplines and tends to be augmented by these changes, and by determining who is responsible for the improvement and where is it seen best. There are some real dangers in this since the treatment atmosphere can be turned into a reward and

punishment type structure, and a system of caste may develop with the inevitable outcasts. This may emerge all too readily around the fact that some children are improving and other children are not. At the same time, the need for a more complex program emerges, so that the varying achievement levels of all the children can be met instead of some being gratified and some deprived. In the face of these difficulties, it is often difficult to decide whether a particular upset means that a child is regressing, or whether he is merely experiencing the pangs of trying to cope with a necessary new experience. One must, therefore, take calculated risks and be ready to accept the public reaction to the inevitable failures that will ensue. This study casts considerable doubt on the value of any type of intensity scale of skills, traits or personality characteristics as a measure of improvement. A serious weakness in our current descriptions is that the language depicting the psychological ingredients of settings and situations is very weak compared to the language we have to describe the changes in behavior itself.

The Biographical Studies

The major area of dedication of the final year's work of the Child Research Branch lies in a series of six biographies, one for each of the resident patients, which are currently engaging the energy and the attention of the senior research staff. It is expected that these biographies will eventually be brought together into a single volume which will be a formal statement of the lives and of some of the clinical thinking that went into the treatment of each of these boys. A second major study now under way is the definitive writing-up of the material on life-space interview. Here we will attempt to summarize all the various experiences we have had with the modality of treatment, to categorize types of life-space interview, to relate this interview to the therapeutic interview, and to explore the differences between the earlier style of life-space interview initiated with the long-term group and the later style developed on the ward with the eight younger patients.

One investigator is making an intensive examination of the learning problem of one of the long-term patients, will attempt to relate this to the youngster's psychopathology. He hopes to correlate the learning difficulties as they were encountered day-by-day in the classroom with the psychotherapy hours and the content and the style of adjustment in the therapy. It is difficult at this point to predict exactly what will emerge from so careful a study with the large amount of data available—certainly the oft-discussed question of the interaction between psychopathology and learning difficulty should receive a searching and throughly documented explication.

A handbook on residential treatment based on cumulative experiences here is being outlined. It will review some of the broader outlines of treatment methods within a residence, the philosophy of treatment, the techniques, the problems, diagnostic issues, staff training, and some of the difficulties and attempted solutions that have emerged in the course of the interactions.

A number of less ambitious projects are also in prospect. Several of the boys have sustained losses of important relatives during their time in residence, and the record of their reactions is being assembled in order to study something about the meaning of such a loss to this type of child with particular emphasis on the forms of expression of this reaction throughout the totality of the child's life.

In reviewing the way some of our patients came to us, and some of the things that happened to their families, both before and during the course of treatment, one of our staff was impressed by the way that the values of the mental-health personnel who handled these families and patients at one point or another became entangled with the issue of the management and treatment of the patients. It is anticipated that a report of these observations will be presented, and that they will prove useful to social agencies in dealing with disturbed families.

Another book in prospect is the history of the educational aspect of the project, i.e., the record of the total experience in attempting to establish the school, the changes in method, the philosophy, and a thorough reworking of the meaning of the educational issues within the total undertaking.

Yet another report is being devoted to a history of the project as a whole with a careful examination of its origin, many of the events that have played a role in it, the administrative issues involved in its structure, the many clinical events

that determined its form, with particular emphasis on the theoretical implications of the development of this type of social structure within a larger institution.

Laboratory of Clinical Science*

The laboratory of Clinical Science was established to straddle what might have become a gap between the basic disciplines, especially the biological fields of biochemistry, physiology, and pharmacology, and the problems of psychiatric disease. Its division into basic and clinical sections represented the stake which each of these programs has in the functions of the Laboratory, but, although the scientific work has represented a broad spectrum of activity from studies on patients with psychiatric disorder, on normal volunteers or on nonclinical and rather basic problems, there is no precise relationship of the scientific activities to the administrative division. In fact, the extent to which the clinical-basic division is not clear represents the success which the Laboratory has had in breaking down the conceptual barriers which often separate these two approaches. For purposes of this summary, the work of the Laboratory in the past year may be divided into certain problem areas: schizophrenia, aging, experimental allergic encephalomyelitis, sleep, and specific problems of metabolism related to the nervous system or behavior.

SCHIZOPHRENIA

The Laboratory has continued its major program of investigation into the possible role of biological factors in the etiology and pathogenesis of schizophrenia and their interaction with social and psychological factors. The Section on Psychiatry, in selecting patients for Laboratory studies, had previously made a broad survey of the family histories of male patients diagnosed as schizophrenics in Maryland and District of Columbia public hospitals. This survey provided an opportunity for an analysis by Dr. William Pollin and his associates of the distribution of schizophrenia and other forms of mental illness in the families of schizophrenics. This revealed a preponderance of schizophrenia in the mothers, as opposed to the fathers, of such patients and was further supported by an extensive analysis of data in the literature. Although this appeared to support the sociologically based theories of the role of maternal influence in the development of schizophrenia, further analyses of alternative explanations revealed that the findings were compatible with a considerably greater marriage and fertility rate found among schizophrenic females in contrast to males.

The Section on Psychiatry is presently engaged in a careful study of the life situations of the selected group of patients under investigation by the Laboratory in comparison with their nonschizophrenic siblings, in an effort to elucidate the special and perhaps highly individualized psychosocial factors operating before the development of the mental disorder.

Among the attractive hypotheses recently formulated to account for schizophrenia in biochemical terms, perhaps the most interesting is that based upon a postulated disorder in the metabolism of circulating epinephrine, entailing production of possibly psychotomimetic substances such as adrenochrome. By virtue of the recent excellent work of Dr. Julius Axelrod in elucidating this hormone's normal metabolism, the Laboratory was uniquely equipped to test that hypothesis. Drs. Stephan Szara, Julius Axelrod, and Seymour Perlin, more than a year ago, had ruled out the presence of abnormal or even detectable concentrations of adrenochrome in the blood of schizophrenic patients, and, at the same time, Dr. Roger McDonald and his associates had demonstrated that the reported rapid *in vitro* oxidation of epinephrine by the serum of such patients was the result of a dietary deficiency of ascorbic acid. An extensive study of the metabolism and the physiological and psychological epinephrine infused into a series of normal controls and schizophrenic patients was undertaken collaboratively by a number of investigators in the Laboratory. Dr. Jay Mann has found that the overall rate of metabolism of this hormone as judged by blood levels achieved and the rate of their decay was identical in the two groups, while Dr. Elwood LaBrosse has demonstrated that the pathways of metabolism and the metabolic products of epinephrine were the same in schizophrenics as in normal man. Studies by Dr. Philippe Cardon on the cardiovascular effects

*Prepared by Seymour S. Kety, M.D., Chief.

of infused epinephrine, by Dr. Louis Sokoloff on the blood-glucose response, and by Dr. William Pollin on the mental effects, tended to confirm the slight differences previously reported. Although the latter findings indicate some differences in response, which are being further studied, the metabolic studies leave little room for the possibility of the generalized disturbance in epinephrine metabolism, which had been postulated in this disorder.

Because of suggested possibilities of a disturbance in histidine metabolism in schizophrenia, Dr. Donald Brown, of the Section on Biochemistry, undertook a study of the urinary metabolites of the amino acid uniformly labeled with C^{14}. Although this study resulted in a number of significant basic findings which are discussed later, it revealed no differences between the normal and schizophrenic patterns.

There is no *a priori* reason to suspect that if a biochemical abnormality exists in some types of schizophrenia, it must be a generalized one. On the other hand, a number of cogent arguments point to the highly differentiated metabolism of the brain as more likely to harbor important chemical mediators of behavior, both normal and abnormal. The inaccessibility of the brain for investigation during life makes it necessary to devise indirect methods of approach to possible cerebral metabolic disorders in schizophrenic patients. Dr. Irwin Kopin has been developing highly original, double-labeling techniques which may be suitable for the study of the metabolic turnover of such substances as serotonin and norepinephrine and other amines in the human brain. Another approach is to produce certain mild alterations in brain chemistry on the basis of biochemical theory or of changes shown to occur by direct analysis in animals, and carefully to study the effect of these discrete chemical changes on mental function as determined by carefully controlled but intensive psychological and psychiatric evaluations. One such study, initiated in the past 3 months by a number of investigators of this and other laboratories, employs the dietary or parenteral administration of certain precursors of possibly psychoactive substances which may occur in the brain in conjunction with certain enzyme inhibitors which may retard their destruction. There has been an opportunity partially to test tryptophan, phenylalanine, histidine, glutamine, glycine, and methionine with and without the possible potentiating effect of small doses of iproniazid in this situation. Certain behavioral changes have been observed and reproduced, and these findings are being actively pursued.

The discovery and characterization of the significant metabolites of epinephrine and norepinephrine make possible for the first time reliable measurement of the endogenous production of these important hormones, not only in schizophrenia but in a large variety of psychiatric states and situations. Dr. Roger McDonald and Dr. Elwood LaBrosse and their associates have undertaken the development of simple and reliable methods for the quantification of 3-methoxy-4-hydroxy-mandelic acid and of metanephrine and normetanephrine, respectively, in the urine. A method for the first of these compounds has already been developed and is being applied in studies of schizophrenia and other mental states and the effects of certain psychoactive drugs.

New information on the properties of certain drugs extensively used in schizophrenia has been obtained in the past year. Dr. Julius Axelrod, of the Section on Pharmacology, in showing that both chlorpromazine and reserpine speed the destruction of epinephrine *in vivo*, has demonstrated one of the rare biochemical effects which these two drugs—both with similar psychiatric effects—have thus far revealed to have in common. Confirming this in man, Dr. Roger McDonald has shown a sharp increase in the excretion of a major metabolite of the catecholamines following a single dose of reserpine. Dr. Conan Kornetsky further replicated his finding of a differential effect of chlorpromazine on the standing blood pressure of normals and schizophrenic patients, and plans, in his new position at Boston University, to investigate the possible mechanisms of this action.

The absence of specific biological criteria in schizophrenia is matched by the paucity of reliable objective psychometric indices of the disorder. Dr. Irwin Feinberg, of the Section on Physiology, has carried out a series of studies aimed at differentiating the mental impairment associated with schizophrenia from that associated with nonschizophrenic illnesses. He has succeeded in designing a psychological test on which the performance of acute schizophrenic subjects differs

significantly from that of patients with an organic mental syndrome. The test in question, a modification of Raven's Matrices, shows that the acute schizophrenic patients make many more unreasonable errors than do chronic schizophrenic patients or patients with organic mental syndrome. These results point the way to a more precise and objective characterization of the nature of cognitive impairment in schizophrenia. The Section on Psychiatry is continuing its work on the development and testing of new methodologies which may provide data both clinically and psychodynamically meaningful and, at the same time, verifiable and quantifiable.

The manifestations of psychiatric illness, more than any other group of diseases, probably represent the interaction of a multitude of factors from the sociological as well as the biological spheres, and a single and sufficient cause for a process like schizophrenia is probably not to be expected. One of the real values of an intensive interdisciplinary study of a selected small sample of patients is the opportunity for relating, in the same patient, findings of one discipline to those of many others. To take full advantage of this opportunity, the Laboratory, with invaluable collaboration by Dr. Samuel Greenhouse of the Biometrics Branch, NIMH, and by the computer facility of NIH, has undertaken a program of data reduction and comparison which will make for maximum utilization of the data obtained by the individual investigators and their intercorrelation with other information.

AGING

Several cogent developments resulted from studies by Dr. Louis Sokoloff and the Section on Cerebral Metabolism in over 50 normal elderly men, carefully selected for their relative freedom from the common degenerative diseases of old age and functioning competently in their communities, which were completed and analyzed in the past year, as part of a large collaborative study in this Institute. This series showed no reduction in cerebral circulation or cerebral oxygen consumption in comparison with healthy young men, indicating that the reduction in these functions usually found in less carefully selected patients is not a necessary concomitant of the aging process. In the presence of arteriosclerosis of varying degrees there is a decreased cerebral blood flow, a decrease in cerebral venous oxygen tension indicative of cerebral anoxia, and a somewhat smaller fall in oxygen utilization, all of which appear to be correlated with the degree and duration of the arteriosclerosis and the psychological deficit, suggesting that one of the primary changes in the mental disorders associated in some individuals with aging is cerebral circulatory insufficiency and the resultant partial cerebral anoxia. Patients suffering from what is known as chronic brain syndrome showed a more marked decrease in cerebral oxygen consumption compatible with the thesis that this syndrome represents parenchymal damage in the last stages of progressive cerebral ischemia.

Drs. Robert Butler and Seymour Perlin of the Section on Psychiatry have studied these patients from the psychiatric point of view. In addition to contributing the psychiatric component of the correlations mentioned above, their studies on the psychiatric aspects of the aging process have revealed the importance of the personal meaning of psychosocial changes in terms of the individual personality as compared with the nature or incidence of the stresses themselves. The psychological defense mechanisms utilized by the volunteers and patients were studied and described in terms of their adaptive or maladaptive consequences.

EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS

This experimental disorder, produced in guinea pigs by the subcutaneous injection of brain tissue with certain adjuvants, offers a useful model for the investigation of multiple sclerosis and other demyelinative or degenerative diseases. During the past year, the Section on Biochemistry, under the leadership of Dr. Marian Kies, has continued its studies on the etiology and pathogenesis of this disease. Purification of a water soluble antigen continues to be of major importance in this project. Encouraging results have been obtained with chromatography on modified starch columns as a means of separating traces of inactive protein from the antigenic material.

Of considerable significance to both pathogenesis and treatment of the experimental disease are the immunologic results obtained with this purified antigenic fraction. All immunologic tests on the antigen (skin and corneal hypersen-

sitivity, serum antibody reactions) have been negative. However, carefully controlled skin testing has led to the observation that the disease can be suppressed by intracutaneous injections of aqueous solutions of the active fraction *after the initial injection.* A study of the significant variables in suppression of the disease in this manner offers exciting possibilities with regard to therapy and prevention of related neurologic diseases in humans.

SLEEP

This universal and mysterious state is an important segment of normal mental function and has associated with it the phenomenon of dreaming with certain interesting parallels to schizophrenic thought processes. Ten years ago, Dr. Sokoloff and Dr. Kety and their associates had demonstrated that normal sleep was not associated with cerebral ischemia, anoxia, nor with the reduction in oxygen and energy utilization associated with coma and anesthesia, providing evidence that sleep consisted of a change in the patterns of activity in the brain rather than a change in their overall intensity.

During the past year, the electrophysiological work of Dr. Edward Evarts and the Section on Physiology has been devoted to studies of the effects of sleep on the electrical activity of the brain. These studies have indicated that sleep has different effects on activity in the brain stem reticular formation as compared to the cerebral cortex. These findings support and extend the theories of previous workers (Magoun and others) concerning the role of the reticular formation in the waking state.

During sleep, potentials evoked by clicks in the reticular formation are reduced, whereas cochlear nucleus and primary cortical potentials remain relatively unchanged. Recordings of single-unit activity from the visual cortex show a considerable increase in total neuronal discharge during sleep as compared to the waking state. These microelectrode studies indicate that during waking there is a selective reduction of spontaneous neuronal discharge as compared to discharge evoked by primary afferent input (electrical stimulation of lateral geniculate radiations). This selective reduction of spontaneous neuronal discharge might be viewed as leading to an increase in the signal-to-noise ratio during waking. This notion involves the supposition that the spontaneous discharge is "noise" and the discharge evoked by afferent stimulation is "signal." Such a change in the pattern of neuronal discharge may be of importance in attention mechanisms associated with the waking state. Studies of evoked potentials recorded from scalp electrodes in man are to be carried out in order to determine the degree to which similar alterations of electrical activity may be found associated with sleep in man. These studies were made possible by an ingenious technique, developed by Mr. Robert Cox, for enhancing the signal-to-noise ratio in such recordings.

Dr. Philippe Cardon has continued his interest in the physiological and psychological effects of sleep deprivation. Subjects whose continuous performance is impaired show characteristic changes in heart rate, respiratory rate and depth, fingertip volume and pulse volume, and forearm volume-pulse form. These changes occur in the course of the test when the subject is not responding to the visual or auditory cues presented, and disappear when the subject is responding. Thus, there seems to be abundant confirmation, at the physiological level, of the current hypothesis that much of the impairment of psychic functioning which accompanies sleep loss is due to "lapses" or "microsleeps."

METABOLISM

Mechanism of Action of Thyroxine. A unique feature of the cerebral metabolism is its apparent lack of response to high circulating levels of thyroid hormone. An understanding of the basis of this unique behavior may reveal information concerning the metabolism of the brain in health and disease. The mechanism of action of thyroxine has been under investigation for many decades, but thus far a satisfactory explanation of how it increases metabolic rate stimulates metamorphosis and growth, or causes the many disturbances in body physiology and biochemistry in thyroid diseases, has eluded investigators.

Dr. Louis Sokoloff and the Section on Cerebral Metabolism have continued to make progress in their investigations of the mechanism of thyroxine action. Their finding last year that L-thyroxine

enhances the *in vitro* incorporation of amino acids into protein has been shown to be a definite stimulation and not a preservative effect. They have uncovered evidence of a latent period of action of thyroxine *in vitro* during which a still unidentified intermediate is probably formed which is then responsible for the stimulation. They have demonstrated that the formation of this intermediate is dependent on the presence of an active oxidative phosphorylating system. Their studies with the physiologically less-active isomer, D-thyroxine, and the physiologically active analogue, L-triiodothyronine, indicate that the thyroxine effect on amino-acid incorporation behaves in a manner to be expected of a physiological effect of thyroxine. They also suggest that D-thyroxine is physiologically inactive, not because the intracellular enzymes involved in the action of L-thyroxine are stereo-specific, but because it does not reach the enzyme sites when administered into the intact animal. Dr. Seymour Kaufman, of the Laboratory of Cellular Pharmacology, has been an active collaborator in many of these studies. The findings of this project represent encouraging progress toward the ultimate solution of the mechanism of action of the thyroid hormone.

Metabolism of Epinephrine and Norepinephrine. During the past year, the Section on Pharmacology was mainly concerned with studies on the metabolism and physiological disposition of H^3-epinephrine. In collaboration with Dr. Hans Weil-Malherbe, the distribution and rate of O-methylation of epinephrine were investigated. The amine was found to be unevenly distributed in various organs and tissues and did not pass the blood-brain barrier except to a small extent in the hypothalamus. Within two minutes, most of the administered catecholamine was O-methylated, while part of the hormone was bound by tissue constitutents and retained in the body for long periods of time.

Dr. Axelrod, in collaboration with Drs. Kopin and Mann, reported a new metabolite of epinephrine and norepinephrine, 3-methoxy-4-hydroxyphenylglycol. This compound was shown to arise from the deamination of (nor)metanephrine, followed by reduction. Subjects with pheochromocytomas excreted large amounts of the glycol.

Inhibitors for catechol-O-methyl transferase *in vitro* and *in vivo* have been found (pyrogallol and quercetin). Since other investigators have shown that these compounds prolong the action of epinephrine and sensitize the sympathetic nervous system, it would appear that catechol-O-methyl transferase is the enzyme chiefly concerned with terminating the action of the catecholamine hormones.

Using C^{14}- and H^3-labeling of various precursors and intermediates in the metabolism of catecholamines, a technique is being developed by Dr. Kopin which enables, in a single experiment, estimation of the relative importance of alternate pathways of metabolism of one substance to an excreted metabolite. The metabolic rate of the precursor substance can also be estimated by study of the rate of change of the H^3/C^{14} ratio in the excreted compounds. The effect of various drugs on the routes of metabolism of the catecholamines and on their rate of metabolism is being studied. By this technique, an estimate of the importance of the pathways of epinephrine metabolism in man has been made. About ⅔ of an injected dose of epinephrine undergoes methylation while the rest is either excreted as such or acted upon by monoamine oxidase. About half of the 3-methoxy-4-hydroxyphenylglycol and 3-methoxy-4-hydroxymandelic acid formed from injected epinephrine is formed by methylation followed by deamination. In the rat, methylation is of lesser importance, but is still a major pathway of metabolism.

Metabolism of Histidine. Incidental to his studies on the metabolism of this amino acid in schizophrenic patients, Dr. Donald Brown, of the Section on Biochemistry, made a number of contributions relating to the normal metabolism of histidine which dwarfed the initiating study in their significance. In collaboration with Dr. Kies, an unstable metabolic intermediate (imidazolone propionic acid), whose existence was postulated but which had not hitherto been isolated, has been stabilized and characterized, and a new metabolite present in the urine of man and other species has been identified (hydantoin propionic acid). In collaboration with Dr. Axelrod, a new methylating enzyme has been demonstrated and party characterized as N-methyl transferase, which catalyzes the transfer of CH_3- from S-adenosylmethionine to the imidazole ring of histamine. The presence

of this enzyme in highest concentrations in the brain suggests the possibility of a significant function for histamine or some related amine in central nervous function.

Metabolism of Other Amino Acids. Because of the relationship to certain amines which may play a central role in the mediation of particular types of behavior or emotion, a number of amino acids are of special interest to psychiatry. Studies by Drs. Elwood LaBrosse, Irwin Kopin, and Shoichi Hotta are under way on certain aspects of the metabolism of tryptophan, phenylalanine, glutamine, methionine, and tyrosine, in an effort to relate the differential aspects of their metabolism to mental and behavioral state, to dietary intake, or to the action of certain psychopharmacologic agents which may operate by an effect on such pathways.

ENZYMATIC ACTIVITIES IN BLOOD

Dr. Roger McDonald in collaboration with Dr. Gary Felsenfeld, a former member of the staff of the Laboratory of Neurochemistry, has been concerned with studies on the chemistry of ceruloplasmin. This approach comes as a logical development of previous studies in his Section on the possible role of ceruloplasmin in mental disease. In the current studies of ceruloplasmin it has been shown that copper exists in both the oxidized and reduced state. Furthermore, when ceruloplasmin is actively functioning as an oxidase, there is an increase in the amount of reduced copper present in the molecule. Other aspects of the chemistry of ceruloplasmin are presently under investigation.

A second area of study has been by Dr. Franklin Evans, of the Section on Medicine, concerning serum cholinesterase. In this study, the effects of psychotomimetic and psychotropic drugs on two forms of human serum cholinesterase have been investigated. The difference in the responsiveness of the two forms of enzyme to inhibition by the psychotomimetic drug, lysergic acid diethylamide, was found to deviate from the pattern usually seen, suggesting a different mode of reaction. In addition, an apparently new and deviant form of cholinesterase was discovered in a screening of mental hospital patients, although there is no reason to believe that its presence is related to mental disease.

Laboratory of Psychology*

As Chief of a combined laboratory, I am again submitting a single report to the two Directors. It becomes increasingly clear, as the program of the Laboratory develops, that the most effective long-time research in our area calls for movement back and forth from studies of the human to the subhuman, from the laboratory to the field, and from the clinic to the laboratory. Some of this trend is reflected in a constantly increasing overlap between the activities of sections from the two programs and within programs.

Since we have recently rethought our programs in the context of long-term plans, it appears wise to introduce the description of the progress of the various sections during the year against a background of what the future program of the Psychology Laboratory at NIMH looks like as at present projected.

Although the areas to be listed seem to be quite disparate, it is surprising how many interchanges and points of mutual fertilization turn up. In this respect, more of the outlined program emerges as actuality than is apparent on the surface.

Child Development. This area is likely to see tremendous activity and advances during the next decade. Child psychology is going through a renaissance. I expect that the work which will be done by our group will be extended and carried out in the context of increasing knowledge of both the physical and social aspects of development. It is here that the combination of field and laboratory studies both in animal and man are so important and for which some of the facilities will have to be extended.

The Psychophysical Parameter. I include under this heading a whole area of research which our present Animal Behavior and Learning and Perception Sections will be involved in during the next decade. Psychophysiology and physiopsychology, psychochemistry and chemopsychology (under which might be included pharmacopsychology) will be going through an unusual development during this time. It is important for psychologists working in this area to keep their eyes mainly on the *psychological* aspects. In the context of sophistication about physiology and

*Prepared by David Shakow, Ph. D., Chief.

chemistry they must persist in the careful and detailed study of the complexities of behavior—learning, especially emotional and social learning, perception, emotion and attention, and perhaps in time, thinking. Whether the approaches are physicopsychological—where lesions, drugs, etc., are the independent variables—or psychophysical—where psychological conditions are the independent variables—the psychologist's primary job is to study the behavior for its tie-in with the physical.

Creativity. This is a new area of endeavor for us which I hope will become a major activity during the decade because it has such important theoretical and practical implications. Our present early thinking is to make a multiple attack on the problem of talent and the optimal use of capacity; to carry out studies on the motivational, environmental and capacity aspects in the clinic, the field, and through experimental techniques.

Psychotherapy. The area of psychotherapy viewed as an important highroad to the understanding of personality will continue to be the preoccupation of the groups now studying this area. Because of the complexity of the data it is to be hoped that new techniques of analysis can be developed capable of dealing at least in part with such multiplex data.

Schizophrenia. A continued and more extensive attack on the psychology of schizophrenia must be made. This area has great importance for personality theory because in so many ways it provides opportunity for study of aberrations from the normal which may eventually enable us to understand normal phenomena better. In addition, of course, this particular disordered group, because of its cost, is one of mental health's greatest problems. A start has already been made, and a broader program is being developed with the use not only of patients at the Clinical Center, but those at St. Elizabeths and elsewhere.

Aging. An active program in this area has been going on for a number of years. Most recently this section's interest in the psychology of aging has begun to emphasize higher thought processes and continuing effective function in older persons. (This latter would tie in with the creativity program.) This is an important area theoretically as well as practically, requiring the cooperation of a number of disciplines represented in part in the Section on Aging.

All the above areas have a common context—one which I emphasized in last year's annual report. I refer to the central concern with underlying capacity and potentiality of the organism, whether it be the residual functions of the aged or the aberrations of the schizophrenic which throw light on the ordinarily unnoticed range of capacities in childhood and adulthood.

Against this general background I shall first consider the work of the three sections in the Basic Research area: Aging, Animal Behavior, Learning and Perception, and then go on to consider the three in Clinical Investigations: Child Development, Personality, and Section of the Chief.

SECTION ON AGING

The general goal of the Section is to observe aging in both animals and humans with the aid of experimental methods in order to reveal and characterize mechanisms of biological and psychological interaction of general scientific importance. Through the research and theoretical efforts of staff members, the subject matter has undergone what in retrospect seems to have been a rapid transition from a descriptive naturalistic phase into a more tightly organized state of knowledge. Knowledge and study of aging now seem to be established as complementary to early development as one of the fundamental scientific orientations.

The research program, while it has several facets, has two major foci. These are: (1) the gathering of systematic information about the behavioral changes of aging, and (2) the psychobiology of aging. Of continuing concern has been the analysis of age changes in learning and in speed of psychological processes, in which areas several advances were made. Cellular components and extracellular relations in the nervous system continued to be studied, with reason to hope for eventual success in identifying some of the biological accompaniments of the behavioral changes of aging.

During the past year the Section on Aging was engaged in a major activity in organizing and edit-

ing the *Handbook on Aging and the Individual: Psychological and Biological Aspects.* Chapters for the Handbook were prepared by staff members on the subject matter of their research. The advances in the state of our knowledge about aging has been made manifest by the completion of the Handbook. The organization of this material in many ways seemed a necessary early step in systematic research effort.

The data obtained in the extended study of healthy elderly men has been analyzed and draft manuscripts have been prepared reporting the results. Data was gathered on 27 men over 65 years, all of whom were community residents and judged to be physiologically normal, "healthy." Also studied was a sample of 20 men who had asymptomatic or subclinical diseases (mostly vascular). Comparison of these two samples, and of these with data from provious studies of young men, gives considerable insight into what psychological changes may be regarded as the normal expectancy of advancing age and those changes frequently associated with disease states in older persons. It is expected that the manuscripts will be combined with those from investigators in other laboratories in the form of a monograph. The monograph will also report the intercorrelation of the experimental psychological, physiological, psychiatric and social psychological data.

In general the results showed that the population studied compared more favorably with test results of young subjects than do the aged men of previous studies less well selected for health status. Two points may be made: (1) healthy men over 65 do better on psychological tests than men unselected for health and (2) age differences in patterns of abilities were found even in a population devoid of apparent disease. These findings are regarded as significant since they clearly point to the fact that age differences in psychological measurements may not be solely attributed to illnesses which frequently occur in aged individuals.

Dr. Alfred D. Weiss reports the results of his measurements of hearing loss, click perception, diotic and dichotic digit span and response to delayed speech feedback. The click discrimination measurements indicate that healthy older men do not differ from healthy young men in two click discrimination and that the less-healthy old tend to have larger discrimination times, i.e., a longer length of interval was required for discrimination between one or two clicks. While all auditory intensity measurements were correlated, none correlated with the two-click discrimination threshold. Subjects were also presented with trains of clicks varying in number from one to ten at several rates of speed. The older subjects differed from young controls in their ability to enumerate the number of clicks. This measure of "perception" did not correlate with intensity thresholds. In the digit-span measurements, distinct age declines were found. The older subjects were relatively poorer in the dichotic situation where they receive different digit series in the two ears. This suggests that the old may have more difficulty than the young in simultaneously monitoring two channels compared with monitoring a single channel. In the relayed speech feedback measurements very little difference was found between groups.

Dr. James E. Birren, in collaboration with Drs. Botwinick and Weiss and Mr. Donald Morrison, analyzed the intercorrelations of the 23 cognitive and psychomotor variables and 9 audition variables utilized in this study. The 32 variables were all intercorrelated and analyzed using Hotelling's Principal Component Method. About 58 percent of the common variance was accounted for on the basis of the first five components. Five independent component scores were then derived for each subject. While all five scores showed mean differences in favor of the "healthy" group, only component I was statistically significant. Component I was interpreted to be a measure of previously organized information. Interpretation of the finding was that late life illnesses result in a loss of stored information, primarily verbal; the healthy aged had higher mean information scores than would be expected in a young adult population. However, on perceptual and manipulative types of tasks, the older subjects were slower than the young. In the present study the health difference was smaller than the age difference. This leads to an unanswered question of why "aging" is more important in speed of psychological functions and "health" more important in measures of stored verbal information.

Dr. Ruth M. Riegel, Visiting Research Associate of the Section, completed a comparison of factorial studies of the Wechsler-Bellevue, the

Wechsler Adult Intelligence Scale of the German translation of the test HAWIE. Her results indicated that there seem to be no essential differences in factorial structure of the different versions of the tests or between results obtained on German and American populations.

Dr. Edward Jerome has been studying higher cognitive processes in aging subjects using the Logical Analysis Device of the Psychological Corporation and some additional classical problem-games. To date about 15 young and 10 elderly subjects have been studied in a sequence of individual appointments which for each subject extends over five or more days. The older subjects have expressed a preference for the automatically controlled device despite the fact that they experience difficulty in its solution. The records are being analyzed to identify the source of their difficulties in problem solution which in some cases reflects a failure to secure pertinent information to solve a problem or a disorderliness in the search for information.

In related studies of learning and transfer, using the rat, a group of 40 additional animals were trained during the current year on two problems of different difficulties. The major findings may be summarized as follows: (1) senescent rats learned as quickly and efficiently as "mid-life" rats on a set of problems representing a wide range of difficulties, (2) senescent rats showed as much positive transfer of training as did younger rats, and (3) no age differences were found in the degree of behavioral stereotypy, or rigidity of performance.

Dr. Jack Botwinick found that when elderly and young adult subjects were compared with respect to performance on card sorting tasks (varied in: extent of perceptual matching or searching, number of stimulus aspects needed to be kept in mind and manipulated simultaneously, and relearning requirements) it was found that the older subjects did relatively poorer with tasks that involved most mental manipulation and perceptual searching. Poor performance was also seen when age differences in motor or movement performance were examined. Relearning rate and practice effects were similar for the elderly and younger subjects.

It was also found that elderly subjects, as compared with young, required more time to prepare or to organize for response. When time intervals were presented regularly but varied between 0.5 and 4 seconds, a disproportionate slowing with age occurred with shortest interval. Much of this slowing was decreased with practice suggesting that at least part of the change with age is related to a difficulty in adjusting or learning to prepare for response during brief time intervals. Increasing the number of stimulus response alternatives had little, if any, differential age effect.

Another study involved general level of activation or reactivity in relation to age. There is reason to think that one aspect of general responsiveness is measured by the GSR. Older adults were found to condition less readily and extinguish more readily than younger adults. This lowered frequency of GSR output was taken as an index of reduced reactivity with age.

Dr. Birren in collaboration with Dr. Klaus F. Riegel designed a study to examine the relations of psychomotor slowing and possible age changes in verbal association strengths and other language functions. In this study, subjects are presented with a variety of stimuli on a standard apparatus, the Psychomet, which permits measurements of individual response times while varying "key-light" relationships. Old and young subjects are being systematically observed in a series of tasks involving the measurement of simple movement times, simple and choice reaction time, and a variety of symbolic (numbers, letters, colors) and syllable and word associations. The general objective of the study is to determine the extent to which certain of the age changes in behavior are the result of habit and overlearned language usage or in endogenous changes in the central nervous system which might be reflected in such aspects of performance as speed of response and perception, but which may have some more pervasive implications for verbal reasoning.

Dr. Eugene Streicher has made extracellular space determinations for brain. To obtain a measure of the extracellular space of rat brain, the distribution of thiocyanate between blood and brain was ascertained. (Thiocyanate is considered to be impermeable to most cells.) The "thiocyanate space" was found to be a function of dosage, and extrapolation of the curve relating extracellular volume to plasma level suggests a space of approximately 5–10 percent. This figure is in general agreement with the estimates of electron micro-

scopists, and indicates that, in contrast to liver or muscle, a large fraction of brain sodium and chloride is localized intracellularly. This conclusion was supported by measurements of brain extracellular space in rats in which cerebral edema had been experimentally induced. In this instance no change was observed in the distribution of thiocyanate, although the sodium content of the brain was elevated 50 percent and that of the spinal cord was doubled. A few experiments were conducted with the congenitally jaundiced mutant strain of Wistar rat. In animals exhibiting neurological symptoms, such as difficulty in walking, the extracellular space of the spinal cord was decreased. The brain-thiocyanate space of 2½-year-old rats was not significantly different from that of young animals indicating that possible brain changes in aging such as loss of cells, dehydration, gliosis, etc., if they do occur in rats, are not reflected in alterations of the extracellular space. However, in preliminary experiments, it was found that thiocyanate was eliminated from the brains of older animals more slowly than from the brains of younger rats.

Dr. William Bondareff completed his analyses of cytological preparations made the previous year from old and young, fatigued, and control animals. The experiment had been designed to investigate whether intracellular deposition of lipofuscin pigment could be initiated in animals by a course of acute muscular fatigue and whether the amount of intracelluar pigment normally present in the neurones of old animals could similarly be increased. In both cases it can be definitely stated that if such short-term muscular fatigue, as results when animals are forced to swim until exhausted, does cause an increase in intracelluar pigment, the increase is not readily detected by cytological methods. It is possible also that pigment produced by this means was not chemically equivalent to that normally present and hence was not demonstrated by the cytological technique employed, but it seems justifiable, however, to conclude that there is no increase in intracellular lipofuscin.

Cytologic examination of the distribution of Nissl material does not indicate a great difference between the 3-month and 20-month animal. The Nissl pattern seen in 20-month animals is more closely related to that found in 3- than in 24-month-old animals. The cytological picture of 20-month-old rats indicates that the metabolic processes of nucleic acid synthesis occur to a degree intermediate between that found in 3- and 24-month animals though more closely comparable to that of 3-month animals.

SECTION ON ANIMAL BEHAVIOR

Broadly speaking, research in the Section on Animal Behavior has been concerned with (a) defining the behavior served by association cortex in monkey, chimpanzee and man (b) determining the neural mechanisms underlying this behavior and (c) specifying the neural mechanisms of attentive behavior in man. Accordingly studies have been designed to elucidate the interaction within the association areas, and between these areas and the basal ganglia, hypothalamus, and brain stem. Participating in various aspects of these studies have been Dr. H. E. Rosvold, Chief of the Section, and Drs. M. Mishkin, A. F. Mirsky, M. K. Szwarcbart. Also participating have been Dr. Elinor Brush and Dr. Charles Butter, NIMH Post Doctoral Fellows; Dr. Karl Batting, a Visiting Scientist from Zurich; Dr. Stefan Brutkowski, a Visiting Scientist from Warsaw; Dr. Bryan Robinson, a Research Associate; and Dr. H. Kuypers of University of Maryland, as a Consultant in Anatomy.

Our earlier findings that, unlike monkeys, chimpanzees with damaged frontal lobes recover from an initial deficit on delayed-response problems has been confirmed in additional animals. Since, in a sense, the chimpanzee has a partical deficit in this behavior, he has been a particularly good subject in which to manipulate variables which may affect delayed-response behavior quantitatively. Early results suggest, however, that it should be possible to specify which variables may be manipulated in the delayed-response test to increase its difficulty so that the chimpanzee's postoperative performance, like that of the monkey's, will be completely and permanently impaired.

Behavioral studies with monkeys have also revealed something of the nature of the deficits following frontal-lobe damage. Findings last year suggested that monkeys with such damage are impaired in their ability to inhibit responses whether or not a delay is involved. More recent studies suggest that this impairment is quite gen-

eral in the sense that frontal animals have difficulty in inhibiting any strong response tendency which develops as a result of training, preference, or novelty, and appears whether the response is dependent on visual, auditory, or tactual stimuli. An interpretation of these findings leads to the notion that in the frontal animal some on-going central process gets locked in and occludes others. Behaviorally, the animal develops a set to respond and perseverates this set to the exclusion of others. Further research will attempt to set up critical tests of this notion.

Earlier work based on limited information provided by results on delayed-alternation testing had suggested that the effects of frontal lobe lesions are similar to those of lesions in the head of the caudate nucleus. Research this year has extended the information to other types of delay problems, and to auditory, tactual, and visual learning tests. The results have been consistent in showing that the effects of caudate lesions, while quantitatively less severe, are qualitatively similar to those of frontal lesions. Preliminary results of special histological studies using Nauta-Gygax silver staining procedures suggest that the behavioral similarities following damage to these two structures may be accounted for by the anatomical connections between them which have now been demonstrated.

Last year we completed a study which appeared to implicate the splenium of the corpus callosum in delayed-alternation performance. More recent studies, however, indicate clearly that this is not so. The earlier effect appears to have resulted from damage either to the posterior columns of the fornix, the mid-line thalamic structures, or to some yet unsuspected structure. On-going research is attempting to determine which of these structures are in fact involved.

Investigation of the role of the inferotemporal cortex in vision continues as a major effort of the section. A recent study in the literature questioned the specificity of the inferotemporal-visual relationship, suggesting that not only inferotemporal but also other temporal neocortical areas serve visual functions, and that these areas serve not only visual but also olfactory functions. A special study undertaken to reinvestigate this problem provided no support for these suggestions, but confirmed instead the original thesis that the focal area for extrastriate visual processes is the inferotemporal area.

It was hypothesized on the basis of earlier work that the inferotemporal area is connected with the primary visual area mainly, if not exclusively, via cortico-cortical connections. This conclusion derived from an experiment in which the cortico-cortical connections between an intact striate area in one hemisphere and an intact inferotemporal area in the opposite hemisphere were cut by the complete sectioning of the corpus callosum. This procedure produced an abrupt and severe decline in visual performance. An extension of this study, now in its early stages, demonstrates that the same impairment may be produced by sectioning the posterior third of the callosum only, whereas sectioning the anterior third is without effect on visual performance. On the basis of these results a detailed picture of a sensory-associative visual system is beginning to emerge. Concurrent anatomical studies, using the Nauta technique for staining degenerated fibers following selective cortical ablations, fully confirms this picture.

The behavioral analysis of the visual impairment produced by inferotemporal lesions has depended until recently on work with monkeys trained by discrimination techniques. In an attempt to broaden the approach, new studies have now been initiated using generalization techniques and the studies designed to extend the analysis to chimpanzees and man are continuing.

This year a new project has been developed to attack directly the problem of relating learning to brain function. The first step has been to establish base-line measures for continuous and periodic food-getting and water-getting responses in the monkey. The next step has been to compare these measures with those obtained by electrically stimulating through implanted electrodes the ventromedial and lateral hypothalamic areas (which are known to be reciprocally related in regulating eating and drinking). The third step has been to study the effects on the electrical activity in these structures of food and water deprivation and satiation. This phase of the study is in its very early stages, but preliminary results are encouraging. The next step, if the first two are successful, will be to study the changes in these measures as the learning of an alimentary condi-

tioned response takes place in order to determine if a change in the activity of these brain centers occurs as the unconditioned and conditioned stimuli become related in the process of learning. The long-range goal of this study is to gain an understanding of the function of the hypothalamic hunger mechanisms in motivation and learning.

The studies of attentive behavior in man during the past year have continued to involve detailed study of epileptic patients with presumed subcortical pathology and patients with electrodes implanted in the temporal lobes. The abnormal electrical activity of the former group may interfere seriously with attentive behavior; that of the latter group does not. Approximately 15 patients with presumed subcortical pathology have been studied exhaustively from the standpoint of phasic interruptions in their ability to perform sustained vigilance tasks. Simultaneous with the behavior, the E.E.G. and seven different autonomic functions have been monitored. Present efforts are directed towards encoding the obtained information and analysis of the relationships among the several behavioral and physiological variables.

Supplementing the study of neurological patients has been the investigation of those conditions in normal individuals which produce impaired attentive behavior. Thus, behavioral, E.E.G. and autonomic variables have been studied in a group of ten normal controls under the influence of prolonged sleep loss and of the drug chlorpromazine.

The research conducted to date suggests certain broad similarities and striking differences among the effects of agents which impair attentive behavior. Studies are currently being planned to test some of the anatomical-behavioral-autonomic relationships suggested by the information that has been obtained.

SECTION ON PERCEPTION AND LEARNING

A great deal of the data of the traditional psychophysical kind of experiment may reflect the operation of the observer's assumptions, attitudes, and language habits rather than elemental sensory processes from which perception is presumed to be elaborated. Instead, many perceptual phenomena which have in the past been interpreted as cognitive or judgmental may be more appropriately characterized as fundamental conditioning-adaptational processes not given directly in conscious experience.

Dr. V. R. Carlson has utilized size constancy as a paradigm for exploring the usefulness of these considerations. This phenomenon refers to the empirical observation that perceived object size remains approximately invariant with variations in the distance of the object from the observer. It is a relationship which obtains under more or less natural environmental circumstances, but systematic deviations occur when the relationship is studied experimentally. Two experiments in progress are concerned with determining whether these deviations have their explanation in terms of the attitudes and motivations which are inherent accompaniments of this kind of experimental situation.

The first involves the effect of an instructional enhancement of the perspective attitude on size judgments. It appears clear that the degree of overconstancy which can be produced by attitudinal determinants is fully as great as the degree of underconstancy which can be produced by alternative instructions. Underconstancy, however, has generally been interpreted in terms of underlying sensory mechanisms rather than as an attitudinal manifestation.

The second size-constancy experiment investigates one aspect of the latter problem. LSD–25 was used to produce a psychological state in which orientation of report toward subjective sensation is greatly enhanced. In this experiment, underconstancy has thus far occurred under circumstances in which the effect cannot be interpreted as due to a change in basic sensory function.

Two other perceptual studies are more directly concerned with the dependence of perceptual response on adaptation to various kinds of stimulation. Dr. Irvin Feinberg (Laboratory of Clinical Science) and Dr. Carlson have accomplished some initial work preliminary to determining relationships between kinesthetic and visual adaptation and to the measurement of adaptation to visual motion.

The animal research program conducted at the Rockville Farm by Dr. John C. Calhoun and Dr. Barbehenn (postdoctoral fellow) has been directed chiefly this past year toward problems in which

group size operates as a variable in the accommodation of the individual to a complex set of environmental stimuli.

Two long-term studies have been completed on the relationship of group size to learning in two kinds of social interaction problems. One of these situations required the proximity of two individuals in order for either to be rewarded ("cooperative" behavior), while the other required that only one rat be present at the response situation for a reward to be received ("disoperative" behavior). The response was a lever press in order to obtain water. For cooperative behavior, smaller group size is associated with greater initial errors and a rapid rate of learning. Larger group size results in fewer initial errors and a slower rate of learning. This trend arises from the fact that in the cooperative situation there is more opportunity for reward in a larger group purely on the basis of random activity. For disoperative behavior, the situation is different. The problem is learned but not rapidly enough to prevent gradual accrual of a deficit in water balance. A point is reached where the motivation to secure water or to respond to the lever-pressing situation cancels our prior learning and produces a situation in which individuals stand side by side at the lever, even though this precludes the possibility of reward.

It has been observed in several studies in which social groups of rats are maintained that wherever there is a high probability of two rats being in close proximity during occurrence of reward, the response situation develops a new definition which requires the presence of another individual. The other individual appears to serve as a secondary reinforcement, and few rats in the group will respond at those places or those times when other individuals are absent. Eventually such an altered pattern of response can lead to highly pathological social aggregations in the sense that the presence of other individuals in great numbers interferes with the execution of sequences of responses which form a behavioral unit. Where the behavior is instrumental in obtaining a physiologically necessary reward, this interference may lead to such an extreme deficit that most of the animals in the group die.

Another observation is that there were clear alterations in the durations of behaviors and in the 24-hour cycles among individuals. This finding suggests a promising approach to further understanding of mechanisms of biological time.

Dr. Barbehenn has studied the influence of litter size on later selection of places of habitation and places of visitation as these differentially offer opportunity for contact with other rats. Of several places to which rats had free access, those individuals who were members of small litters tended to seek out those situations in which contact with other individuals is minimal. Rats from litters of larger size tended to maximize opportunity for contact with other individuals.

SECTION ON CHILD DEVELOPMENT

The program of research in the Section on Child Development is concerned primarily with studying the characteristics of the infant under one year of age, and with those aspects of his environment that are likely to be significant in their effect on the infant's development and the formation of personality characteristics.

Several current theories of personality development and of the etiology of mental pathologies, as well as of normal variations in personality and in other aspects of mental functioning, have emphasized the importance of the infant's early experiences. We hear of the devastating effects of maternal deprivation and of environmental impoverishment, and of such things as hostile rejection and of overprotection of the child by his mother; we hear of the conditions which foster in the child feelings of security or insecurity, and of the effects of the child's early emotions and experiences on the course of his development. It becomes important, therefore, to explore in some detail just what is meant by these rather vague generalizations: Specifically, what behaviors and conditions does the infant in different settings experience, and just how does he react to them? How lasting are the effects of early experiences and how persistent are behaviors and response-tendencies that are learned (or at least manifested) in infancy? Can we determine which behaviors are species-specific, or within species genetically determined, and the differential effects of given experiences on children who are differently constituted?

Our studies take several forms, as they approach a number of the different facets of these

indicated conditions in the immature, developing organism as it interacts with different features in the environment. Within this framework, the nature of our investigations is determined to a considerable extent by the specific interests and preoccupations of the investigators involved.

Dr. Harriet L. Rheingold has made one approach by studying the comparative aspect of mother-infant interaction. She has so far made observations of maternal behavior and infant response in several mammalian species of widely differing degrees of complexity: the hamster, the dog, the monkey, and the human. In these comparisons she will seek to differentiate aspects of maternal behavior specific to humans and those general across species.

In the human infant, Dr. Rheingold has studied 3-month-old infants in two widely different environments: first-born children in middle-class homes, and infants in an institution. She finds the same kinds of caretaking occurring in both environments, but great differences in amounts of these: the home baby has much more care, and care primarily by one person, whereas the institution baby is left more to itself, but is cared for by many different people. At 3 months of age the behavior of the two groups of babies is about the same in many areas. The institution infants, however, proved to be more socially responsive to the examiner than the home infants. Subsequent research will attempt to uncover the causes of this difference. For example, were the institution infants more sociable because they were more deprived of stimulation, or because they did not discriminate the examiner as a strange person?

Other studies of Dr. Rheingold's are relevant in working toward answers to these questions. These studies are in infant learning. She has, for example, shown that 3-month-old institution babies quickly learn to vocalize more frequently when their vocalizations are rewarded regularly by a smiling social response of an adult. As a result of these research efforts Dr. Rheingold has developed some hypotheses about the genesis of social responsiveness and emotional attachment in the human infant. At a very early age there are already developed in him both a *responsiveness* to stimuli in the environment and a *searching* of the environment for stimulation. The sight of some objects in his environment brings about smiles and vocalizations and other signs of delight. Of all the objects which arouse these responses the most potent appears to be the social object, that is, another human being. More than any other object the other human being brings to the child not only complex stimulation, but also stimulus change, and especially stimulation *in response* to the infant's own behavior. Because of the large role of vision in these interactions, Dr. Rheingold proposes the thesis that human sociability develops primarily from visual but also to some extent manipulatory exploratory behavior.

Dr. Jacob L. Gewitz is also interested in the acquisition of social motives and attachments by the human infant. He believes that a large variety of stimuli can function as unconditioned postive reinforcers of the infant's attachment behavior, in addition to those which meet the organic needs of the infant, and that reinforcing stimuli are likely to be provided also by nonhuman as well as human environmental changes. He plans to test these hypotheses by the technique of operant conditioning, and has been developing an apparatus that is suitable for use with infants, to provide stimuli and record responses and thus measure the processes of conditioning. He plans to continue these studies while on leave in Israel for an extended period.

Another facet of the study of early personality development is that of variations in maternal behavior. It is evident that normally a predominant part of the infant's environment is furnished by the mother. Therefore, her predispositions, expressed attitudes, and behavioral habits in regard to her infant may have a strong influence on the kinds of response habits the infant develops. Certain types of maternal behavior have been given prominence in theories of the causes of, or at least strong causal components in, schizophrenia. Dr. Earl S. Schaefer has been working on a series of rating devices for use in classifying maternal attitudes and behaviors. His current scales and hypotheses are outgrowths of the Parental Attitude Research Instrument and the Maternal Behavior Research Instrument scales that he and Dr. Richard Q. Bell developed, and which have been described previously. In statistical analyses of the maternal behavior scales, Dr. Schaefer has found two factors and also a Guttman type of cir-

cular order of neighboring that fit, not only the data from the Berkeley Growth Study, on which the scale was devised, but also a large number of other published data on maternal bahaviors. The two main orthogonal dimensions (i.e., factors) in this circumplex are autonomy-control and love-hostility. He has used this model, and the descriptions of maternal behaviors in the Berkeley study, as a basis for constructing a short-form Maternal Behavior Rating Scale and a Maternal Personality Rating Scale that can be used in evaluating the mother-child interactions as seen during a standard developmental testing situation. These scales are now being tried out, will be tested for reliability and validity, and revised on the basis of these preliminary trials. The relationship of these rated maternal variables will then be used to test out some hypotheses on mother-child relationships that Dr. Schaefer and Dr. Nancy Bayley have derived from the analyses of the Berkeley Growth Study data. For example, they found there some evidence that the lower-class mothers of boys were more punitive and authoritarian than the upper-class mothers, and that these relations in the mothers of girls were much less clear. There is also some evidence that the children of punitive, hostile mothers are more active and score higher on the developmental scales in the first year or two, but become less active and make poorer scores as they grow older. The happier, less excitable, inactive babies tend to be slow in their early development, and to have mothers who are more affectionate and generally accepting. There is also tentative evidence that the punitively controlling mothers have children who develop withdrawn, more maladaptive, kinds of social adjustment.

Dr. Bell has conducted a series of researches based on evidence that the mother-child processes of interaction and the resultant maternal behavior and infant personality characteristics grow out of individual differences in the child as well as in the mother. He has observed and recorded the behavior of 3-day-old infants, each for a 3-hour period with a standard set of stimuli. From a careful analysis of his data he has been able to derive five factors which differentiate newborn infants before they have had more than minimal experience with their mothers. These factors he has called: skeletal muscular strength, skin sensitivity (these two are negatively correlated with each other), level of arousal, depth of sleep, and oral integration. In a further analysis of strength and skin sensitivity he finds both sex differences and within-sex variability in these factors. Males tend to be stronger, females more sensitive to skin stimulation. There appear, thus, to be genetic differences that are identifiable in the newborn infant. If these remain stable, they may well form a genetic basis for both individual and sex differences in personality variables.

In following through with the research into other aspects of maternal characteristics as they affect the child, Dr. Schaefer is now testing out a scale of psychosomatic symptoms and psychological reactions of women, before, during, and after pregnancy, and the relations of the latter two to difficulty of labor. In another approach to parent-child relationships he is testing out a scale for measuring children's perceptions of their parents' attitudes. Preliminary analysis show differences between normals, delinquents, and schizophrenics in their perceptions.

The interaction of the infant with his environment is determined to an important extent by the characteristics of the infant himself. Some of these have already been mentioned, such as individual differences in sensitivity to stimuli, and there are also evidences of differences in vulnerability to stress. Furthermore, the degree of development in an infant is a limiting factor both in his perceptions of his environment and in his ability to cope with the stimuli to which he responds. It is therefore necessary to evaluate these factors in the studies of early personality and learning as they develop. With a view to improving the tools for these evaluations, Dr. Bayley is revising and standardizing her mental and motor scales of infant development. These scales are designed to extend from 1 month through 30 months of age. Another dimension which she is adding to the scales includes the appraisal of emotional and other reaction-tendencies that should prove useful as rough measures of the variables of sensitivity, vulnerability, and emotional tone, among others. At present, in cooperation with the NINDB collaborative projects, about half of the 1,500 standardization tests for the first 15 months have been completed. With the use of such scales it will become possible to study learn-

ing and evironmental adjustments in infants in relation to their developmental and "personality" scores.

SECTION ON PERSONALITY

The conduct and investigation of the process of psychotherapy continues to be one of the most fruitful sources of hypotheses for members of this section. This is illustrated in the work of Dr. Donald Boomer. He and Dr. Wells Goodrich have recently completed their replication of George Mahl's study of the relationship between instances of speech disturbances and global judgments on patients' anxiety level. It was found that (1) the appropriateness of the speech disturbance index as a general measure of anxiety for all subjects is doubtful; (2) the therapist appears to be the best judge of anxiety in those patients who react to stress with some form of speech disruption; and (3) the reliability between judges concerning the identification of high and low anxiety phases is very poor.

The significance of this research lies not only in the fact that the investigators have provided a more sophisticated analysis of the speech disturbance instrument, but that it has raised basic questions regarding the conceptualization of anxiety and its expression. Dr. Boomer now intends to test out some of his clinically derived hypotheses in the laboratory. He will attempt, under controlled conditions, to extend the investigation of tension indicators to include not only speech disturbances but other measures of autonomic lability. Issues of preferred modalities and the interrelationships among modalities under specified conditions will be investigated. Further study of the phenomena of brief intercurrent speech pauses will be made to determine the part they play in what Mahl now defines as speech disturbances. It is to be noted that Dr. Goodrich's participation in this project has been reduced with his assumption of new administrative responsibilities.

Drs. Joseph Handlon and Morris Parloff also have utilized their experience in group and individual therapy in their work concerning families of schizophrenics. This experience was relevant to the following activities: (1) The development of a test instrument devised to distinguish patterns of inter-family relationships which may correlate with mental health of the family members. (2) Dr. Handlon prepared a paper in which he derived and organized assumptions and hypotheses implicit in existing theories of schizophrenia. This paper makes clear the implications for research strategy of the various concepts currently in vogue regarding schizophrenia. (3) Dr. Parloff was coauthor of a paper read at the American Psychiatric Association concerning recent experience with family group therapy. (4) Drs. Handlon and Parloff are currently preparing a paper to be delivered at the annual meeting of the American Group Psychotherapy Association contrasting group therapy and family group therapy.

Dr. Handlon has taken an increasingly active role in a research program of the Section on Psychosomatic Medicine in the Adult Psychiatry Branch entitled, "Relation of Emotional Behavior and Adrenal Function." He has participated in the planning and execution of a study which involves observing changes in emotional state in response to three critical situations: (1) adaptation to a new environment (Clinical Center); (2) spontaneously occurring events of personal significance in the life of each subject; and (3) standard commercial films that effectively present common human problems. Two of the most significant findings to date related to the work of Dr. Handlon are the following: (1) the identification of two Rorschach patterns that correlate with two patterns of endocrine responses and (2) the identification of individual affective response patterns to specified situational stresses. The technique of the self-rating questionnaire also assisted the investigators in focusing their interviews and ward observations.

The area of research in creativity is becoming a central concern of the Section. In general, the ultimate aim of the research will be to describe the conditions, internal and external, which are necessary and sufficient for productive thought. Our more immediate aim will be to learn about basic psychological processes involved in creative thinking. One of the basic assumptions made in the work thus far is that the process is qualitatively the same at all levels of productive thinking and therefore can be studied not only in individuals designated as "creative" by their peers but also in generally talented subjects. It is the intention to

attack the problem from two directions: (1) To undertake intensive clinical investigations with (a) scientists who have been designated as "creative", and (b) individuals who have demonstrated talent but as yet have failed to be judged as creative. Hopefully this will provide hypotheses for experimental study. (2) To select for intensive study basic psychological processes believed relevant to creative thinking.

To date this section has undertaken four approaches to the investigation of creativity.

(1) The testing and interviewing of talented individuals. The sample has included Research Associates selected for the NIH training program. Efforts have been made to integrate the current testing program with that of Dr. Morris Stein in the hope that this will facilitate comparison and interpretation of the findings.

(2) The testing of the hypothesis that intuitive thinking is a significant element in creative thought. To this end, an instrument for measuring "intuitive problem solving" has been devised and is currently being tested and improved.

(3) An investigation of the hypothesis that problem solving may be enhanced under conditions of reduced inhibition. This is being conducted by use of the Kleitman technique of monitoring the dreams of individuals who have been given a problem-solving task which they failed to solve before retiring.

(4) A pilot study has been conducted regarding the role of social-influence factors involved in collaborative problem-solving. The variables included are (a) degrees of respect for the opinion of others and (b) the degree of approval received from such individuals in the course of collaborative efforts to solve open-end problems. Although the analysis of the data is as yet incomplete, there is evidence to suggest that (1) approval is an important condition in stimulating an individual to produce original and plausible ideas and (2) the less accepting the individuals are of each other the greater the impact of receiving approval or criticism.

It is hoped that it will soon be possible to undertake the intensive psychoanalytic investigation of a highly gifted but as yet "uncreative" individual to provide clinical data about inhibition and other hypotheses relevant to this project.

SECTION OF THE CHIEF

The major areas of research in this Section remain schizophrenia and psychotherapy. Another involves the psychological aspects of physical illness. In the area of psychotherapy there is some overlap with studies in the Section on Personality.

In addition to Dr. David Shakow, the members of the Section concerned with the problem of schizophrenia are Drs. David Rosenthal and Theodore Zahn. Dr. William Lawlor of Fordham University and Dr. Rue Cromwell of Peabody College participated in this program during the summer. One continuing study by Dr. Shakow involves the analysis of a large body of already existing experimental data on the psychology of schizophrenia as a basis for development of theory in this field. During the year further progress was made on the analysis of this material. A paper on discrimination reaction time and a paper on the tautophone are among the many studies in an advanced degree of analysis. It is hoped that eventually this work will result in several monographs on the psychology of schizophrenia.

Besides the above, studies in schizophrenia carried out during the year fell into three areas: heredity, psychophysiological responsivity, and simple adaptive behavior (reaction time).

In the area of heredity, Dr. Rosenthal has continued to examine the relative contributions of heredity and life experience factors to the severity and forms of expression of schizophrenia. To deal with this problem he has carefully analyzed the relevant literature in this area and has marshalled strong evidence for the following points:

There is a group of schizophrenics in whom heredity factors probably play a strong part. In males, the form of the illness is mainly catatonic and the illness is severe. There is a group of schizophrenics in whom hereditary factors play a minimal part or none at all. In males, the form of the illness is mainly paranoid and the illness is relatively mild in that onset is late and outcome non-deteriorating. In the major twin studies of schizophrenia, chronic patients have constituted the bulk of the large samples, a factor which tends to yield overestimates of concordance rates. In the major twin studies, there are differences between investigators with respect to sampling methods, determination of index cases, and diag-

nostic criteria. These findings, plus the probability that too many twins are classified as dizygotic who are probably monozygotic, suggest that the present estimates of concordance rates are not reliable. Differences in concordance rates between female and male pairs, and between same sex versus opposite sex pairs occur with respect to schizophrenia and mental illness generally when the relationship is in the nuclear family, but not when the relationship is avuncular. This finding provides strong support for psychological influences playing a significant role in the manifestation of schizophrenia.

Continuing experimentation went on in the area of psychophysiological responsivity as measured by GSR and heart-rate reactions to repetitive presentations of simple stimuli. The purpose of these studies is to investigate the autonomic reactions of schizophrenics under conditions which are minimal both in the quality of the stimuli and in the demands put on the subject. By intragroup comparisons of these autonomic measures with measures of overt behavior assessed by both ratings and more objective means such as experimental tasks, and by intergroup comparisons of schizophrenics with normals on these measures, the attempt is being made to evaluate the contribution of differences in autonomic functioning or arousal to deficits in the behavior of schizophrenic patients. This minimal stimulus-minimal response situation is to serve as a "basal" condition for further studies in which the affective quality and/or demands on the subject will be increased.

Preliminary findings on the GSR data indicate that, with respect to normals, schizophrenic patients give about the same number of specific responses to 40 repeated presentations of an auditory or visual stimulus, but the normals start at a higher level and habituate more rapidly. In addition, the patients give more nonspecific responses relative to specific responses, have a lower baseline resistance and give responses with less amplitude than do normals. Data on a previous sample of patients indicated a significant correlation between response frequency and "mental health" or "ego intactness". This was not replicated on the latest sample of patients, but "mental health" correlated negatively with baseline resistance and response magnitude. The data from the two samples indictate, therefore, lowered autonomic responsivity to external stimuli by patients with poorer "mental health," but each sample shows this via a different measure.

The studies of reaction time are being continued in a variety of settings and under various experimental conditions in order to answer some important theoretical questions about factors relating to this response.

A number of findings have come out of our most recent studies in this area. These are: (1) Severity of schizophrenic disorganization is more reliably measured by any of three different methods than are other traits and behaviors of such patients, and this measure correlates highly with various aspects of their performance on tests and experiments. (2) When preparatory intervals (PI's) are administered in regular series, increasing in duration from 1 to 10 seconds, or decreasing from 10 to 1 second, normal S's do equally well under both conditions. Schizophrenic S's show poorer R T with longer PI's in the ascending series, but do poorly on all PI's in the descending series. (3) When PI's are administered irregularly, schizophrenic S's are more influenced by the previous PI than are normal S's, who are also influenced by it. (4) R T of schizophrenic S's is better when S is given a verbal "Ready" signal as compared to when PI's are programed automatically. (5) If two relatively short PI's are administered irregularly so that the probability with which each PI occurs is predetermined, then for normal S's, R T for the short PI increases as its probability of occurrence decreases. (6) In a R T situation where S is permitted to select his own PI's and where he is not, normal S's prefer the "autonomous" condition where they select their own PI's, whereas schizophrenic S's prefer the "controlled" condition where the PI's are determined for them. (7) Preliminary findings suggest that normal S's have better R T's under the "autonomy" condition, whereas the R T's of schizophrenic S's are better under the "controlled" condition. (8) Normal S's who perceived their mothers as more protective tended to see events as externally controlled, but schizophrenic S's did not. But schizophrenic S's tended to see their mothers as more hostile than did normal S's.

In collaboration with the Clinical Science Laboratory the effects of various precursors of serotonin and of low dosages of Marsilid on autonomic

556044—60——15

responsivity, reaction time, and ability to maintain a set are being studied in schizophrenic patients. The data are still being analyzed, but it is planned to relate changes in these measures produced by the drugs to changes in behavior as measured by ward ratings, interviews, and other experimental techniques as well as to biochemical changes.

In an attempt to explore some of the conditions influencing reaction time and the maintenance of sets in normals, a reaction-time experiment was carried out on normals who had been deprived of sleep for 48–52 hours. The results showed that at the beginning of the half-hour session, if the preparatory interval was short and the subject could control the onset of the trial, there was no impairment. If any of these conditions were not met, sleep loss produced an impairment of reaction time.

Dr. Rosenthal has continued to carry the administrative responsibility for the general aspects of the scientific investigation of a group of identical quadruplet schizophrenic girls who have been under study by investigators from various NIMH laboratories for a period of several years. A large amount of data in different areas has been accumulated and considerable progress was made during this year in its collation and analysis. The integration, evaluation, and write-up of this data have been begun by various investigators. The psychological material is in final stage of analysis by Drs. Rosenthal and Parloff.

At the general theoretical level, Dr. Shakow has revised the paper presented at national and international meetings: "The Recorded Psychoanalytic Interview as an Objective Approach to Research in Psychoanalysis." This is an attempt to provide the general rationale for the approach used in the major psychotherapy project. As an outgrowth of the research group's activity in the analysis of the first hour of the therapy, Dr. Shakow drew up a systematic account of modes of adaptation, particularly modes of defense, as they reveal themselves in the psychotherapeutic process.

Dr. Dittmann has continued his microanalytic studies of communication in the therapy process through the investigation of foot movements and speech disturbances. Two samples were collected and analyzed, the first finding association between foot movement and speech disturbance at the .01 level, the second finding no association. A third sample has been collected, and is now being analyzed for speech disturbance. The variability in movement from interview to interview is so great as to cast doubt on its usefulness beyond a characteristic of the interview as a whole: the spread is from 1 to 54 in 18 interviews so far studied. Thus, while in those interviews with many movements, the occurrence of a movement may mean something about the events within the interview, interviews with very few movements may be best characterized as lacking information, not lacking in the sort of disturbance we are interested in. It also is very clear that in later interviews there are more movements than in earlier ones, the first two samples having been selected within the first 40 interviews, and the third from 50 to 100. Work now in progress is sampling much later interviews for simple count of foot movements.

Dr. Dittmann together with an assistant has also been involved in the indexing of the interviews. About a dozen interviews have now been checked by them. This work is chiefly to develop a standard level of abstraction, but has the side purpose, of course, of amassing a list of topics and referents which may be categorized. The progress is such that after one or two more interviews the two will be able to index an interview independently and check agreement.

Another important facet of the work on psychotherapy is one with which Dr. Bergman is concerned. During recent years he has been gradually developing "A General Theory of Psychotherapy." He presented a short formulation of this theory at the annual meeting of the American Psychological Association this year and is now setting up the project for the clinical testing of the evolving hypotheses. Since counter-conditioning is a fundamental part of his theory, Dr. Bergman is at the same time planning a series of experiments in which the attempt will be made experimentally to counter-condition so-called "anxiety" stimuli.

Another area of research being carried on in the Section is Dr. Kendig's study of psychological factors related to physical disease in association with various of the other Institutes and with some outside agencies. Her major project is on the self-concept and body-image as related to disease

susceptibility and organ choice. In this study she is exploring attitudinal factors insofar as they affect health and longevity. She is particularly interested in early childhood attitudes which may be instrumental in determining the nature of the self-concept and the body-image, especially in relation to susceptibility to illness, organ choice, course and outcome of disease. Dr. Kendig has developed an elaborate detailed questionnaire or interview schedule which is aimed at uncovering the attitudes towards the self and the body inculcated in early childhood, explicitly by direct instruction and implicitly by the emotional climate of the home and family reactions to illness. After pretesting on two patient groups and on one group of normal controls, the scales have been drawn up so the data can be coded and treated quantitatively. The coding of the interview schedule which has already passed through a number of forms has been completed and a weighted scoring system devised. The two notable developments of the past year have been the extension of the project to include the study of a group of leukemia patients (NCI) and the selection and interviewing of a group of "normal" controls. With the approval of the Medical Board, NIH personnel are drawn upon for the selection of the latter. The immediate goal is to complete the interviews. To achieve the desired n of 50 rheumatoid arthritics and 25 leukemia patients, 10 more subjects must be obtained in each group and patients continue to filter in slowly. About 50 more controls, carefully matched for age, race, sex, education, occupation, and religion must also be selected and interviewed. Meanwhile arrangements have been made for the blind scoring of the protocols. As soon as the first 25 arthritics and their controls are scored, analysis of the data can begin, the second 25 arthritic cases then serving as a replication of the first.

Dr. Daniel E. Berlyne, here as a Visiting Scientist for a year, is assigned to this Section but actually involved in projects in several sections. He has only recently come so that his projects are in the planning or about-to-be-embarked-upon stage. They comprise the following: (1) The psychophysiological effects of conflict. Experiments would deal with these questions: (a) the effects of conflict on autonomic measures, (b) the effects of conflict on E.E.G. measures, (c) the effect of number of competing response-tendencies on reaction-time, (d) the effects of complexity and incongruity of visual stimuli on orienting responses and autonomic measures. (2) As part of the creativity project of the Section on Personality, Dr. Berlyne plans to do some experiments on motivational factors underlying creative thinking, especially on the role of conflict and uncertainty in intellectual (epistemic) curiosity. This may lead to work on aesthetic behaviour. (3) In association with Dr. Rheingold, Dr. Berlyne plans to work on the determinants of perceptual curiosity in human infants, especially the role of novelty and complexity.

Laboratory of Socio-environmental Studies*

The development of the Laboratory's program in 1959 has perhaps been most impressive in the area of family studies, both in previously undertaken and in more recently initiated studies. These researches touch upon substantive problems such as (a) the structuring of interpersonal relationships in selected samplings of normal families, (b) the influence of maternal employment upon the mother's attitudes and her performance of the maternal role, and (c) husband-wife communication and interaction in the period antecedent to the hospitalization of either spouse for mental illness. They also touch upon methodological problems such as (a) the validity of retrospective data on early parent-child relationships and (b) the development of observational techniques to supplement and cross-validate interview techniques for the study of family relationships. This concentration upon the description and analysis of family patterns (though by no means to the exclusion of other areas of research) is in part a reflection of the strategic importance of the family as the group through which broader social and cultural influences upon personality development and behavior are mediated, and in part a matter of phasing of the long-range Laboratory program.

The Laboratory's ultimate goal is to delineate and study the social norms and processes which (a) influence the development of personality and the distribution of mental health and illness, (b)

*Prepared by John A. Clausen, Ph. D., Chief.

affect the individual's ability to carry out normal family, occupational, or community responsibilities and activities, and (c) govern the ways that disturbed or ill persons are perceived, defined, and dealt with at various stages of the life history. Research has indicated a number of significant correlations between grossly classified social-cultural phenomena and the phenomena of mental health and illness, but the specific linkages remain for the most part obscure. For example, rates of treated schizophrenia are inversely related to social status in the United States and Great Britain. Parent-child relationships exhibited by families which contain schizophrenic patients appear to differ from those of families with normal children; and family patterns also appear to differ by social-status groups. But our knowledge of the distribution of such family patterns in the general population is meager, as is our knowledge of the specific effects upon personality development of various combinations of family patterns. More systematic study and more adequate specification of what goes on in the normal family is necessary, then, as a prelude to more sophisticated investigation of the epidemiology of mental illness. Hence, at the present stage of our knowledge, methodological studies and systematic analyses of the intercorrelated networks of variables that constitute family patterns seem especially strategic approaches to the pursuit of our long-range goals.

Hand in hand with the description and analysis of social structure and interpersonal networks must go the development of more adequate theories of personality development. While the social scientist looks to the psychologist and the psychiatrist for aid in conceptualizing personality organization and dynamics, he has a special responsibility for analyzing and formulating the relationships between social structure and personality development. If certain combinations of relationships and experiences lead to distinct behavioral responses (delinquency, drug addiction, aggressive striving, etc.), linkages will require conceptualization of both the social-experiential input and the behavioral output.

Thus far the Laboratory's program has been focusing on the analysis of the "input" variables, and for some years our primary efforts are likely to continue here. It may be noted in the descriptions below, however, that the research of Campbell and Yarrow on children's peer-group relationships, the new study by Burton on processes of internalization of standards and values in children, and Rosenberg's research on self-images in adolescence deal to a considerable degree with social behaviors and psychological processes on the "output" side.

Before turning to consideration of the specific projects under way in the several sections, it may be noted that during 1959 the Laboratory was fortunate in recruiting four well qualified younger social scientists representing a broad range of skills in social psychology, sociology, and anthropology. Their research is still largely in process of formulation or early phases of data collection, but each man comes with broad research goals well envisoned.

OFFICE OF THE CHIEF

During 1959 the field work on the long-term study of the impact of mental illness on the family was substantially completed, as was the coding of data relating to family status and functioning, and to the spouse's perceptions of the patient's behavior antecedent to hospitalization. An initial, analytical report on the marital relationship traced the sequence of behavioral changes and the efforts of the spouse to communicate with the patient and cope with the family crisis. The sharp difference between confronting physical illness and confronting mental illness in a spouse is pointed up by the fact that, in many of the families studied, the patient's symptomatology entailed a direct assault upon the marital ties as such; conflict or progressive alienation characterized the marital relationship after the onset of mental illness even where the marital tie had been close and happy. Subsequent analyses will attempt to assess the consequences, for the family unit and for the patient, of various modes of defining and coping with the illness.

The Laboratory chief attended the Fourth World Congress of Sociology in Stresa, Italy, and subsequently visited a number of social psychiatry research centers in Scandinavia, France, and Great Britain. Out of this experience will come a general examination of the interrelationships

between theoretical orientations of psychiatry and the organization of the psychiatric profession, on the one hand, and the development of community mental health services and of psychiatric research programs, on the other.

Dr. Melvin Ember, who joined the Office of the Chief in September, will apply the research techniques and concepts of the anthropologist to the study of social influence in interpersonal behavior.

COMMUNITY AND POPULATION STUDIES

The long-range interests of this Section include the study of social organization; social differentiation and dominant cultural emphases (values, life styles, etc.) as these influence the development of personality; the distribution of various forms of deviant or problematic behavior; and the development or utilization of various techniques for coping with problematic behavior.

Drs. Kohn and Clausen, and Miss Eleanor E. Carroll, have for the past 4 years been studying the relationship of social class to child-rearing values and practices in 400 Washington families, each with a child aged 10–11 years. Following the previously reported analyses of social class differences in values parents hold for their children and in the ways in which they exercise their authority over the children, the past year's work has focused on the patterns of limit setting and emotional support vis-a-vis the 10-year-old child. Of considerable potential significance is the finding that while mother-child and father-daughter relationships do not markedly differ by social status, the father-son relationship seems more often close and emotionally supportive in the middle class than in the working class. Whether one considers reports of relatively objective phenomena such as the amount of time spent by the father with his son or more subjective feelings on the part of both parents and sons of closeness and understanding, the working-class father seems to be less involved with his son—at least at age 10 to 11—than does the middle-class father. This finding runs counter to previously accepted statements suggesting that the son of the working-class father identifies or feels closer to him because he can more readily comprehend the father's occupational activities and because the father is more often at home. Further analysis of these data will examine other characteristics which seem to influence the perception of authority and affectional patterns; consensus among mother, father, and child with reference to such perceptions; and the probable implications of these patterns for personality development. An effort will be made to secure some additional data by direct observation in selected households.

A second study, dealing with adults rather than children and the occupational realm rather than the family, attempts to look at the relationship of social structure to personality from quite a different perspective. Dr. Stephen Boggs has developed and pre-tested a questionnaire for use in a study of the ways in which the meaning of a man's job and work career is related to his emotional and physical health. He is now in process of administering this questionnaire to a sample of 180–200 men in their middle working years.

Dr. Erwin Linn continued analysis of data on patient characteristics, treatment, and duration of hospitalization among functional psychotics first admitted to Saint Elizabeths Hospital in the District of Columbia from 1953 through the first half of 1956. Dr. Linn this year concentrated his attention on the question of whether there have been changes, following the advent of drug therapy, in the relationship between patients' social characteristics and the probability of their release from the hospital within 1 year of admission. Various factors—such as marital status, race, sex, job stability, education, rural vs. urban background, and social mobility—which had been highly associated with the probability of release in the period before the advent of drug therapy, were found to be of diminished significance in the later period.

Dr. Gordon Allen, the geneticist attached to this Section, has continued the analysis of the data collected in his twin study of mental deficiency. He has also served as genetics consultant to other laboratories and in connection with such consultation has developed quantitative methods for the analysis of zygosity.

SOCIAL DEVELOPMENT AND FAMILY STUDIES

The work of this Section has been largely directed to two major areas of research on the

family—methodological problems of data collection and problems of extending and refining variables conceptualizing parent, child, and family as a group. A third area has been the study of the impact of immediate settings outside the family upon family members.

The particular problems tackled in the Section's current family research stem from a number of deficiencies of methodology in this field: (1) Research on parent-child relations is rarely done in the natural setting of the home and rarely deals directly with intrafamilial behavior; it relies heavily upon interview and questionnaire procedures. (2) The data are generally the reports of a peculiarly involved reporter, the mother. (3) Research often requires of the mother remarkable synthesizing and interpretative powers in providing the interviewer with the data he desires; she is asked to give her modal parental behaviors, and to summarize highly complex and variable interactions and feelings extending over long and indefinite time periods. (Is the home permissive, is she strict in the exercise of control over her child, etc.?) (4) Research on child-rearing has been channeled into an unvarying set of variables which tend to dominate research designs.

In 1958 research was begun on observational techniques in the natural family setting. It has continued in the past year. Practical and technical factors in the observer role are being examined (gaining access to the family, developing appropriate observer-observed relations, developing specific techniques of observing). In a small group of families, data was gathered on areas of authority, discipline, and responsibilities, using participant observations, interviews, and questionnaires. Preliminary work with the data attempts to combine observational and interview data in ways which will make it possible to analyze disciplinary processes as to philosophy of parent, actions of parents, and what it is in the situation which translates (or prevents the translation of) philosophy into behavior. Further, efforts are being made to include dimensions of the child's definition of the situation and his participation in the process—in evoking parental response and in responding to parental actions.

Much of our work on observational methods to date has been preliminary to extensive data-gathering and definitive project design. This exploration will continue in the next year with the expectation that a design will be developed, combining technical data-gathering problems and a substantive problem of child development. Mr. Thomas Gillette and Dr. Yarrow have been engaged in this work. Dr. Roger Burton, who joined the staff this fall from the Laboratory of Human Development, Harvard University, will also be working on problems in this area.

A project concerned with problems inherent in data-gathering which relies on mother's retrospective reporting on child and family relations has progressed during the past year under the direction of Dr. John Campbell and Dr. Yarrow. The research questions we are asking are: Is it possible for the mother to reconstruct accurately the earlier periods in her child's life and her relationships with her child? What are the changes which take place in the recall, and of what are they a function? Of the project sample of 240 families, data-gathering has been completed on 85 families. This includes processing of data collected at earlier periods (2 to 30 years ago) in tests, observational records and interviews, and conducting interviews with mothers to obtain their reconstructions of the past. Data collection will require 9 to 10 more months; during this time analysis will also be proceeding.

Several projects (Yarrow and Gillette) focus on the mother role. Specifically, the focus is on how the mother's personal goals in her various adult roles (wife, career, woman) relate to family functioning and child-rearing, and how social factors (social class and race) modify the mother role. During this year, data collection was completed on a questionnaire sample of 700, and an interview sample of 100 mothers. Coding and machine analysis of questionnaires have been substantially completed. It is anticipated that interpretations and reports of findings will be completed in the next 6 months. The coding of interviews will require much of the coming year. Initial interpretations of questionnaire findings in limited areas suggest that variables of social class and of race within matched classes are more powerful than the variable of maternal employment status in explaining differences in family role performance, mother's confidence regarding her understanding of the father's point of view in family issues, atti-

tudes of the mother regarding career and mother role combinations.

A new project begun this year is an investigation of processes of internalization of standards and values in children. In this area, Dr. Burton is using experimental situations for measuring the child's resistance to temptation and guilt. Child-rearing variables will be investigated, through interview and observational methods, as antecedents of the child's superego behavior.

The interest of members of this Section in the impact of immediate social settings and of peers upon the child has received less attention this past year. Investigation of the nature of the child's perceptions and understanding of his human environment (the characteristics and motives of the persons with whom he interacts) was completed by Drs. Campbell and Yarrow. Reports are in preparation on developmental aspects of cognitive processes, sex differences in social perceptions, and interactions of children's social perceptions and behavior in establishing relationships in peer groups. Boys show more highly organized initial impressions of their peers than girls, and are more likely to make inferences about personality and motivational factors in others. Boys more than girls are selective in their perceptions of peers in terms of aggressive, rebellious, nonconformity aspects. Girls tend to stress patterns of nurturance in others. Sex differences and individual differences in social perceptual characteristics are more pronounced than age differences between 8 and 13 years. The child's skill in social relations (defined in terms of his reputation among his peers) is related to his ability to synthesize more and often divergent cues in the social environment and to assimilate them into organized impressions of persons.

The full professional staff of the Section has been recruited. The research workers now represent considerable variation in theoretical background and methodological experience and have at the same time common interest in the problem areas of the Section.

SOCIAL STUDIES IN THERAPEUTIC SETTINGS

The work of this Section has been devoted largely to the study of the social environment within which therapy takes place. Three general approaches are utilized in our mental hospital research: broad attitude-survey methods, represented by the work of Dr. Leonard Pearlin; the method of controlled experimentation, represented by the work of Dr. Carmi Schooler; and the method of participant observation and unstructured interviewing (akin to the anthropological method) represented by the work of Mr. Yngvar Löchen.

The survey approach has been utilized by Drs. Pearlin and Rosenberg in their investigation of staff attitudes and behavior in a large mental hospital. While there is clear evidence that the mental patient's contact with nurses, psychiatric aides, and nursing assistants has an important bearing upon the course and outcome of the mental illness, relatively little is known about the factors which contribute to the attitudes and behavior toward patients of nursing personnel on the ward. Hence, a questionnaire for nursing personnel has been designed to provide information about staff perceptions of patients, preferences for types of patients, methods used to influence patient actions, conceptions of causes of mental illness, sense of personal efficacy in treating patients, social distance or intimacy with patients, receptivity to change in hospital practices, belief in the efficacy of hospital treatment, attitudes toward authority relationships, salient problems in work, work satisfactions and dissatisfactions, and other information relating to a custodial or humanistic approach and to job morale. In addition, the questionnaire contains items on personal background and demographic characteristics, as well as certain simple personality measures. After three pretests (one involving a 10-percent probability sample of the hospital), the questionnaire was administered to the total nursing staff of Saint Elizabeths Hospital. One initial administration and three followups yielded a return of 1,138 questionnaires, representing 87 percent of the nursing population. In addition, ward information surveys have been collected on all 156 wards in the hospital, and data is being collected on staff behavior patterns. The survey- and ward-information data has been processed and is now being subjected to statistical analysis. Throughout this study the research team has had the wholehearted cooperation and unqualified encouragement of the supervisory nursing personnel at Saint Elizabeths Hospital.

The method of controlled experimentation is represented by the work of Dr. Schooler. Dr. Schooler, who joined the Laboratory in April, received his training in experimental social psychology in the research program of the Montrose Veterans Hospital. Since his arrival, he has initiated an experimental study of affiliative behavior among 60 chronic schizophrenics at Springfield State Hospital. Response to the experimental stimulus will be related to the patient's present intellectual and emotional functioning, as measured by the Wechsler Adult Intelligence Scale, the Rorschach, and a word-association test, and to his premorbid level of social adjustment, as determined by a study of his records. Data collection was very largely completed in 1959.

Mr. Löchen, who joined our staff as a research associate in the Visiting Scientist Program in July, had previously used the methods of participant observation and unstructured interviewing with great effectiveness in his work at the Dikemark Mental Hospital in Oslo, Norway. He is interested in comparing certain aspects of organizational structure in Norwegian and American mental hospitals. In his work in Norway, he observed that staff members performing different functions in the hospital often interpreted the success or failure of the introduction of new therapeutic programs differently. Mr. Löchen reasons that the likelihood that such therapeutic programs will be introduced into mental hospitals, or, if once introduced, will be effective, will depend upon how people occupying different functional positions in the hospital interpret or react to these innovations. He plans to conduct a small-scale study of this problem in a local public mental hospital.

The work of this Section includes not only the investigation of the social organization and functioning of the mental hospital, but also interdisciplinary research on a variety of mental-health phenomena. In particular, Drs. Rosenberg and Pearlin have collaborated with several members of the Personality Development Section of the Adult Psychiatry Branch on a study of how competent adolescents cope with the problems generated by the transition from the senior year of high school to the freshman year of college. The aim is to define, during the period under study, those factors in the subject's early experiences, present personality structure, and current environment which are related to his methods of coping with stress. Interviews with students and data analysis are being conducted by personnel from the field of psychiatry, psychology, psychiatric social work, and sociology.

Dr. Rosenberg has been engaged in a study of self-images and self-ideals in normal adolescence. He is studying the impact of cultural background, roles and statuses, family experiences, and unique experiences upon the adolescent's level of self-esteem, and attempting to determine the relationship of self-esteem to measures of tension, depression, and neuroticism. He is also concerned with learning about the impact of self-esteem upon interpersonal relationships and socially significant opinions, attitudes, and values. In the basis of preliminary studies with small samples, it has proved possible to develop reasonably satisfactory Guttman scales of the following dimensions: self-esteem, preoccupation with self, interpersonal inhibition, certainty of self-image, stability of self-image, imagination, and depression. In addition, questionnaire items dealing with a number of other relevant areas have been developed. The administration of this research instrument to a large sample of normal adolescents during the coming year is planned.

During the year the Section was host to Dr. Cyril Sofer, a visiting scientist from Tavistock Institute of Human Relations in England. Dr. Sofer served as a consultant to several Laboratory projects for a period of 3 months. His broad experience and keen intelligence proved most helpful in the formulation and clarification of research problems, and he applied his knowledge of group processes to an analysis of problems of research administration and the implementation of research findings in program operations both at Bethesda and in the research program at Saint Elizabeths Hospital.

Addiction Research Center, NIMH*

Administrative

As in previous years, the time and effort of scientific personnel at this Center have been

*Prepared by Abraham Wikler, M.D., Acting Chief (Harris Isbell, M.D., Chief, on leave).

conserved very substantially by the superb performance of the administrative staff, even though the administrative workload has increased at a rapid rate due partly to assumption of duties previously performed by the USPHS Hospital and partly to increased volume of scientific reports and correspondence.

Studies on Addictive Properties of New Analgesics

These studies are designed primarily to provide information on the human addiction liabilities of new drugs (chiefly potent analgesics with morphine-like properties) for use by authorities responsible for recommending measures for control of manufacture, distribution, and dispensation of such agents at national and international levels. They also assist the medical profession in the evaluation of therapeutic and toxic properties of new drugs for clinical use, and provide opportunities for basic research on the mechanisms of tolerance, addiction, and habituation.

During the current year, advances were made in the improvement of methodology for measuring the overall abuse-liability of new drugs. Thus, "subjective" and "objective" rating scales of behavioral change (with particular reference to "euphoria") were developed and tested with a large number of new drugs under double-blind conditions. These quantitative data revealed a high degree of concordance between the subjects' self-ratings ("subjective") and those made by other observers ("objective") in the cases of the more potent analgesics, but considerable discordance when weaker analgesics were tested; hence, both types of measurement have to be taken into account in the final evaluation. Another interesting finding was that only 39 percent of the subjects reported they would like to continue the daily use of one of the drugs tested (morphine subcutaneously). Subsequently it was found that this was also true for heroin, subcutaneously (42 percent for morphine, 39 percent for heroin). These studies will be extended later to include intravenous injections to which addicts are more accustomed under "natural" conditions.

Also it was found in a double-blind procedure involving testing of eight analgesic drugs that reliable information on their ability to produce tolerance and physical dependence could be obtained with much shorter periods of chronic administration, e.g., 18 to 20 days, than has been assumed heretofore. This appears to be definitely true for the more potent analgesic drugs, but further work is necessary to establish its usefulness in evaluating less potent analgesic agents. The method promises to be of considerable value in shortening the time required for evaluation of the addiction liability of new compounds. Currently the double-blind procedure is being applied also in the use of the 24-hour substitution method for testing addiction liability—i.e., the substitution for morphine, over a period of 24 hours, of a new drug in morphine-tolerant subjects, with measurement of the extent to which the morphine-abstinence syndrome is suppressed. As part of this program, work was continued on four compounds about which preliminary results were described in the last annual report, and five new drugs were also investigated. The outcome of these studies may be summarized as follows:

d-Methadone. Euphoriant dose, 250–400 mg orally; suppression of abstinence from morphine, 1/14th as potent as morphine; nalorphine precipitation test, positive; abstinence syndrome after direct addiction, mild (toxic effects at high chronic doses—weight loss, neutropenia, lymphocytosis, positive thymol turbidity test). These results will be reported to the Committee on Drug Addiction and Narcotics of the National Research Council, and submitted for publication.

R–1132. This is a complex compound with a piperidine nucleus developed by a Belgian firm for use as a constipating agent. Euphoriant dose, 60 mg orally, comparable in effectiveness to 120 mg of codeine in producing morphine-like behavioral effects; suppression of abstinence from morphine, by the oral route, about one-half as effective as morphine subcutaneously; abstinence syndrome after direct addiction, orally, milder than oral morphine or oral codeine. The work on this compound has been completed and the results will be presented to the Committee on Drug Addiction and Narcotics, NRC, and prepared for publication.

NIH–7525 (An N-phenacylmorphinan compound). Euphoriant dose, 2–3 mg (subcutaneously), equivalent to 20–30 mg of morphine, administered by the same route (as little as 0.5 mg intravenously identified as "like heroin"); suppression of abstinence from morphine, 1 mg equiv-

556044—60——16

alent to 10 mg of morphine; abstinence syndrome after direct addiction, moderately severe, but significantly less than after direct addiction to equivalent intoxicating doses of morphine; nalorphine precipitation of abstinence, positive. Full controls under the narcotics laws have been recommended by the Committee on Drug Addiction and Narcotics, NRC.

NIH–7519 (A substituted benzmorphan compound). Euphoriant dose, 3–4 mg subcutaneously (equivalent to 20–30 mg of morphine); suppression of abstinence from morphine, 1 mg equivalent to 8.1 mg of morphine (in this respect action in man differs markedly from that in monkey, in which species this drug is only one-sixth as potent as morphine in suppression of abstinence); abstinence syndrome after direct addiction, moderately severe but significantly less than after equivalent intoxicating doses of morphine; nalorphine precipitation of abstinence, positive. Full controls under the narcotic laws have been recommended by the Committee on Drug Addiction and Narcotics, NRC. Studies on both this compound and NIH–7525 have been completed and the results are being prepared for publication.

NIH–7296A (A substituted morphinan compound.) Euphoriant dose—"euphoria" not consistently reported even after administration of as much as 1,000 mg orally, but three of four subjects reported morphine-like effects when 500 mg were administered orally four times daily, although toxic effects (gastric disturbances, mental confusion, anxiety) supervened after 1 or 2 days; suppression of abstinence from morphine, only $\frac{1}{25}$th as potent as morphine; abstinence syndrome after direct addiction, none found but dose level achieved during chronic intoxication was equivalent to only 48 mg of morphine per day. Work on this compound has been completed and will be reported to the Committee on Drug Addiction and Narcotics, NRC, and prepared for publication.

NIH–7590 (A substituted piperidine carboxylate congener of Demerol). Euphoriant dose, 15–20 mg (equivalent to 20–30 mg of morphine); suppression of abstinence from morphine, 0.5 mg equivalent to 1 mg of morphine (contrasting with findings in monkeys, in which species NIH–7590 is 18 times as potent as morphine). No further work is planned on this compound. Full control under the narcotic laws has been recommended by the Committee on Drug Addiction and Narcotics, NRC.

NIH–7586, ARC I–G–1 (A substituted chlorobenzyl benzimidazole). Euphoriant dose, 100 mg orally; suppression of abstinence from morphine, partial (2.62 mg equivalent to 1 mg of morphine). Since it is unlikely that this compound will ever be marketed, no further investigations of its addiction liability are planned. The results obtained will be reported to the Committee on Drug Addiction and Narcotics, NRC, and prepared for publication.

NIH–7607, ARC I–G–2 (A substituted ethoxybenzyl benzimidazole). Though closely related to NIH–7586 in chemical structure, this compound is far more potent. In mice and rats its analgesic potency is said to be 1,500 to 1,700 times that of morphine; it is also 1,500 times as potent as morphine in suppressing the morphine abstinence syndrome in the monkey. In man, the euphoriant dose appears to be about 0.25 mg orally (80–120 times as potent as morphine), and 60 times as effective orally as morphine is subcutaneously in suppressing abstinence from morphine. In a "short" (18-day), direct addiction study it was possible to achieve a dose level of 4 mg daily without alarming effects and, after abrupt withdrawal of the drug, an abstinence syndrome ensued which was comparable in intensity with that produced by equivalent doses of morphine. The results of these studies will be reported to the Committee on Drug Addiction and Narcotics, NRC, and prepared for publication. It is expected that, because of its effectiveness in producing morphine-like changes when administered in minute quantities by the oral route, this drug will find many uses in experimental studies on addiction and habituation in animals. (See below.)

NIH–7446, ARC I–B–19 (An N-allyl derivative of morphinan). In clinical studies done elsewhere it has been reported that the analgesic potency of this compound is comparable to that of morphine and that, like morphine, its effects can be antagonized by administration of nalorphine. Studies carried out at the Addiction Research Center to date indicate that, both with respect to euphoria production and suppression of abstinence from morphine, this compound is very similar to morphine, and therefore possesses a degree of addiction liability comparable to that of the latter

drug. These results will be reported to the Committee on Drug Addiction and Narcotics, NRC.

Studies on the addition liabilities of new drugs constitute a major part of the long-range program of the Addiction Research Center, and will be continued as heretofore.

Chronic Intoxication With Barbiturates, Alcohol, and Related Drugs

During the year, work on drugs of this type was concentrated mainly on the problem of alcoholism. Minnesota Multiphasic Personality Inventory profiles were obtained on 600 institutionalized alcoholics, narcotic addicts, and criminals without alcoholism or narcotic addiction (200 in each group). Factor analysis of the data revealed that the profiles of these three groups were very similar to each other and that most of the subjects could be classified in one or another of the following categories: (1) undifferentiated psychopath, (2) primary psychopath, (3) depressive psychopath, (4) schizoid psychopath, and (5) neurotic psychopath. Significantly, the alcoholics were more paranoid and neurotic, while the criminals were more schizoid than the narcotic addicts. Although addicts, alcoholics, and criminals have been impressionistically described as psychopathic personalities the present study is the first to demonstrate this in an objective and quantitative manner. Further statistical evaluation of the results will be carried out before publication of the data.

A Habit and Attitude Inventory was developed and applied to 350 alcoholics and 100 narcotic addicts. A large number of differentiating items were found and their discriminatory reliability was established by cross-validation tests on another set of 50 subjects in each of the two groups. Items and procedures require refinement before further data is gathered.

A study of the subjective effects of acute alcoholic intoxication was initiated this year, using the Addiction Research Center Inventory (ARCI) for this purpose. Two pilot studies were completed which were designed at three dose levels, with the aim of attaining and maintaining appropriate degrees of intoxication at three dose levels (high, intermediate, and low), for a period of at least 2½ years (well over the time required for administration of the ARCI). For this purpose the Purdue Pegboard (a measure of psychomotor performance) was employed in addition to an abbreviated form of the ARCI. By measuring changes in performance on both tests, beginning at various times after administration of the initial dose of alcohol, it was demonstrated that this dose plus the "maintenance doses" were sufficient to produce graded changes in the measures employed. Therefore, these dose schedules are now being used in systematic studies of the subjective changes occurring during acute alcohol-intoxication periods. It is also planned to delineate the effects of alcohol in amounts comparable to those of social drinking on various measures of behavior, with concomitant monitoring of blood-alcohol concentrations.

Clinical studies on barbiturates and other hypnotics were held in abeyance this year. However, a study of the addictive liability of gluethemide (Doriden) in dogs was completed. Of 11 dogs, in which chronic intoxication at dose levels up to 3.5 grams per day was attempted, 6 died during the chronic intoxication period; of the remaining 5 dogs, one exhibited convulsions on withdrawal. Though Doriden appears to be much more toxic than barbiturates in this species, its addiction liability appears to be less. No further work with this compound is contemplated.

Acute and Chronic Intoxication With Drugs Other Than Analgesics, Barbiturates, or Alcohol

Studies in this area, during this year, were primarily concerned with the psychotomimetic drugs. As noted in the previous annual report, some of the congeners of LSD–25 (with substitution of alkyl, hydroxyl, or alkoxy groups of the acid amide radical) exert hypnotic effects in man contrasting with excitant effects in rodents and dogs. Of these, 4 mg of Lilly 23194 proved to be more efficacious than 200 mg of secobarbital in increasing hours of sleep after a morning dose. Unlike secobarbital, Lilly 23194 did not produce fast activity in the electroencephalogram and patients receiving the former were more readily awakened and exhibited less impairment of motor function. When administered simultaneously with LSD–25, neither Lilly 23194 nor secobarbital reduced significantly the mental effects of LSD–25. Actually, Lilly 23194 seemed to intensify the LSD reaction, although this was not statistically significant. This finding suggests either that Lilly

23194 has different sites of action from LSD–25, or that in sufficiently high doses it may produce psychotomimetic effects.

Another drug, elymoclavine, related to LSD–25 structurally, was also found to exert hypnotic effects in man, though excitatory in animals. In doses of 1 to 25 mcg/kg, this drug produced no psychotomimetic effects, and drowsiness was reported by 9 of 12 subjects. A new supply of this drug has been obtained and further studies are planned.

Studies on two new congeners of LSD–25 (*d*-dihydro-lysergic acid diethylamide and 1-methyl-d-lysergic acid butanolamide tartrate) yielded data confirming previous conclusions on the lack of correlation between antiserotonin and psychotomimetic potencies, and hence the lack of support for the "serotonin-deficiency" hypothesis of natural psychoses.

Of special interest in connection with theories of drug-produced or natural psychoses are the results of studies with psilocybin (O-phosphoryl-4 - hydroxy - N-dimethyltryptamine). This compound, isolated and first studied pharmacologically by Swiss workers very recently, appears to be the active substance in the psychotropic mushroom used by Indians in Mexico. It is closely related to serotonin and bufotenine, the chief difference from the latter being that the hydroxyl group on the indole ring is on the 4 rather than on the 5 position. Because of such structural similarities this compound may prove to be of value in the investigation of the possible role of serotonin and its precursors or metabolites in the production of psychoses. It was found that in adequate dosage psilocybin produces effects indistinguishable from those of LSD-25, though of shorter duration. Statistical analysis of dose-effect curves for peak pupillary (dilatation) and subjective (positive answers on questionnaire) changes reveal that LSD–25 is about 100 times as potent as psilocybin. Studies on cross tolerance between psilocybin and LSD–25 were also made on five subjects who were tested on single, relatively high equipotent doses of both drugs before and after 1-week periods of daily administration of either drug, in amounts increasing from a very low dose to the control dosages. The results strongly suggest that, as in the case of LSD–25, tolerance to psilocybin develops rapidly and that after such tolerance has been established the subjects are also cross-tolerant to either drug. However, the results did not reach statistical significance on all measures. This study is now being replicated with a view to increasing the total number of subjects for greater statistical reliability, or, if necessary, to determining whether higher doses of psilocybin or longer periods of administration can produce greater degrees of cross tolerance to LSD–25.

Biochemistry of Addiction

As noted in the previous annual report, over 50 percent of an injected dose of normorphine is recoverable in the urine in the "free" form, contrasting with only 10 to 15 percent of "free" morphine. This possibly accounts in part for the greater sedative effects of normorphine on chronic administration. Work on this compound during the current year was devoted to further study of the nature of the "binding" of normorphine excreted in the urine. As reported previously, it has been found that, unlike morphine, normorphine is not "bound" as a beta-glucuronide. Failure to release "bound" normorphine with strong acid at room temperature indicates that it is not bound as an N-glucuronide. Studies with mylase P (an ethereal sulfatase) also indicate that normorphine is not "bound" as a sulfate. Currently attempts are being made to isolate larger quantities of excreted normorphine from the urine by two methods: paper chromatography and precipitation with potassium carbonate with subsequent purification of the tarry precipitate by use of anion and cation exchange resins. Preliminary studies on urines with added normorphine indicate that the ion-exchange technique may prove satisfactory.

Work was also continued during the year on methods for determining blood levels of epinephrine and norepinephrine and urinary levels of metanephrine, with a view to investigating the effects of opiates, chlorpromazine, chlorpromazine sulfoxide, and other drugs on catechol-amine activity and metabolism. Many difficulties have been encountered in attempting to use methods, such as that of Weil-Malherbe and Bone, reported in the literature. Currently, two other methods are being used: a modified form of a Von Euler and Floding oxidation technique, and the method

of Crawford and Low in which manganese dioxide is employed as the oxidizing agent and amberlite resin IRC 50 as the adsorbent.

A sample of absolutely alcohol-free DPN was obtained and preliminary results with this purified hydrogen acceptor indicate that the enzymatic method (alcohol dehydrogenase) can be used successfully in determining levels of alcohol concentration in minute quantities of blood. Further studies on this technique and comparison of the results obtained with the standard dichromate method are being continued with a view to future applications in clinical studies on alcoholism.

Neurophysiology and Neuropharmacology of Addiction

These studies were designed to investigate primarily the loci of origin and neurophysiological mechanisms involved in the production of the barbiturate-abstinence syndrome. As an initial phase of this project, a study was made of the changes in cortical thresholds for electrically induced seizures during chronic barbiturate intoxication and after abrupt withdrawal of the drug in cats. The data, completed this year, indicates that daily production of seizures by transcerebral electrical stimulation gradually increases the threshold for induction of seizures in control animals, thus complicating interpretation of data obtained by this method during addiction cycles to barbiturates or other drugs. Furthermore, since no clear-cut fall in threshold was found to occur during the acute barbiturate abstinence period in this species, it appears highly unlikely that further studies with this technique will yield positive results, at least at the cortical level. However, it is planned to extend these investigations to studies at subcortical levels in the future.

Another phase of this project has been concerned with the modifications of the barbiturate abstinence syndrome produced by ablations of the cerebral cortex. This has been attended by great difficulties because of the apparently increased susceptibility of decorticated cats to the deleterious effects of chronic barbiturate intoxication. Of 7 chronic decorticated cats, 6 died during the period of chronic barbiturate intoxication. The remaining cat exhibited no convulsions after abrupt withdrawal of barbiturates but displayed marked restlessness, tremulousness, and insomnia during the first few days of acute abstinence. Attempts to use rats for this purpose were unsuccessful. Of 12 intact rats started on 20 mg/kg of barbituric acid daily, 10 died and the remaining 2 exhibited no withdrawal phenomena after acute withdrawl of the drug. Currently attempts are being made to continue the project in dogs. Three completely decorticated beagle dogs have been started on barbital sodium, and 3 other dogs have had hemidecortications so far. When completely decorticated, these animals will also be placed on barbital sodium daily.

The Mode of Action of Central Nervous System Depressants

The major purposes of this project are to investigate the pharmacological and the electrophysiological homogeneity or heterogeneity of the neural systems responding to electrical stimulation of the midbrain reticular formation, and the functional relationships between the activities of these systems (as reflected in measurements of electroencephalographic, electrocardiographic and vasopressor changes), and behavioral arousal.

To these ends, stimulus-response curves pertaining to electroencephalographic, electrocardiographic, and vasopressor changes have been obtained in succinyl choline chloride curarized intact cats on artificial respiration before and after intravenous injection of graded doses of atropine (0.2–5.0 mg/kg); chlorpromazine (1–10 mg/kg); chlorpromazine sulfoxide (5–25 mg/kg); pentobarbital (4–12 mg/kg); epinephrine (1–3 mcg/kg); norepinephrine (1–3 mcg/kg); and a number of other drugs. For purposes of comparison with drug effects, stimulus-response curves (under nondrug conditions) were obtained in uncurarized cats on artificial respiration with transections of a spinal cord at the first cervical level (encéphale isolé) and electroencephalograms were recorded in uncurarized cats with transections through the midbrain at the intercollicular level (cerveau isolé).

In all these studies the electroencephalograms derived from anteroparietal to midparietal calvarial leads were analyzed automatically in the frequency range of 1.5–30 cps by the use of the Offner analyzer, while blood pressures were recorded through transducer tracings derived from intraarterially implanted polyethylene catheters,

and cardiac rates by concomitant electrocardiographic tracings. In experiments involving electrical stimulation of the midbrain reticular formation, measurements were made of the changes produced by drugs in "delta percent," defined as the ratio of the mean amplitude of the Offner pen deflections in the 1.5- to 3.5-cps range in the drugged animal to that of the undrugged animal. These measures were used in the calculation of changes in "threshold" of electroencephalographic response to reticular stimulation by a method that takes into account the changes in "base line" produced by these drugs (specifically, the increase in slow activity). Thus, "change in threshold" is defined as the difference between the stimulating voltage necessary to produce an infinitesimally small reduction of delta percent in the undrugged animal and that necessary to reduce the delta percent in the drugged animal to values obtaining under undrugged, unstimulated control conditions. Also "change in reactivity" is defined as the difference between the slopes of the stimulus-response curves obtained in the drugged and undrugged animal—i.e., the difference in the magnitude of decrease in delta percent produced by a given increment in stimulating voltage. The significance of all differences observed was calculated by appropriate statistical techniques.

The results may be considered in relation to the primary objectives of this project. Atropine produced marked slowing of the electroencephalogram, elevated the threshold of activation and increased the reactivity of the EEG to electrical stimulation of the midbrain reticular formation. This drug also elevated the threshold for, and increased the reactivity of the vasopressor response to, such stimulation. A close parallelism was demonstrated between such central changes and the peripheral muscarinic blocking effects of graded doses of atropine, suggesting that the neural systems involved in the central responses to midbrain reticular stimulation are mediated in part through muscarinic synapses, although transmission through non-muscarinic synapses must also be assumed since graded electroencephalographic and vasopressor responses could be obtained under atropine in spite of complete peripheral muscarinic blockade. Also a closer parallelism was found between the norepinephrine potentiating actions of chlorpromazine and chlorpromazine sulfoxide and the central depressant effects of these drugs than between their adrenergic blocking actions and their central effects. The data suggest that norepinephrine may also be involved in neurohumoral transmission, but, if so, such transmission is more likely to be inhibitory rather than excitatory.

In the intact curarized cats, reticular stimulation produced two kinds of changes: reduction of the amplitude of all activity and the acceleration of frequencies. However, these normally associated changes could be dissociated by certain of the drugs employed. Thus acceleration of frequencies by reticular stimulation was not observed under atropine while reduction of amplitude at all frequencies (below 22 cps) did occur. After administration of pentobarbital, acceleration of frequencies was observed but the reduction of amplitude at lower frequencies was accompanied by augmentation of amplitudes at higher frequencies. From these studies it appears that the neural systems responding to midbrain reticular stimulation are not homogeneous either pharmacologically or physiologically.

As already noted, atropine, while elevating the threshold of electroencephalographic activation, enhanced the reactivity of the systems involved in this response to midbrain reticular stimulation. This was found to be true also for chlorpromazine and pentobarbitol although these drugs elevated the threshold of the vasomotor response and decreased the reactivity of the systems involved in its mediation. Furthermore, comparisons of the electroencephalographic responses to midbrain reticular stimulation in the encéphale isolé preparation (without drugs) with those obtained under atropine, chlorpromazine, and pentobarbital in the intact curarized animal revealed that the augmentation of reactivity produced by these drugs in the latter was absolute—i.e., the electroencephalographic responses to midbrain reticular stimulation in the drug-treated animals were greater than would be expected in an undrugged animal, with slow activity comparable to those produced by the drugs in the resting state. Still, it is known that in contrast to the other drugs, atropine does not elevate perceptibly the threshold of the *behavioral* response to "arousing" stimuli. Therefore, it would appear that neither elevation of threshold of EEG activation nor augmentation of the reac-

tivity of the EEG to reticular stimulation can be correlated in an invariable way with changes in behavioral arousability, nor would it appear that either simple reduction of amplitude or acceleration of frequencies of the EEG is an invariant correlate of behavioral arousal. On the other hand, it was found that the effects of high cervical transection (encéphale isolé) and intercollicular midbrain transection (cerveau isolé) could be mimicked by chlorpromazine and pentobarbital respectively. Therefore, it is conceivable that actions of these drugs on the input into the reticular activating system and/or their effects on the caudal projections of this system, may be more closely correlated with their effect on behavioral arousal. To test this hypothesis it is planned to investigate the effects of midbrain reticular stimulation on behavior and the effects of drugs thereon, as well as on evoked potentials in the midbrain, in the chronic decerebrated cat preparation.

Psychological Studies of Addiction

In addition to the work on alcohol already described (see Section IV above), extensive studies on human subjects were conducted with the Addiction Research Center Inventory for measuring subjective effects of drugs (ARCI). The drugs employed in these studies include placebo, LSD–25 (in two doses), pyrahexyl compound (in two doses), morphine, pentobarbital, amphetamine, chlorpromazine (multiple daily doses), as well as alcohol. Thus far, 100 subjects have been studied under all conditions except alcohol and morphine, and it is hoped to complete the quota of subjects receiving all drugs shortly.

Currently, arrangements are in progress with the Statistical Processing Servicing Center and the Psychopharmacology Service Center of NIMH for the development and cross validation of empirical scales for each drug. After these steps in the analysis of the data are completed, it is planned to carry out a factor analysis of the empirically derived scales. This project is of great potential importance, not only because it provides a reliable instrument for quantitative measurement of the subjective effects of drugs of particular interest to this Center, but also because of the opportunities the data provide for testing basic theories of behavioral change in general and drug-induced changes in particular. Thus, the data obtained with the ARCI on LSD–25 (1.0 and 1.5 mcg/kg) have been used to investigate a factor-analytic theory of "causation." According to this theory positively scored items on the ARCI reflecting the "primary" effects of LSD–25 should be more highly intercorrelated than positively scored items reflecting "secondary" effects of the drug, and these in turn more highly intercorrelated than positively scored items selected at random. Also, the loadings of the "secondary" items on an empirically derived factor should be dependent upon the loadings of the "primary" items on that factor. Using items that differentiated between control and LSD–25 conditions at or beyond the 0.0025 level as a class representing the "primary" effects of LSD–25, and items for which the frequency of "true" responses under LSD–25 was greater by 10 than for control conditions as a class representing the "secondary effects" of LSD–25, with the application of appropriate statistical techniques, confirmatory evidence was obtained for both predictions from general factor analytic theory.

Applications of factor analytic methods to the data obtained with the ARCI on schizophrenic patients and on postaddicts under control and LSD–25 conditions revealed that with respect to the main factor, the schizophrenic subjects tested significantly higher than the postaddicts without drugs, but not when comparisons were made with postaddicts under the influence of LSD–25. Whether or not the apparent similarity between schizophrenia and the changes produced by LSD–25 is specific for that drug remains to be determined by extension of similar analytic methods to the data obtained with other drugs. In addition, factor analysis provided some evidence that certain personality characteristics found on the control conditions are intensified or accentuated by LSD administration. It may be noted too that, in addition to detecting the well-known subjective effects of LSD–25, there is some evidence that this instrument can bring out changes concerned with tactile, thermal, olfactory, appetitive and sexual changes as well as interpersonal and attitudinal alterations which have previously not been emphasized in the literature.

Other studies on human subjects during the year included continued measurements of Minnesota Multiphasic Personality Inventory profiles on

addict physicians and preparations for investigations on probability learning in man. The work on addict physicians has been progressing very slowly, because, in the past, 30 days or more were allowed to elapse before the tests were administered to physician patients in the hospital after admission. It has now been decided to administer the tests to such patients within a week after admission, as well as after 30 days, to determine whether such early profiles can be used—a procedure which would facilitate the acquisition of a suitable number of subjects. The purpose of the projected study on probability learning in man is to compare the variables controlling decision making in psychopaths with those in normal human subjects, particularly with reference to whether or not the behavior of the psychopath is controlled to a greater extent than normally by the objective "odds" in a given situation, and whether the psychopath is more responsive to negative than to positive reinforcement. Later it is planned to study the effects of drugs upon these variables. Apparatus for this study has been completed and a few pilot investigations have been made. However, the results to date are not sufficient to warrant any conclusions.

In animals, work has continued on the problem of the possible interaction of effects of drugs upon auditory discrimination with their effects on tone-shock-evoked conditioned inhibition of bar-pressing for food ("anxiety", "conditioned emotional response"). Attempts to train rats to discriminate "tone-on" and "tone-off" by use of a shock-escape technique proved to be unsatisfactory. The design has been changed in several ways, and it is planned also to try appetitive reinforcement should satisfactory results with aversive reinforcement not be forthcoming. A great deal of time has been devoted during the current year to statistical analysis of the enormous body of data obtained in previous years with various drugs. In general, the previous conclusions have been upheld. Although the effects of drugs on the conditioned inhibition appeared to depend to a considerable extent on the particular characteristics of the tone used as the conditioned stimulus, the opiates and opioids produced the greatest reduction in this measure of "anxiety," and (except for pentobarbital) it is this class of drugs only for which significant dose-effect curves can be obtained. Pentobarbital produces a pattern somewhat similar to that of the opiate series, but only at 20 minutes after injection. Chronic reserpine medication produced much less effect even when administered at three times the dose-level reported to be effective by other investigators. Nalorphine, amphetamine, cocaine, LSD–25, and chlorpromazine did not yield significant dose-effect responses. However, final interpretation of the data must await the outcome of the studies on the effects of drugs upon auditory discrimination.

During the year a replication was made on a part of a study, conducted previously, which was concerned with the role of "internal stimuli" produced by drugs in the rate of acquisition and extinction of conditioned responses. In studies with morphine and amphetamine on rats conditioned to press a Skinner bar for food, it had been shown that extinction occurred faster when drug conditions (or no-drug conditions) were the same during acquisition and extinction than when they were different. Another interesting finding was that in rats trained to press the bar for food under placebo conditions, bar-pressing rates under amphetamine during extinction were equal to or lower than the rates found under placebo extinction, a finding which suggests that under certain conditions amphetamine may be a "depressant" rather than a "stimulant." This finding was confirmed in the present study.

In addition, a study was initiated this year on the effects of autonomic blocking agents upon traumatic avoidance conditioning. The hypothesis to be tested is that while autonomic effector discharge is not essential for the acquisition of a traumatic avoidance response, it serves to increase the resistance of the response to extinction. To test this hypothesis it was planned to compare rates of acquisition and extinction of a traumatic avoidance response in normal rats and in rats treated with an autonomic blocking agent, "Ecolid." In preliminary studies an attempt was made to determine the autonomic blocking dose of Ecolid for this species. Surprisingly, it was found that even in large doses (5 mg/kg) this compound did not produce slowing of cardiac rate in the normal animal. Since the basic studies on this drug in the literature had been made in animals under barbiturate anesthesia, the effects of Ecolid on cardiac rate were investigated in rats pretreated with a

small dose of pentobarbital (5 mg/kg). Under such conditions, it was found that Ecolid did indeed reduce cardiac rates significantly. At present the reasons for such interaction between Ecolid and pentobarbital are not clear. Further studies are in progress in which the effects of Ecolid upon vasopressor responses to tetramethyl ammonium chloride in rats pretreated with methyl atropine will be used as a measure of its potency as an autonomic blocking agent. It is conceivable that the failure to demonstrate autonomic blockade by Ecolid in normal rats is due more to the unsuitability of cardiac rate as an indicator of such effects than to any necessary dependence of these effects upon pretreatment with pentobarbital. However, if the latter should prove to be the case, the projected study on traumatic avoidance conditioning may have to be redesigned to make provision for "balancing out" the effects of pentobarbital.

Conditioning Factors in Addiction and Habituation (Relapse)

This project was designed as an attempt to test, insofar at it is possible in animals, some of the basic postulates of a theory of relapse to the use of addicting drugs in man. Briefly the theory holds that, in part at least, relapse represents a conditioned response to environmental stimuli that were previously associated with the periodic reduction of abstinence distress consequent to addiction. It is postulated that once physical dependence on a given drug is established, each dose of the drug serves to reinforce whatever behavior is instrumental in bringing about the administration of the drug. Furthermore, it is postulated that the strength of such "primary" reinforcement, as well as the strength of "secondary" reinforcement by environmental stimuli regularly associated with the former, varies directly with the "effort" expended in the performance of the instrumental response. In man, special social conditions may provide sources of tertiary and even higher orders of reinforcement, so that the original basis for inveterate recidivism may become greatly obscured. However, there appears to be no reason why animals cannot be used in the study of "primary" and "secondary" reinforcement, provided the species selected is capable of generating physical dependence on drugs and of making the required discriminations.

In the present investigation the rat has been used because it has been reported that this species develops physical dependence on morphine, and because of the wealth of information available on instrumental and operant conditioning in this species. In studies initiated last year it was found that reliable evidence of physical dependence on morphine administered subcutaneously once daily could be obtained only when the daily dose was maintained at a level of 200 mg/kg. Also several methods were tried in which differential preferences for goal boxes identified with visual cues might be developed by addicted, but not by nonaddicted, rats, as a consequence of reinforcement by subcutaneous injections of morphine. However, these were unsuccessful, because in the design, trapping of the rats and subcutaneous injection of morphine were interposed between the start and the goal boxes.

During the present year, an attempt was made to circumvent the above difficulty by subcutaneous injection of morphine (or saline) before placing the animal in a small compartment, egress from which could be accomplished by pressing one of two levers, the "correct" one depending on the substance injected. The animals learned to make this discrimination readily, but comparisons of the number of lever presses made (up to a total of 10 presses) on "test" days during which no egress was permitted revealed that they developed a distinct aversion to the lever associated with morphine injections. The most plausible explanation for this unexpected result is that because of the highly hypertonic (8 percent) solution of morphine that was used, pressing of the "morphine" lever was associated with pain rather than relief of abstinence distress. Because subcutaneous injection of morphine introduced such difficulties, an attempt was made to train rats to operate a Skinner bar which in turn activated a pump mechanism that delivered intraperitoneal injections of morphine through an implanted polyethylene catheter. After about 2 weeks, however, it was found that the intraperitoneal end of the catheter was completely occluded by a thick fibrous membrane. Hence, this technique was also abandoned.

Following receipt of information of the extraordinary potency of the morphine-like benzimidazole derivative, I–G–2 (NIH–7607, see section II above), preliminary studies were made

which revealed that water-deprived rats will drink a 5-mcg/cc solution of this drug as readily as pure water and that effects identical with those of morphine can be observed in these animals within 4 to 7 minutes after commencement of drinking. In view of this discovery the project was redesigned completely. Rats are now maintained on morphine at a dose level of 200 mg/kg (100 mg/kg twice daily) and oral ingestion of a 5-mcg/cc solution of I–G–2 is used as the reinforcing agent each morning (several hours before the first daily injection of morphine) in two types of experiments. In one of these, rats are trained to press a bar for continuous liquid reinforcement after water-deprivation, the liquid being either pure water or the I–G–2 solutions on different days, with provision of discriminatory tactile cues for each kind of reinforcement and a clicker activated by the bar as a secondary reinforcer associated with the delivery of I–G–2. On "test" days the animals are satiated with water prior to presentation of the bar with the discriminatory cues. Control rats are treated in exactly the same manner, except that instead of morphine they are injected twice daily with saline. The critical dependent variable in this experiment is the differential bar-pressing (and liquid drinking) rates of addicted and control animals when *water satiated.* In its present form this experiment has been in progress only about 1 month, which has provided opportunities for only 5 test days. At present there appears to be a slight trend in the direction of higher bar-pressing rates for the addicted animals on water satiation days compared with the controls. However, there is as yet no evidence of discrimination between the "water bar" and "I–G–2 bar." If clear-cut evidence of discrimination (in favor of I–G–2) does develop the experimental and control rats will be divided into two subgroups each, one of which will continue on the present schedule of one-to-one reinforcement, while the other will be placed on a fixed ration or variable interval schedule with a view to subsequent examination of this "effort" variable in the determination of "relapse."

In the other experiment, groups of morphine-addicted and nonaddicted rats are deprived of water for 20 hours daily and are then placed in cages permitting the choice of one or another of two compartments, at the end of which tubes are available for drinking, one of them identified by visual and tactile cues. In the first phase of this experiment, either tube may contain water or the 5-mcg/cc solution of I–G–2, the purpose being to determine whether or not, without training, rats in either group exhibit a preference or an aversion for the I–G–2 solution, or the discriminatory cues, independently. This phase, which has extended over about 50 days, is now almost completed and the results indicate that without training the rats show neither an aversion nor a preference for either the solution or the cues. In the second phase of this experiment, which has just begun, rats will be presented with only the cue-identified tube containing I–G–2 solution daily for four days, and tested for tube preference by presentation of both I–G–2 and water tubes on the fifth day, and such cycles of 5 days' training and testing will be continued indefinitely. The critical variable here is the relative amount of drinking from the two tubes on the test days as a function of the number of training trials. It is expected that this experiment will serve to provide an additional measure of the "primary" reinforcing potency of the I–G–2 solution and the relationship of this to the presence or absence of physical dependence on morphine.

Effects of Drugs on "Mental Set"

This complex procedure, which may be regarded as a method for quantifying "attention," involves measurement of auditory-manual reaction times following visual "warning" signals at different foreperiods under "irregular" and "regular" conditions. In previous years it was found that a number of drugs, including LSD–25, morphine, and pentobarbital, produced changes in "mental set" of nonpsychotic postaddicts which were similar qualitatively though not quantitatively to those obtaining in schizophrenic patients under no-drug conditions. This study was extended during the present year to an investigation of the effects of chlorpromazine on "mental set" in 6 nonaddict schizophrenic patients, employing a balanced design in which half of the group received the drug (up to 600 mg/day for 6 weeks) and the other half a placebo during the first treatment period, following which the treatment conditions were reversed. One of the subjects had to be dropped from the study because of the

development of toxic side-effects. The data obtained in the remaining 5 have not yet been analyzed statistically, but in its raw form affords no evidence for gross change in mental set which can be attributed to chlorpromazine. The statistical analysis of the enormous body of data acquired on "mental set" this year and previously will be completed before any additional work with this method is undertaken.

*CLINICAL INVESTIGATIONS—NINDB**

At the time of completion of the seventh Annual Report of the Clinical Investigative Unit, an increase in all activities of the Unit was noted. A total of 749 inpatients were admitted during this calendar year, an increase of 143 over the past year. The total patient days were 22,156; this is an increase of 2, 878 over the previous year. Six hundred and eighty-two outpatients were seen, and 1,275 consultations were rendered. The average patient stay was 29.6 days.

Studies centering around these patients, and other activities of the Clinical Investigative Unit, directed towards further understanding of neurological and ophthalmological disorders, resulted in the reporting of 104 projects and 92 publications (either published or in press) for the current year. Where such publications reflect results obtained from previous years, the given investigator has noted such in his report.

The design problems arising from the development of the new neurosurgical suite have occupied many hours. The ground for this building, which will house this suite, has now been broken, and the final plans committed to the hands of the architect. This suite will constitute a new departure in operative design for neurosurgical procedures. Both in the neurosurgical operating rooms and on the ward, intensive studies are being undertaken in a control of air-borne infections, and these have been incorporated by the Neurosurgical Branch into the report which is being studied by the Surgical Administrative Committee for the National Institutes of Health.

Three investigators of the permanent staff were lost during the year. Dr. Glenn Drager departed in July, to assume the responsibilities of Associate Professor of Neurology at Baylor University, Houston, Tex. Dr. Curtis, of the Section of Neurochemistry, has resigned to return to teaching. Dr. Gunter Haase will be leaving in January, to accept the responsibility of creating a neurological unit at the University of Oklahoma, where he will hold the rank of Associate Professor. This will leave vacant the position of Associate Neurologist, until July, 1960, when Dr. King Engel returns to take over this position. In January 1960, Dr. Bonting will arrive to establish the Section of Cellular Chemistry in the Branch of Ophthalmology.

Again, the Unit has benefited from the numerous visiting scientists and guest workers from abroad. From England came two senior investigators: Dr. Tansley, who has just departed to return to her Institute in London, and Dr. William Rushton, from Cambridge, England, who has joined the Ophthalmological Unit, and who is studying the regeneration of rhodopsin in the mammalian eye. During the year, Dr. Fritz Buchthal, from Copenhagen, Denmark, where he occupies the Chair of Director of the Neurophysiological Institute, was with the Medical Neurology Branch. Dr. John Caughey, who occupies the Chair of Neurology at Otago University, New Zealand, was with the Medical Neurology Branch for 6 months.

Dr. Wherrett and Dr. Humphrey, both from Toronto, Canada, are also with the Medical Neurology Branch; Dr. Wherrett is in the Section of Neurochemistry, and Dr. Humphrey is spending his time in the pathology and electronics of muscle disease.

In the Branch of Neurosurgery is Dr. Chou, from Minneapolis, who is working with microelectrodes with Dr. Choh-luh Li. Dr. Chou will be returning subsequently to the professorial staff at the University of Minnesota. Dr. Strang, from Australia, who was also with the Neurosurgical Branch, has moved on to Stockholm, Sweden, for a period of 6 months, and thence to return to Australia.

In the Ophthalmology Branch, Dr. Lele and Dr. Tasaki (the former from India, the latter from Japan), have now departed, as has Dr. Gerin from France, who was in the Electroencephalographic Branch. At the time of this report there

*Prepared by G. Milton Shy, M.D., Clinical Director.

remains, in the Branch of Electroencephalography, Dr. Morillo, from Colombia, and Dr. Widen, who is returning to Sweden.

As always, the Unit has benefited by such visiting scientists and guest workers, and the specific research undertaken by these investigators and their contributions to the research projects may be found in the Branch reports.

Specifically, the **Branch of Medical Neurology** reports as follows: During the period covered by this Report, 269 patients were admitted. This is an increase of 54 patients over the previous year. The total patient days were 6,981, an increase of 1,007 patient days; the average was 26.0 days, a decrease of 1.8 days per patient. Two hundred and thirteen outpatients were seen; an increase of 57. Thus, there was an increased turnover and an increased number of patients both on the inpatient and outpatient service.

The activities of the **Section on Neurochemistry** are centered about the amino-acid metabolism of *in vitro* and *in vivo* studies of normal and epileptogenic cortex, the electrolyte and energy metabolism of normal and epileptogenic cortex, and the relation of pyridoxine to certain seizure abnormalities. This includes studies of protein metabolism and turnover rates, the effects of certain anticonvulsant drugs on the alteration of such metabolic processes, and the utilization of certain amino acids in therapeutic trials with seizure patients.

In muscle disorders, studies are continuing on the distribution of action and tropomyosin in normal and diseased muscle. Studies of alterations of actomyosin tensile strength in muscle disorders, as well as a comparative study of contractile proteins in smooth and striated muscle, are being carried on, and finally, attempts to produce muscle lesions in animals injected with various protein contractile preparations and adjuvants in both animals and tissue culture are being done.

The studies on new physical methods to determine minute quantities of macromolecular constituents in C. S. F. and urine have been terminated with the resignation of the senior investigator, Dr. Curtis. His results are summarized below.

Finally the Section has indicated its interest in studying the formation and turnover of PNA in the C. N. S. This will be started in July 1960, when Drs. Sporn and Dingman join the Unit.

Specifically, Dr. Tower has shown by tissue-slice technique that there is a very rapid formation of glutamic acid and subsequently a slower decline in rate, the later presumably by metabolic conversions to other compounds such as γ-aminobutyric acid and glutamine. When cortical slices were incubated with 40 m*M* malonate the glutamic acid increase remained at higher levels. Glutamine did not subsequently rise nor did ammonia decrease in amount. However, the γ-aminobutyric acid increased 3X in the above studies. Thus malonate appears to block glutamic acid and is an effective "stabilizer" of the free glutamate pool.

Since L-2,4-diaminobutyric acid has been recently reported to block metabolism of γ-aminobutyric acid it was also used in *in vitro* slices. No effect on glutamate metabolism, glycolysis, or oxygen consumption, was noted in concentrations of 40 m*M*. When given *in vivo* to mice, this compound produced seizures, and the subsequent *in vitro* study showed a reduction of O_2 consumption and an elevation of the γ-aminobutyric acid level. This was repeated in cats, injecting 8 mM/kg, and seizures occurred in 6 hours. Here the *in vitro* O_2 consumption decrease could be corrected by giving pyridoxal phosphate but *not* by giving γ-aminobutyric acid. The latter level was 2X the normal and increased during incubation. Thus, Dr. Tower feels this confirms a block of γ-aminobutyric-acid metabolism by L-2,4-diaminobutyric acid, but he also suggests ammonia intoxication of the brain consistent with the hepatotoxic effects of this substance. Studies using aspartic acid by the Lowry fluorimetric procedures were unsuccessful.

Dr. Tower has also undertaken a study on electrolyte and energy metabolism in normal and epileptogenic cortex *in vitro*. This problem carries with it the perennial difficulty of the quantitation of the intra- and extra-cellular fluid compartments. Dr. Tower utilizes the chloride space and concurrently checks this with the spaces calculated by sucrose. By these techniques, the chloride and sucrose spaces showed close correspondence in the normal and epileptogenic cortex. When incubated with glutamic acid, glutamine or asparagine, the slices exhibited a marked increase of the nonchloride space. Gamma-aminobutyric acid produced no change in this space, however. When incubated under hypoxic conditions, the chloride and sucrose space were no longer similar, and indicated swell-

ing of the non-sucrose space and influx of chloride into this space. This is consistent with recent reports and places emphasis on the probable non-reliability of the chloride method for determining extracellular space in damaged tissue.

In nondamaged tissue, however, the Elliott-Heller formula may be used to estimate the cation distribution in the nonchloride space (? intracellular). It appears that neurons account for 75 percent of cortical nonchloride space and cortical potassium, but only 45 percent of sodium. Extrusion of Na and uptake of K is confined to the neuron. This is presumptive, and is based on the fact that cortical and subcortical glial elements react similarly. This then allows an estimation of the O_2 consumption of cortical neurons in terms of nonchloride space volume. The neurons showed almost double the metabolic activity of glia. It is of interest that the "extra" metabolic activity of the neuron almost exactly equalled the fraction of neuronal oxidative metabolism attributable to γ-aminobutyric acid.

Drs. Wherrett, Tower, and Heinrich Waelsch (New York State Psychiatric Institute), have carried out a study on the incorporation of labelled amino acids into protein fractions of cerebral tissue. They have shown that L-glutamine-U-C^{14} is incorporated into protein-bound glutamine, the specific activity being 0.5 percent the specific activity of the free pool glutamine. When 40 m*M* maolonate is added (blocks glutamic acid metabolism, see above) there is a decrease of protein-bound glutamic acid. Similar experiments with 10m*M* NH_4Cl (this elevates free glutamine and depresses free glutamic acid) showed a striking rise of protein-bound glutamine. These findings combined with the demonstration of slow C^{14} turnover in protein glutamate, lead Drs. Wherrett and Tower to conclude that a portion of cortical proteins, involved in the active amino group transfer, utilize the carboxy and amide groups on protein glutamic acid and glutamine. This is one approach to Waelsch's thesis that cerebral proteins may participate in amino nitrogen metabolism and Drs. Tower and Waelsch feel possibly that the transfer of amide from protein glutamine to free glutamic acid may be important to neuronal activity. The findings with NH_4Cl have an obvious bearing on the chemical basis of hepatic coma.

Dr. McKhann, since joining the Section of Neurochemistry, has also worked with γ-aminobutyric acid and pyridoxine, and has finished a study on a patient with pyridoxine dependency. A decrease in cerebral oxygen consumption was found *in vivo* during depletion which was corrected by adding pyridoxine. Thus, this condition varies from other types of seizures in which oxygen consumption increases. Two types of pyridoxine deficiency have been found by Dr. Coursin; in one type there is an abnormally rapid conversion of pyridoxine to 4-pyridoxic acid, and in the other there is an abnormally rapid excretion of ingested pyridoxine through the renal apparatus. This patient was shown to be of the second type. Experimental animal studies in kittens on pyridoxine-free diet precipitated ataxia and seizures and death. A study of the brains of these kittens showed a decrease of γ-aminobutyric acid. This latter defect could be reversed *in vitro* by addition of pyridoxal phosphate or γ-aminobutyric acid. This adds to the evidence that pyridoxal phosphate acts as a coenzyme necessary for the enzyme glutamic decarboxylase, and correlates with the study above on electrolyte and energy metabolism in showing the "extra" metabolic activity of the neuron is dependent upon the oxidative metabolism attributable to γ-aminobutyric acid.

These investigators continued their studies on the quantitative contribution of γ-aminobutyric acid pathway to total oxidative metabolism at the Krebs cycle stage, using pyruvate-3-C^{14} as a tracer. These studies showed that 44 percent of the total substrate metabolized from α-ketoglutarate to succinate was via the γ-aminobutyrate pathway. Since the latter pathway appears from the above to be almost exclusively neuronal it is estimated that nearly 60 percent of neuronal oxidative metabolism proceeds via γ-aminobutyric acid. Since this varies in a sigmoidal fashion during 1-hour incubation there is, in the minds of these investigators, a suggestion of a reciprocal metabolism via the parallel succinyl-Co A pathway. Here studies were done with cortical mitochondria and γ-aminobutyric acid-1-C^{14}, comparing the $C^{14}O_2$ evolved to the total O_2 consumption. In the presence of arsenite, which blocks α-ketoglutarate to succinyl-Co A, the inhibitory was not seen. Thus, as levels of the common precursor, α-ketoglutarate, rises the latter pathway (succinyl-Co

A) is referred. *In vivo* studies using pyruvate-3-C^{14} show that, in initial studies, the labelling of glutamic acid and γ-aminobutyric acid is extremely rapid. Using seizure preparations (i.e. thiosemicarbazide and L-2, 4-diaminobutyric convulsants) these investigators feel *the metabolism through* the γ-aminobutyric acid pathway rather than the level of the γ-aminobutyric acid itself is the significant factor.

Dr. Tower and his group have utilized γ-aminobutyric acid to treat 10 seizure patients. Fifty percent of these patients improved. The B.B.B. (blood-brain-barrier) seems to be the chief factor in the variability of response as the permeability of the B.B.B. between seizures appears to be limited. Dr. Bushnell Smith, who is now entering his second year, is in the laboratory with Dr. Tower, carrying on a study as to the effects of anticonvulsant drugs on cerebral electrolyte metabolism. He has found a slight effect in reducing the non-chloride space sodium concentration during incubation with "Diamox" at a concentration of 3μM/L. This is approximately the concentration known to effectively inhibit blood carbonic anhydrase. Other concentrations of "Diamox" are now under study. It is planned to do similar studies with diphenylhydantoin (Dilantin).

Dr. Horvath is continuing his studies on the molecular architecture of muscle in normal and diseased states, and is now devoting much of his time to immunochemical methods. Thus, in the study of tropomyosin A he has developed tests and standardized quantitative determinations of immune precipitins. These include an adaptation of the Kabat method in which immune-precipitates are washed and the nitrogen content measured by direct nesslerization. Semiquantitative measures may also be obtained by chromatographic technique, developed by Dr. Miquel, in which antibody and antigen are paper electrophoresed together. The soluble protein moves but the immune-precipitate remains at the site of application. This may then be stained with bromphenol blue, eluted, and determined spectrophotometrically.

Both of the methods just listed provide information on quantitative bases as to the amount of antibody circulating and on the stoichiometry of the antigen-antibody reaction. Thus, with clam tropomyosin the system was found to have an equivalence point in which two antibody molecules combine with one tropomyosin A molecule, while in the antibody excess region four or more antibody molecules combine with one tropomyosin A molecule. Titers rose as high as 2,000 μg/ml. after a second set of injections 2 months following the first. Circulating antibodies were identified by electrophoresis as γ-globulins. These antibodies were found to fall within 1 month after immunization to about 10 percent for the maximal level, and booster doses raised the titers in approximately 10 days. Dr. Horvath carried out a more rapid extension of this method by using the passive hemoagglutination test of Vorlaender. This method uses tannic acid-treated sheep red cells. Immediately after such treatment the cells bind tropomyosin A and are agglutinated by high dilutions of anticlam tropomyosin A. Normal sera would not do this. This test does not, however, have the accuracy of the quantitative precipitin reactions, but it is very much faster. In addition to this, diffusion agar plates have been used for testing the homogeneity of the antibodies. After the first set of immunization injections, only a single precipitin line appeared on the agar plate, and it was estimated that impurities could not have exceeded three percent. However, similar tests after a second set of injections revealed multiple precipitin lines. The causes of this are now under study.

Cat muscle myosin apparently is more complex than that of clam tropomyosin A, and the agar plates show several precipitin lines. The quantitative precipitin reactions fail to show clear-cut optima. These observations are consistent with the concept that myosin possesses several antigenic groups and that the subunits of myosin possibly consist of a complex of tropomyosin and actin. A study of the cross reaction of cat myosin and clam tropomyosin reveals a 50 percent cross-over. Considering the remote relation between the two species, Dr. Horvath believes this demonstrates a close relationship of the two proteins.

Dr. Horvath has also continued his studies on alterations of actomyosin tensile strength and of muscle proteins in neuromuscular diseases. He is thus immunizing rabbits with muscle proteins prepared from rabbit muscle. The initial studies on immunization of rabbits with rabbit actin were done probably with preparations which were 50

percent impure. Under the present methods, which are 99 percent pure by the criteria of polymerization, antibodies could also be developed in rabbits. Most of the impurities were presumed to be tropomyosin B. When tropomyosin B was prepared from rabbit muscle it was found rabbits could be immunized with it, with titers obtained as high as 300 μg. antibody N/ml. Similarly rabbits could also be made antigenic to tropomyosin A. That this reaction is not due to chemical manipulation may be indicated by the fact that fluorescent antimyosin staining of muscle samples confirmed the site of the antibody reaction, and the biological activity of myosin isolated from muscle, in terms of ATP-ase action and the combination of contraction with myosin, is not altered in the chemical or biological sense. Attempts to produce muscle lesions in guinea pigs injected with various muscle preparations and adjuvants have not been uniformly successful so far.

These studies in immunochemistry have led to a temporary suspension, during the calendar year 1959, of Dr. Horvath's comparative biochemical study of smooth and striated muscle. However, a dystrophic mouse colony is being maintained to provide one type of source material for such studies.

Dr. Curtis left the Branch of Medical Neurology to return to teaching. His primary interest during his stay at the Institute was that of developing physicochemical methods for determining minute amounts of material in organic fluids, such as spinal fluid and urine. This was directed particularly to the identification and characterization of macromolecules, such as polypeptides, pyrogens, etc., which occur in the urine and other biological fluids. One such study was directed towards the presence of any specific substance which might be liberated or produced in association with primary or secondary demyelinization in the central nervous system. Dr. Curtis, at the time of his leaving, was studying this by methods of bubbling benzene, under controlled conditions of rate and drop size, through a column of cerebral spinal fluid. With this method apparently complete removal of lipids appears to occur. Subsequent chromatography of such lipid-containing benzene on silicic-acid-impregnated paper demonstrated the presence of lecithin, cephalin, sphingomyelin, cholesterol, and cerebrosides. His preliminary studies indicate elevation of some substances, particularly the latter three, in cerebral spinal fluid samples from multiple sclerosis patients. No quantitative data was possible however by this method, but the lipid "profile" seemed consistent with the recent report by Tourtellotte. Complementary studies were carried out on a model protein, which was guinea pig serum asparaginase, as noted in the last Annual Report, which yielded considerable information. This is a globulin which has readily assayable enzyme activity, and can be isolated as a crude fraction from serum by ammonium sulfate precipitation and can subsequently be partially purified with calcium phosphategel. The crude fraction contains two visible contaminants by electrophoresis and at least one by ultarcentrifuge analysis. The previous studies reported in 1958 indicated the enzyme activity was associated with the fastest component in the ultracentrifuge analysis. However, purification by these means was unsuccessful. In 1958 it was found that the cellulose resin developed by Peterson, et al, would completely absorb this enzyme. Studies this year on elution of the enzyme clearly indicated that, under the appropriate pH and ionic strength, four separate protein-containing fractions could be eluted from these cellulose columns. Good separation of the four fractions was obtained, but only one contained asparaginase activity. This suggests that complete purification had been achieved without loss of enzyme activity, and represents the first such isolation of an enzyme protein in this manner.

The **Section on Biophysical Applications** has now transferred the collimating scintillation scanner and its instrument console to the Department of Radiology of the Clinical Center, where it will now be used as a diagnostic tool for future studies. Due to the amount of instrumentation technique involved in this apparatus, however, the technicians from the Section of Biophysics are working with the Radiological Department on each patient scanned. The scans in turn are read both in the Department of Radiology and in the Section of Biophysics; the reason for the latter being that the Section is attempting to obtain 500 confirmed scans before dropping the research interest in this given technique. At the present time the Section has 359 scans. We are suggest-

ing to Dr. Di Chiro that methods now be used in the scanning technique in which the posturing of the patient might bring out lesions of the parasellar area and lesions in midline of the posterior fossa, such as is being done in standard radiological techniques. It is probable that this part of the study will be transferred to the Section of Neuroradiology.

In the meantime, histopathological and chemical investigations on neuromuscular diseases are continuing, and this is a continuation of a long-term study initiated at the inception of this Institute. Over the past years the Institute has elucidated the pathology of muscle disease, the role of certain cations, and contractile proteins in muscle disease, and at the time of the 1958 Report had finished a prolonged study on the endocrine and metabolic aspects of muscle disease. During the past year attention has been paid largely to regeneration of muscle in various neurogenic and primary muscle disorders. An inclusive study of the primary pathology of peroneal muscular atrophy has been concluded, and the final studies of the various interrelated factors in cationic paralysis have also been concluded.

To follow the regeneration of muscle, tritium (H^3) labeled thymidine has been now used in some 20-odd cases of different neurological disorders in which there is subsequent wasting of muscle, and autoradiology is now underway on these sections. Proliferating cells will manfacture desoxyribonucleic acid (DNA), and in doing so incorporate the labelled thymidine. The low energy range of tritium allows the precise localization of such DNA.

The cationic disorders were studied in relation to thyroid metabolism, to K–42 exchange, and to aldosterone by means of double isotope derivative methods, and in relation to certain pharmaceutical agents such as SC–8109, a steroid-17 lactone, and 2-methyl-9-alpha-fluorohydrocortisone, which deliberately shift monovalent cations.

Major findings to date are that over 80 percent of cases of Charcot-Marie-Tooth's disease demonstrate myopathic findings as well as neuropathic findings on pathological examination and on electromyography. No such findings were found in cases of amyotrophic lateral sclerosis. Two of these cases which were clinically classical and demonstrated sensory abnormalities showed only myopathic changes. Eighty percent of the total demonstrated some myopathic involvement, whereas 100 percent of cases of amyotrophic lateral sclerosis showed only neural involvement.

In the cationic diseases, three separate entities have now been separated which may cause sudden flaccid skeletal paralysis. These are familial periodic paralysis, paramyotonia congenita, and primary aldosteronism. The pathophysiology of each is different. In familial periodic paralysis there is retention of both potassium and sodium, and there is a hypokalemia. The potassium which is lost from the serum enters the cell. Enough water enters the cell, however, to hold the concentration of potassium approximately the same. This has been confirmed by intracellular electrode recordings. Sodium depletion aids in the recovery of the attack or prevents the onset of such an attack, and sodium loading precipitates the attack. Exactly the reverse is true for paramyotonia congenita, although in this disease also d-1 aldosterone does not precipitate an attack. Studies are continuing in the physiology and pharmacology of myasthenia gravis, and as stated in last year's Annual Report, the objective was that of intracellular recordings of resting potentials in patients with myasthenia gravis. The difficulties of this procedure were pointed out in the last Annual Report, in which descriptions of the percutaneous method of introducing microelectrodes blindly inside single cells were given. Approximately 18 such patients were studied in the current year, with over 200 penetrations. It has become apparent, with our present technique at least, we will not be able to successfully carry out this procedure through the intact skin. Such patients would have to have surgical exposure of the muscle to carry on such a study. This phase in the program, hence, has been temporarily abandoned. However, the program itself, like many others, has now separated into two different components. The availability now of agents which influence thyroid formation and TSH formation, as well as methods of influencing each independently, has allowed the Unit to make an extensive study of the problem which has long baffled neurologists, i.e. the relation of thyroid metabolism to myasthenia gravis. This facet has now been worked out by Dr. Andrew Engel, and will be recorded in a separate project.

At the beginning of this reporting year, the Institute was fortunate in having with it Dr.

Fritz Buchthal from Copenhagen, and his method of electromyography is now extensively used within the Branch. The recording instrumentation for the intracellular work in myasthenia gravis has been converted, therefore, to this particular project. The methods now used are those of multiple concentric electrodes so that synchronization and motor unit areas may be mapped. The studies at the present time include a study of the internal ocular muscles in which nystagmus is present in cooperation with the Ophthalmological Unit. This study has just been initiated. It is of interest that a characteristic myasthenic response has now been seen in patients with generalized sarcoid disease, and it may well be that this technique may be of use in the diagnosis of this disease. The reason for this, however, is still not clear and is being followed at this time. The duration of the action potential in varying groups of muscles at different ages of patients, which as yet have not been charted, is being undertaken by the Fellow assigned to the project. A direct correlation of the EMG with the muscle pathology is continually carried out on each patient admitted to the muscle projects in this Institute.

It is of interest that electromyography is in disrepute in many medical centers in the United States today. This institute is now convinced, after analyzing some 110 of our own patients using the Copenhagen type of study, that this conclusion was reached because such studies were not carried out with the pathophysiology of the various disorders in mind. It is our belief, after this series of patients, electromyogram will be of definite value when correctly done and interpreted, and has many times given us information of real importance in the understanding of our patients.

The clinical pathological correlative study of the nervous system in cases of orthostatic hypotension has now been completed, with the serial sections done on the postmortem case listed in the 1958 Report by Drs. Drager and Shy. In this case symmetrical lesions were found bilaterally on each side of the brain stem, in the ventral, intermediolateral column, and Clark's cell column of the spinal cord. Moderate gliosis was also present and most evident in the dorsal funiculus. In the medulla, degenerative changes of the inferior olives, the nucleus ambiguus, the dorsal nucleus of the vagus, the median Raphe of the tegmentum, and the lateral cuneate nucleus were also found. In the cerebellum and pons, neuronal changes were found in the Purkinje cells and in the locus caeruleus of the pons. In the midbrain, lesions were found in the substantia nigra, the third nerve nucleus, the Edinger-Westphal nucleus, and the central aqueductal gray. Some changes were also found in the posterior hypothalamus. It is of interest, however, that the cerebral cortex was normal. Since the cerebral cortex is the most likely to be affected in cases of anoxia, and since no thrombotic lesions were seen in the cerebral blood vessels, it would thus appear that this lesion could not be explained on either of the latter two postulants. A review of the literature indicates similar neurological findings in the majority of cases reported, and it is highly suggestive therefore that so-called orthostatic idiopathic hypotension is, in fact, a degenerative disorder of the central nervous system involving the above centers, and could be corresponded to the so-called motor neuron disease long recognized as a system disease.

The **Section on Clinical Neuropharmacology** continues its study around the choline esters found naturally in biological systems, and the study of muscle cholinesterase and its inhibitors, and studies of depolarizing compounds and their effects on myoneural junctions. They are, in addition, studying the anatomical physiological correlation of the motor unit in mammals.

As discussed in previous years, one of the factors of most importance in determining the etiology of myasthenia gravis would be the identification of a substance in diseased muscle which would act not unlike choline esters, but not be hydrolized by the muscle cholinesterase as is the acetylcholine. Such a substance could be present in muscle in abnormal amounts, and thus pronounced effects on muscle function could occur. Thus, for example, butyrylcholine, imadazoleacrylychloline, and dihydro-acrylylcholine are all nearly equal to succinylcholine in depolarizing striated muscle. In an attempt to study the action of such choline esters and chemically related substances on muscle membrane, and to isolate and identify such choline esters, these investigators used the following methods:

First, a traveling fluid electrode is used to measure depolarization of the muscle membranes. This technique has the advantage of recording the

differential depolarization of a large number of intact muscle fibers. Secondly, paper and column chromatographs are used for the isolation and identification of choline esters and other quaternary compounds. Finally, the heart of the clam *Venus mercenaria* is used for identification and assay of acetylcholine.

These investigators have found that the accurate concentration response relationships could not be obtained since one depolarization compound reduced the sensitivity of subsequent tests and the test muscles varied considerably in their response. Therefore, by selecting a narrow range of depolarization to be produced, and the use of one muscle for each test, a comparison of potency was possible. Their studies appear to indicate that if choline esters were not hydrolized by the muscle cholinesterase, as is acetylcholine, and were hence present in the muscle in abnormal amounts, pronounced effects on muscle function could occur. The identification of such substances in diseased muscle would apparently require muscle samples no larger than 1 gram. This study is of extreme importance in the disorder, myasthenia gravis, and will be followed through the next year.

This Section is continuing its studies also of muscle cholinesterase and its inhibitors. Thus, they find that the cholinesterase content of muscle is low and not uniformly distributed throughout the tissue, and muscle has not been adequately studied in respect either to the type of cholinesterase it contains or as to the substrate and inhibitor specificity. The approach in the current year is that of adequately characterizing this important muscle enzyme in relation to its substrate specificity. Another objective is to examine the activity of the enzyme in the presence of well-known inhibitors which are in wide use clinically, and to correlate this activity with their usefulness. This could then form a basis for testing newer compounds having a potential in the treatment of myasthenia gravis. In studying this the standard Warburg manometric technique is used for determination of muscle cholinesterase activity. The depolarizing properties of cholinesterase inhibitors were determined by the use of the travelling fluid electrode system. These investigators have found that Ambenonium had the greatest inhibitory activity on muscle cholinesterase of any of the compounds tested. Neostigmine had the greater activity in this respect than did pyridostigmin. The major part of this work has centered around the testing both *in vivo* and *in vitro* of compounds which seem likely to have a potential use in the treatment of myasthenia gravis.

Galanthamine is an alkaloid which has recently been isolated from plants of the Amary–llidaceae family in Russia and Japan, and in this country by the Laboratory of Chemistry of the National Heart Institute. Another substance of much promise is Lycoramine, which is an alkaloid with a chemical structure closely related to that of galanthamine. This laboratory investigated both of these compounds and their related derivatives as to their muscle potentiating and acetylcholinesterase inhibiting properties. These compounds are both derivatives of phenanthrene and are of interest because chemically they differ markedly from other compounds currently in use for the treatment of myasthenia gravis. The compounds tested were galanthamine hydrobromide, galanthamine methyl iodide, lycoramine methyl iodide, deoxylycoramine methyl iodide, deoxydemethyl-lycoramine methyl iodide, neopine and neopine methyl iodide. In this study the quaternary forms of the alkaloids were found to be more active in muscle than the tertiary forms. Lycoramine methyl iodide produces about the same amount of inhibition of cholinesterase as pyridostigmin. Galanthamine, both the tertiary and quaternary form, produces more inhibition than does pyridostigmin. Deoxydemethyllycoramine methyl iodide was the most active compound found, and this compound produces cholinesterase inhibition equal to that of neostigmine or physostigmine. The *in vivo* muscle potentiating activity of the compound correlated well with the inhibiting activity. Plasma, red blood cell and brain cholinesterase are also inhibited. The potency of these compounds strongly suggest that they will offer advantages in the treatment of myasthenia gravis. Galanthamine has already received such trial in the USSR, and more recently in Sweden. The biological activity and use of lycoramine has not been previously described, and it would appear that we are about ready to move into clinical trials of this compound after toxicity studies have been carried out.

To better understand how the depolarizing agents work on muscle membranes, a study of the efflux of enzymes from such muscle has also been

carried out in this laboratory, using standard biochemical procedures to estimate enzyme activity in plasma and muscle. Here again, the travelling fluid electrode is used to indicate the amount of depolarization. This laboratory finds that the muscle loses aldolase when kept in a solution containing depolarizing concentrations of potassium. It has not been determined, however, if this depolarization of the cell membrane is the causative factor or whether the loss of enzyme is due to activity of potassium at another site. In an attempt to find out whether aldolase leaves the muscle under the influence of succinylcholine, experiments were done on pairs of rat muscles removed prior to and after the administration of this drug. Changes in the aldolase content of the experimental muscles were variable and not pronounced, at the end of brief periods of depolarization. The laboratory has also found that substances which depolarize muscle membranes also inhibit muscle cholinesterase, but that such inhibitory concentrations are much greater than those necessary for depolarization, and thus esterase inhibition by these compounds appears to be unrelated to their depolarizing activity.

Finally, in this laboratory, Dr. Irwin and Dr. Norris are doing a microdissection of peripheral nerve and electrophysiological studies to determine the actual extent of the physiological anatomical motor unit. The two studies on the action of neuromuscular blocking agents on directly stimulated innervated and denervated muscle, and on blood and tissue choline esterases in neuromuscular blockade which were discussed in full in the 1958 Annual Report, have been completed and are now published. Both were excellent studies. The papers published in reference to these studies were on the contractual response of directly stimulated muscle after administration of neuromuscular blocking agents, and the effect of certain neuromuscular blocking compounds on directly stimulated muscle. The paper on the effects of selective inhibition of muscle and plasma cholinesterase on neuromuscular block has been reported at the Second International Symposium on Myasthenia Gravis, and the methods and findings may be found in the 1958 Annual Report. These projects are now completed.

The **Section on Neuroradiology** has carried on studies as to the skeletal changes accompanying Dystrophia Myotonica; the correlation of brain scanning with standard contrast studies; a study of fractional encephalography, which is anticipated will culminate in an Atlas; a study of the sella and pituitary in the horizontal plane, which is a combined X-ray and postmortem study; and a study on the encephalographic changes in the temporal lobe. In addition studies are being carried out on a bilateral angiographic evaluation of the superficial veins and sinuses of the brain, and radiological study of the soft tissues in different muscle diseases is being carried out to correlate with the overall large program in muscle disorders.

In collaboration with Dr. John Caughey, Associate Professor at the National University of New Zealand, Dr. Di Chiro carried out a study of the radiographic changes of the skull in 18 cases of dystrophia myotonica. Eighty-nine percent of these cases showed "hyperostotic" changes of the skull vault, which these authors have grouped into four different categories. They arrived at the conclusion that the longer the duration of the disease, the more marked are these changes. They suggest there is also a possible correlation between the amount of hyperostosis and the proof of hypogonadism, and interpret these findings as due to increased circulating growth hormones. This project is now completed and will be published in the *Acta Radiologica.*

In correlation with the study in brain tumor scanning, Dr. Di Chiro has picked the confirmed tumors in which adequate studies, both by means of radioactive scanning and by means of arteriography and pneumography, have been accomplished. One of the chief criticisms of the previous work on the brain scanning, which is reported in book form and in the 1958 Annual Report, is that no definite correlation had been done between this and other standard neuroradiological techniques. Dr. Di Chiro has now over 60 cases which have adequate studies in all three such techniques, i.e., radioactive scanning, arteriography, and encephalography. It is hoped that this study, upon its completion, will better elucidate the advantages, limitations, and future possibilities of the brain scanning technique in relation to standard contrast techniques now available.

The superimposition of different anatomical structures makes difficult the interpretation of

many gas contrast studies. This is particularly true in the area of the posterior fossa. To obviate these difficulties, Dr. Di Chiro is undertaking a combined laminagraphic study combined with the ordinary encephalogram done by the fractional method. Laminagraphic studies have been done during the period of time that the passage of air through the different sections of the ventricular system and subarachnoidal spaces is occurring. The laminagraphic cuts were taken in different projections and in different planes. Approximately 30 patients have so far been examined with this technique. The initial study is limited to cases without intracranial space-occupying lesions. This study will be combined with routine fractional encephalography in the preparation of an Atlas devoted to fractional encephalographic anatomy. Combined with this, brain specimens are now being studied to establish a correspondence with the structures seen in the encephalogram. For this purpose different structures, especially in and around the brain stem, are being coated with radiopaque fluids, and afterwards examined with laminagraphic techniques.

Many studies of the size and shape of the sella have been accomplished in the past, and recently a monograph on this subject has appeared in the British literature. None of these studies, however, indicate the true volume of the sella in that a reconstruction in the horizontal plane has not been attempted. Volumetric studies of the sella are becoming more necessary with the increasing surgical approach to the pituitary and sella. Thus, in recent years, hypophysectomy is being carried out in numerous clinics for endocrine carcinomas. Intrasellar implantation of Yttrium ^{90} seems to be a highly promising technique, both in pituitary tumors and in other conditions in which the destruction of the pituitary is indicated. The transsphenoidal route is preferred by some authors, and a preoperative knowledge of the lateral borders of the sella and the pituitary is of utmost importance for this type of approach. To carry out this study, the base of the skull at postmortem, with the dura, pituitary, and the parasellar vessels still *in situ*, is studied with different radiological techniques, including laminagraphy. Metal wire markers and X-ray opaque fluids are used to outline the different border lines of the sella. These in turn, then, are compared with the standard X-ray films of normal cases with intra- and para-sellar pathology.

The venous drainage of the brain, as elsewhere in the body, is extremely variable, and no systematic angiographic study has been done on this subject. This Institute is perhaps in a unique position in that we have a comparatively large amount of bilateral carotid arteriograms, in conjunction with the temporal lobe program, in patients with no intracranial space-occupying lesions. Since such angiograms are carried out seriographically, the different phases of the superficial venous drainage may be demonstrated. Dr. Di Chiro has found, in a significant number of cases, that the venous drainage takes place through different vascular channels in the two hemispheres of the same patient; while the venous drainage on one side may be, for example, through the vein of Trolard, it may, on the other side, be in the large vein of Labbe or the superficial Sylvian veins. Dr. Di Chiro has undertaken a statistical study of the drainage of the two hemispheres through the right or left transverse sinuses as part of this project. It is of interest that these sinuses also show on the radioactive scans, and this may be of some value, also, in correlating these two techniques in this study.

The last two studies of the Section on Neuroradiology are attempts to further elucidate the problems which are encountered in two of the large clinical areas of the Institute. The first of these studies deals with the encephalographic changes in temporal lobe epilepsy in which every case of the latter is now studied encephalographically, in such a way that a reliable comparison of the two temporal horns is obtained. Each temporal horn is filled separately, and afterwards examined in two orthogonal planes. Appreximately 20 patients have now been studied with this encephalographic technique, and it is obvious that many more such studies will have to be carried out for statistical evaluation.

One of the main problems in muscle diseases of the young infant is the masking of muscle atrophy by large amounts of adipose tissue. In certain cases of dystrophy this is also true, in that the muscle is replaced by such adipose tissue. Soft-tissue techniques, determined radiographically, are frequently of aid to the investigator in determining the site of muscle biopsy, as well as the

extent of the disease, so that adequate chemical and other studies may be carried out. Dr. Di Chiro points out the technical problems that are involved in the optimal X-ray visualization of soft tissues and his present impression is that in addition to adequate X-ray techniques, some type of good reproduction apparatus is necessary. He has suggested that the Logetron equipment be chosen for such reproductions. The amount of contrast and detail obtainable with this equipment is unsurpassed. It may also be used in other X-ray studies throughout the Institute, and will be placed in the Photographic Unit of Clinical Investigations.

Dr. Gunter Haase has, during the past 2 years, continued his study of the intramuscular motor and sensory nerve endings in biopsies from normal controls, and in patients with neuromuscular diseases. The method employed is that of injection of methylene blue at the time of biopsy, as indicated by Coërs; the acetylcholinesterase stain and silver stains, in particular the modifications of Bielschowsky and Winkelmann. The materials were obtained in the course of routine biopsies, and only slight variations imposed by the intravital injection of methylene blue. In all of these specimens routine staining methods were also employed, and reviewed at the weekly myopathological conferences.

Dr. Haase finds the concentration of the motor end-plates in a narrow zone at about the center of each of the individual muscle fibers within a fascicle. This confirms what has previously been described. Changes in the terminal innervation and in the motor end-plates of muscles of patients suffering from muscular diseases and diseases of the lower motor neuron, have been found. Thus, marked alterations of the motor end-plates in myotonic dystrophy have been found in which there is a marked ramification of the terminal innervation and multiple end-plates on single muscle fibers. This has also been found in cases of Huntington's Chorea, which were originally biopsied for control purposes. Some cases of Parkinson's disease showed, on orthodox stains, evidence of a mild myopathy; this was reflected by changes suggesting degeneration of the motor end-plate by methylene blue stains.

In reference to the muscle spindle, it is thought that this method does not promise to bear fruit. Spindles, even in the normal muscle, apparently show great variations, and it is impossible to clearly define pathological changes in these structures.

Dr. Haase will be leaving the Clinical Investigative Unit to assume the chair of neurology at the University of Oklahoma, and this will terminate this study at this Institute. It is hoped that he will be able to continue along these lines in his new position, and that an ultimate summary of a larger number of patients will be available.

Dr. Andrew Engel has carried out a thorough study of the thyroid function in myasthenia gravis. It is well known that 5 to 10 percent of patients with myasthenia gravis are also hyperthyroid, and when these two diseases coexist the prognosis appears to be grave. Some observers in the past have reported an inverse relation between these two disorders, and this view has been challenged by others. The study was undertaken by Dr. Engel to define the interaction of the two disorders, and to evaluate the respective roles of pituitary TSH and of the thyroid gland. The methods employed were utilized on five myasthenic patients. Medication was held at constant levels during this study, and the clinical evaluation consisted of daily measurements of the pre-determined group of muscles. The thyroid status was evaluated as follows: Daily BMR, weekly PBI, cholesterol and I^{131} uptake. In three patients thyroidal secretion rate was also measured by direct daily counts of thyroidal radioactivity, corrected for physical decay. Steps taken were as follows:

1. Triiodothyronine (T3) was given at a dose level insufficient to induce hypermetabolism, but adequate to suppress pituitary TSH secretion.

2. T3 was administered at progressively higher dose levels until hypermetabolism was induced.

3. After a labeling dose of I^{131}, thyroidal iodine accumulation was blocked with Tapazole and TSH (Armour) was administered in graded doses, while the fractional release of hormonal I^{131} was measured from day to day.

4. Same as Step 3, but with concurrent administration of sodium iodide to offset the thyroid hormone release-accelerating effect of TSH.

5. The prolonged administration of Tapazole, until depletion of thyroidal hormone stores, re-

sulting in hypometabolism with concurrently high pituitary TSH secretion.

In the myasthenic study, the myasthenia could be seen to become worse in all patients who became thyrotoxic. No see-saw relationship could be seen, although minor fluctuations occurred until the BMR reached plus 20. In one patient who became hypometabolic on Tapazole, the myasthenia improved while the endogenous TSH level was still elevated. Exogenous TSH-induced acceleration of hormonal secretion was followed by elevation of the BMR, and this by worsening of the myasthenia. When this acceleration was blocked by sodium iodide, the effect on the disease was also blocked, and only to the extent that the rise in the BMR was blocked. It is thus apparent that TSH, per se, has no significant extrathyroidal effect on myasthenia, and that there is a direct relation to the metabolic rate and the severity of the myasthenia.

Similar studies were carried out in one patient with familial periodic paralysis. In familial periodic paralysis, as high as 30 percent of patients have been reported with exophthalmos and hyperthyroidism. Induced hypermetabolism did not worsen the clinical status of the patient with familial periodic paralysis. Withdrawal of T3 was followed by a severe and prolonged worsening, and this was relieved by TSH administration. TSH withdrawal again resulted in an exacerbation. Repeated TSH studies could not be performed as the patient became resistant to TSH. Thus, there may be a difference between thyrotoxic nonfamilial and familial nonthyrotoxic forms of this disease, namely the response to abnormal levels of thyroid function.

Dr. Darwin Prockop, of the Heart Institute, and Dr. Bushnell Smith have continued their studies on the use of monoamine oxidase inhibitors (namely JB 516), as an anticonvulsant medication for centrencephalic seizures. Patients were admitted and anticonvulsant medications were reduced to a minimum, whenever possible without danger. After a short baseline period of observation, the patients were given either a placebo or JB 516 daily for 4 to 6 weeks, in addition to the other medications they had been taking. This was done by the double-blind method. Electroencephalograms were run each week and compared to the baseline EEG. Determinations of the amount of 5-hydroxytryptamine in the urine were done during the baseline period, and on the sixth and seventh day of medication.

In the major findings, only two of the patients received JB 516 under this double-blind study. One developed drowsiness, followed by generalized convulsions after reduction of barbiturate. Before reduction the seizures were fairly well controlled. One patient became hypotensive and had syncopal attacks after 4 weeks of medication. During the fourth week, the patient's seizures were fully controlled. Four patients received placebos—one patient showed improvement. At this time statistical significance of this series is not such that JB 516 could be shown to give any improvement in patients with this type of disorder.

The study of other monamine oxidase inhibitors, with more specificity for the central nervous system, will be undertaken now that a protocol has been designed for such patients. Dr. Smith is also continuing his study initiated with Dr. Drager on the pathological substrata of the Heidenhain form of Jakob's disease. Serial sections of the brain were accomplished in a patient dying of this disorder, which is a relatively rare progressive degenerative disease of the central nervous system, the etiology of which is quite obscure and the pathological process not well documented. A cortical biopsy was obtained during the third month of this disease and the diagnosis confirmed at this time. Findings at the present time are those of a widespread diffuse loss of neurons in the brain, particularly the occipital lobe, as marked by status spongiosus and astrocytic proliferation. The cerebellum shows a marked loss of Purkinje's cells and granule cells. The lateral columns showed degeneration. In addition to this, nodules of acute inflammatory cells with foci of necrosis were scattered throughout the brain and spinal cord, and may be due to the terminal infection.

Finally, Dr. Altrocchi, in the Clinical Investigative Unit, and Dr. Krooth, in the Branch of Epidemiology, are joining in a combined study of tissue culture and chromosome counting in human subjects with various neurological disorders, and from specific tissues in which local tissue abnormalities are found, such as in Von Recklinghausen's disease. This study has just been undertaken and will include tissue culture of the removed tissue, chromosome counting of

café au lait spots, local tumors, etc. Studies of quantitative chromosomal abnormalities in patients with mental deficiency, spinal deformities, etc., will also be done.

The methods employed will be in the removal of fresh bone marrow, which will be incubated overnight and treated with colchicine, and then fixed and stained by the modifications of the methods described by Ford, Polani, et al. Squash preparations will be searched systematically for cells in the metaphase of mitosis, which will be analyzed both by direct microscopy and by microphotography for quantitative and qualitative abnormalities. Tissue-culture methods will be used to grow skin, muscle, marrow, and neoplastic tissue in the laboratory, which will then be treated in a manner similar to that above for chromosomal analysis.

The **Branches of Electroencephalography** and **Clinical Neurophysiology** report seven projects. These projects are centered about the clinical electroencephalographic problems in the field of epilepsy; the effects of hypothermia and blood pressure on electrical activity as recorded from exposed normal human cortex; experimental projects in animals elucidating thalamo-cortical and cortico-thalamic connections; and an analysis of various unitary elements activated within the visual cortex, following stimulation of the optic radiations. Other projects relate to an experimental approach to basic mechanisms underlying seizures, in particular with microelectrode investigations of "self-sustained" activity which is produced following repeated electrical stimulation of the cerebral cortex.

During the current year, one visiting scientist, one guest worker, one research associate, and three clinical associates collaborated with the Branch chief in the investigations reported below. In addition to the investigative work, the Unit carried out a total of 1,579 electroencephalograms, during the period of 11 months covered by this report, and 24 electrocorticographic studies, during cortical exposure in the surgical treatment of epileptic patients or on patients in whom operations such as hypophysectomy were being done as a therapeutic measure for carcinoma.

More specifically, Dr. Cosimo Ajmone Marsan and Dr. Kristof Abraham have finished a pictorial study, for an atlas, of 250 epileptic seizures, observed from beginning to end, of which 43 have been selected. Each seizure, in turn, makes up to four large plates of the atlas, and the different components of the seizure are synchronized with the corresponding time interval in the electroencephalogram. This atlas will, in fact, be a companion to the book by Drs. Ajmone Marsan and Ralston, entitled *The Epileptic Seizure*, based upon metrazol-induced seizure patterns. The various patterns described in this monograph are here illustrated and coordinated with the electroencephalogram, and the varying components of the seizure—such as the loss of consciousness; focal movements of body, face or limbs, with or without electrographic correlates; automatisms, and seizures produced through chronically implanted electrodes—are all correlated with the presence or absence of specific electrographic changes during each phase of a given seizure. This atlas has been accepted for publication and will appear as the Fifteenth Supplement of the *Journal of Electroencephalography and Clinical Neurophysiology*. This will, in effect, complete the research portion of this project. As an adjunct diagnostic procedure and screening, in cases of patients considered as possible candidates for surgical treatment of seizures, this type of recording, however, will be continued.

Dr. Ajmone Marsan and Dr. John Van Buren are continuing their studies in depth electrography in epileptic patients. This is part of an overall, long-term project to map out the functional correlates and anatomical substrata of the deeper nuclei of the brain, and particularly those of the temporal lobe, i.e. amygdala and hippocampus.

Electrocorticographic studies in temporal-lobe epilepsy, and in focal cerebral seizures at time of exposure during operations, have continued. Much of the original portion of this work has been reported in the book, published by Thomas, titled *Temporal Lobe Seizures*, edited by Baldwin and Bailey. These findings are considered, in some detail, in Dr. Baldwin's reports on the Branch of Neurosurgery, as are the studies on the autonomic nervous system and electroencephalographic correlations, reported by Dr. Van Buren of the Branch of Neurosurgery.

Dr. Lennart Widen and Dr. Ajmone Marsan have completed a study on the unitary analysis

of response elicited in the visual cortex of cat. The object of this study was to determine the temporal and special distribution of individual unit activity, within the visual cortex and underlying white matter, during the course of the evoked potentials responses elicited by stimulation of the optic radiations. The purpose of this is to understand better the nature of the various components of the cortical response. This response is rather artificial and different from that elicitable with physiological stimuli; on the other hand, it is a typical and characteristic form of evoked potential. These investigators find that there is a correlation to the number of spikes recorded in the presence of a discharge during the course of a specific evoked surface response, and that such spike discharges may be temporally related to the various components of such a surface response. Thus, on the basis of the latency of the recorded spike, and the behavior of such potentials, when tested with double shocks and repetitive stimulation, they find that all such spikes may be divided into two groups, i.e. "presynaptic" and "postsynaptic." They find that all spikes temporally related to the first wave of the surface response, and almost all spikes firing during the second wave, are recorded from the white matter, and all of these have presynaptic characteristics. Those temporally related to the third wave have a more extensive spatial distribution, and are picked up in both the cortex and the white matter. Some of these behave as presynaptic, and others as postsynaptic. The spikes related to the fourth wave of the surface response, and the rare spikes associated with the fifth wave, are all of postsynaptic type, and are found only within the cortex.

A positive correlation existed between the presence of a unitary spike and the amplification of the surface response. Thus, for example, the spikes associated with the first or second wave appear when such waves have reached a critical size. Such spikes then remain stable, responding to every stimulus. A third wave spike, of a presynaptic type, behaves similarly. Post-synaptic spikes, related as seen above to waves 3, 4, or 5, also may appear when the first wave has reached a certain size above this threshold value. They are, at this time however, "unstable" and respond irregularly to stimuli of constant strength. The probability of their discharging is greater, the larger the amplitude of the late surface waves.

These authors conclude that this further confirms some of the current concepts of the various components of this five-wave surface response. They also suggest that this may resolve some of the conflict in regard to the nature of the third wave, in that they find both pre- and post-synaptic elements in this wave.

This study, along with those of Dr. Li in the Branch of Neurosurgery, further emphasizes the attempts being made to understand the slow cortical events in relation to single cell activity. These two investigators have also continued this unitary study in reference to the lateral geniculate nucleus, in an attempt to understand the effects of corticofugal and corticopetal impulses upon single elements of this nucleus. This was an attempt to see whether either specific or association cortical areas could exert any influence upon incoming sensory messages at a thalamic level. The visual and suprasylvian (which is an association area) of the cortex were chosen. These investigators state that the interest in this project was aroused by anatomical work which stated that, in the visual cortex, about three-quarters of the fibers are corticofugal, although their actual destination is presently unknown.

Tungsten microelectrodes were used in this study, and placed within the lateral geniculate nucleus, and cortical electrodes were used to monitor the arrival of the specific visual response. Single pulse stimulation of the optic tract, and of various points of the visual and suprasylvian cortex, were carried out. This project is still being analyzed. It appears, however, that a fairly large percentage of unitary spikes were activated by both optic tract and visual cortex stimulation. In each instance attempts were made to influence each recorded spike by cortical as well as by tract stimuli. In general these investigators found that cortical stimulation tends to inhibit the spikes elicited by the optic tract stimulation, but that stimulation of the optic tract tends to facilitate the spikes elicited by cortical stimulation. However, examples with opposite effects were found, and these investigators point out that some of the cortical effects are likely to be interpreted as the result of antidromic stimulation. However, the

extremely long latency of many spikes, elicited by cortical stimulation, strongly suggests that these must be orthodromically activated elements.

Dr. Paul Gerin, in his year of tenure in this laboratory, carried out microelectrode investigations of the mechanisms of the electrically induced epileptiform seizure, i.e. "afterdischarge", with Dr. Ajmone Marsan. The object of this study was to explore systematically, with microelectrodes, the cellular phenomena characterizing the onset, development and end of the electrical afterdischarge elicited by repetitive stimulation of the cortical surface, and to attempt an interpretation of the mechanisms at the basis of this self-sustained activity. This study was performed on cats, and stimulation was applied to the cortex of the suprasylvian gyrus with various parameters, (i.e. pulse duration, frequency, voltage, etc.). Pickup cortical electrodes were silver-silver chloride, for the slow surface events, and tungsten microelectrodes for the unit recordings. The movement of the latter was controlled by a hydraulic micromanipulator. Dr. Gerin found that repetitive stimulation, capable of eliciting an afterdischarge, is accompanied by progressive and characteristic changes in the spikes activated by each such pulse. The units tended to fire repetitively, and the amplitude of each successive spike decreased until the spikes eventually disappeared. In the absence of such changes, the development of an afterdischarge was improbable. During the EEG afterdischarge, the unitary spikes, generally absent at the very beginning, progressively appear, and their voltage and frequency vary inversely. As the rate of the firing slows down, the amplitude reaches, gradually, the prestimulation value, at which point the afterdischarge ends. This was interpreted as an expression of different membrane polarization levels, the characteristic ones consisting of an excess of depolarization levels, and of long-lasting repolarization. Since the electrical afterdischarge, elicitable with repetitive stimulation, is morphologically similar to the spontaneous discharges during epileptic attacks in humans, the understanding of the intimate mechanism at the basis of this particular type of self-sustained activity is of importance in the understanding of the physiopathology of seizures. This project has been completed, and the results were presented to the Atlantic City meeting of the American EEG Society, and will be reported in the *Archives Italian Biology*, in 1960.

Dr. Morillo and Dr. Ajmone Marsan are continuing the Branch's long-term study of thalamocortical mechanisms, and a comparison between the specific, association, and nonspecific systems. This continuing study can be seen, in previous years, through the studies of Enomoto, Ralston, and others associated with Dr. Ajmone Marsan in this interest. The purpose of this study was that of a unitary analysis in three thalamocortical sectors, by stimulation of different thalamic nuclei. The cortical areas were the suprasylvian (i.e. association area), as well as the primary visual cortex. Within the thalamus the following nuclei were specifically stimulated: The lateral geniculate, the nucleus lateralis posterior, and the nuclei belonging to the nonspecific system, i.e. centralis lateralis, centre medianum, the ventralis anterior. Systematic survey of depth probing with the microelectrodes was carried out by a hydraulic micromanipulator. Surface cortical electrodes were also used to monitor the gross evoked surface response.

To date this study has been done in over 50 cats, and observations concerning differences in latency, variability in responses, changes of unit activation by different stimuli, and the intereffects between different stimulations upon the same sensory unit are being made. It will still be several months before final results are obtained in this study.

The Branch of Surgical Neurology, during the past year, has admitted 231 patients on the ward, and 279 patients were seen in the outpatient area. In the operating theater, 108 major procedures were completed; of these, 50 were concerned entirely with the surgical treatment and investigation of functions of the cerebral cortex and deep nuclei of the brain in patients suffering from seizures. Forty-eight operations were performed for space occupying lesions which came to operation through either investigation of seizure patients, or patients referred from other programs, in particular the scanning program. There were 10 miscellaneous major procedures, the majority of which were performed for the relief of pain. Thirty publications were prepared during the period of this report.

556044—60——17

Twelve patients were referred from the National Cancer Institute and were studied and eventually treated by hypophysectomy or stalk section. The endocrine and antomical studies of this group may be seen in the report of Dr. Van Buren.

Studies were continued by Dr. Dekaban on disorders in the perinatal period, and by Dr. Li on unitary recordings of both central nervous system and peripheral structures, both *in vivo* and tissue culture.

The **Section of Neuropathology** continued its studies on tissue culture, hypothermia, immunochemistry, and histochemistry. Some studies in primate neurology continued, with particular reference to the deep nuclei of the temporal lobe, and studies of effects of radiofrequency energy in the megacycle-range upon the central nervous system. Psychological studies on patients with temporal lobe abnormalities are reported below by Dr. Lansdell and his colleagues, and investigations directed towards a technique by which the brain can be selectively cooled through shunting inflow blood through an appropriate type of cooling apparatus are also reported. Specifically, the Branch reports the following studies:

One hundred and forty-three patients with temporal lobe seizures were studied during the past year. Of those that came to operative treatment, six demonstrated a peculiar bony deformity of the middle fossa, consisting of bony spicules protruding into the overlying temporal lobe through dural defects. This deformity could not be related to inflammation or neoplasm, or to significant head injury. It was related to the electrographic abnormality. Dr. Baldwin points out that in extremely local lesions on the lateral surface of the temporal lobe, the language abnormality observed in more generalized temporal lobe diseases, and described in previous reports, was not present. On the other hand, one patient, with a small focal lesion on the mesial surface of the temporal lobe, exhibited such language characteristics to a marked degree. These investigators conclude that when the lesion is mesial or deep within the lobe, the language difficulty seems more marked and characteristic, and that it is unrelated to the duration of the lesion. Such patients indicate a satisfactory response to standard aphasia tests, but have great difficulty in describing relationships between space, time, and action. These investigators feel that there is no cerebral dominance in relation to this deficit.

Clinical observations seem to indicate the chief difficulty in memory abnormalities associated with temporal lobe disease is not in memory, per se, but in relating given facts to given periods of time. Perceptual aberrations associated with temporal lobe disease seem more marked when the lesion is mesial and when it is bilateral.

The phenomena of automatism associated with a seizure is being thoroughly investigated by means of time photography during a seizure period. During this period of time the patient is given numerous stimuli and his response recorded; his speech also is recorded during this time. Dr. Adamkiewicz has been studying this systematically and correlating the clinical findings with a systematic study of the autonomic concomitants carried on by Dr. Van Buren. Dr. Laskowski and Dr. Otenasek are engaged in the study of the relationship of cerebral dominance to the occurrence of automatism.

Dr. Van Buren, in his autonomic studies of 22 seizures in 20 patients, has found a stereotyped response in which blood pressure was seen to rise. The change in pulse, as it occurred, was towards the tachycardia. Early changes were commonly esophageal peristalsis and fall of skin resistance, with the vascular and respiratory changes occurring later in the sequence. Dr. Ajmone Marsan, in correlating the electrographic phenomena with these autonomic changes, finds about half a bilateral symmetrical aspecific metrazol burst associated with brief autonomic response. A sudden loss of voltage, or a tendency to increase in frequency of the record, was in all cases associated with overt-seizure activity.

A study on centrencephalic seizures was carried out by Dr. Van Buren and Dr. Mirsky of the National Institute of Mental Health, in which the patient's level of response was determined by a continuous performance test, and a simultaneous autonomic record was made in all these seizures. To date some 300 spike-and-wave attacks have been fully recorded, and it is hoped to draw correlations between electrographic features of the attack, the patient's response, and the autonomic characteristics. Isolated observations of interest during this study are that there is an infrequency of autonomic changes with the attacks. The pa-

tient may still be able to carry out rhythmic monotonous mechanical movement during such an attack, while he is unable to distinguish auditory or visual stimulus.

Seizure studies in primates, and in particular to the frontal cortex of the chimpanzee, are continuing by means of penicillin lesions. It is interesting that the electrographic seizure spreads in an almost concentric pattern across the ipsilateral hemisphere and then seems to cross the opposite side, spreading from mesial to lateral. The penicillin lesion was placed in the intermediate frontal region. It was of interest that bilaterial electrographic abnormalities, which began in one frontal cortex, could result in a unilateral focal motor seizure. These studies on seizure mechanisms resulted in four publications, as listed under project NINDB–43(c).

In the operating room, per se, functional representation in the temporal lobe of man and higher primates has been continued by Dr. Baldwin and his colleagues. Twelve unilateral temporal lobectomies were done on patients, and the general effects of unilateral lobectomy in the human are still not clear. Depth electrode studies in the mesial temporal region, by Dr. Norris, have reproduced behavior which is reminiscent of human automatism. Dr. Baldwin feels in his patients there is a change in mood and language, as listed above, and there may be some change in libido, and there may be progressive weight gain. In chimpanzees, during the past year, the effect of unilateral ablations and bilateral ablations of the temporal lobe areas has been studied. The parasylvian region in the chimpanzee has demonstrated a motor representation of tongue and lips. Bilateral excerebration of the Broca's area, i.e. the third inferior frontal convolution, in the chimpanzee does not materially effect the communication pattern. The chronic effects of bilateral temporal lobe ablation have continued, and Dr. Heinrich Klüver, at the University of Chicago, has cooperated in these studies, and he was unable to see any stigmata of his "syndrome" in such animals. This Branch now has six chimpanzees whose bitemporal ablations have now been studied for five years. Their development continues within the norms established by other investigators.

Dr. Baldwin and Miss Lewis are continuing their study of hallucinogenic substances, and psilocin and various isomers of lysergic acid are being tested. There appears to be an apparent similarity between psilocin and LSD–25, and it would appear that continuous administration of either drug provides a considerable resistance to further administration of either drug.

Dr. Baldwin, Dr. Bach, Miss Lewis, and Dr. Klatzo are continuing a correlative study of the effects of radio frequency energy on primate brain mechanisms. As of now, 50 Macaca rhesus monkeys have been exposed to radio frequency ranges of 200 to 400 Mc. The energies are transmitted to the head by a ground-to-air transmitter through means of an antenna placed in the roof of a resonating cylindrical cavity. The antenna in this cavity is adjusted to lie directly above the animal's head, which was positioned at the lower pole of the cavity. Below the base of the skull, the animal was screened from radio frequency energies. Nineteen phantoms containing Elliot's solution, gelatin, Ringer's saline, and other suitable test media were also positioned so as to imitate the critical head positions. The amplitude of the signal received at the head area was monitored by a probe, and the signal was continued long enough to raise the temperature of the test medium 5° C., when frequencies of 200 to 400 Mc. were used. However, it was only between 380 and 390 Mc. that clinically discernible effects were seen, though the temperature elevation was seen over 200 Mc. During this 10 Mc. range of 380–390, the animal became increasingly drowsy, did not respond to pain, but did respond to light and noise in the usual manner. At critical frequency, the animal will arouse from anesthesia when the generator is on, but returns to the anesthetic state after the frequency is turned off. If such a frequency is continued at the critical level, i.e. 380–390 Mc., alterations of pupillary response, paradoxical pupillary response, disorganization of eye movements, nystagmus, grimacing, autonomic changes, and finally generalized seizures will occur. If the animal is carried to this latter stage, recovery is unlikely and he usually expires. Some animals develop focal neurological signs following long exposures at critical frequencies. A long exposure here is defined as an exposure longer than three minutes. Such focal signs consist of facial palsy of the central type, loss of pain and touch in areas sup-

plied by the fifth cranial nerve, weakness of one leg or arm, transient Babinski responses, ataxia, and continuing nystagmus. Such signs, although transient, could continue for periods of 4 to 48 hours after exposure.

The histological and histochemical analysis of the brain was carried out by Dr. Klatzo, in the Laboratory of Neuropathology, and in this study sodium fluorescein was used to study the permeability of the blood-brain-barrier, and various cellular histochemical and histological procedures were employed. Dr. Klatzo finds, in the acute experiments where the animals are sacrificed within a few minutes after the exposure, that changes in the blood-brain-barrier permeability can be found in the pons, medulla and extending through the white matter into the cerebral hemispheres. Sometimes such changes are localized lower and involve the dorsal areas of the cervical cord. The histological changes are confined to a striking tigrolysis in the tegmental neurons of the pons, medulla and the dentate nucleus of the cerebellum. No neurofibrillary changes can be seen. In the chronic experiments only a few animals showed changes in the blood-brain-barrier, and they were localized in the dorsal portions of the cervical cord. In a few animals, changes in the medulla and pons were such that small cystic cavities with well-preserved neurons in the vicinity were found.

These findings suggest that there may be a frequency specific effect with radio frequency energy of certain characteristics when applied to the primate brain, provided such a brain is placed in a resonating chamber so that the sinusoidal waves may actually algebraically summate at a given focus. This technique may be useful in the future in that it can affect the arousal systems and other brain stem mechanisms, and yet leave the test animal apparently unharmed otherwise.

Dr. Van Buren is continuing his studies on hypophysectomy, and during such surgery is making physiological observations in reference to connections of the human temporal lobe, the response of the cortex to hypothermia, autonomic responses, and clinical and endocrine effects of hypophyseal stalk section in man. He is correlating these with the anatomical evidence found at postmortem. A total of nine pituitary stalk sections were carried out for palliation of metastatic breast carcinoma. The clinical experience, however, has been discouraging, and the patients have shown a considerable morbidity. A continuation of this series is not contemplated. Such patients demonstrate marked sensitivity to water intoxication, not related to the intake. In two such cases routine I.V. fluids were given, and the patients became unconscious and had convulsions apparently due to water intoxication. Following this experience, all subsequent parenteral fluids were stopped following surgery and patients allowed to take only what they desired by mouth. Although such disturbances of water metabolism proved reversible, the comparison between the clinical course of those subject to pituitary destruction and stalk section was striking. In the latter case, the patient's recovery from operation was rapid. The evaluation of the hormonal and tumoral response to hypophyseal stalk section as opposed to pituitary removal is still being studied.

Dr. Van Buren is continuing his collection of postmortem material from hypophysectomy cases, and calculating the total cell populations of the various hypothalamic nuclei, and has noted a 6–8 X decrease in the number of cells in the supraoptic nucleus after such procedures. Patients undergoing such hypophysectomy, at the time of the operation, were subject to exploration of the orbital surface of the frontal lobe, the adjacent temporal lobe, and the ventral aspect of hypothalamus by electrical stimulation. Records were made, at this time, of the respiration, blood pressure, pulse rate, esophageal and gastric motility and peripheral circulation with the plethysmograph. Nine of these patients were maintained with hypothermia and three with normothermia. With one exception, there was no evidence of disease of the nervous system. Under these conditions, the predominant electrocorticographic frequencies usually lay in the alpha range or faster. Hypothermia appeared to protect the cortex from after-discharge from repeated electrical stimulation while after-discharge with the same parameters appeared in one quarter of the stimulations at normothermic levels. In nearly all patients, both normothermic and hypothermic, cortical stimulation was followed by a depression of voltage of fast activity and an augmentation of voltage of slow activity, which was compared with the spreading depression of Leao. Hypotension under light anesthesia, in both normothermic and hypo-

thermic patients, induced a fall in the voltage of both the slow and fast activities. In two cases the addition of Fluothane resulted in a greater depression of electrocorticographic activity with progression to intermittent activity, and in one case to electrical silence.

Dr. Van Buren has also continued his studies on the anatomical connections of the human temporal lobe in cooperation with Dr. Paul Yakovlev at Harvard University. Two contrasting lesions to the human temporal lobe were studied by whole brain serial sections and template reconstruction methods. Lesion in the posterior portion of the temporal lobe resulted in a subsequent degeneration of the posterior portion of the nucleus lateralis posterior and nucleus medialis dorsalis, and in the medial half of the lateral geniculate body, while anterior lesions caused degeneration in the lateral half of the lateral geniculate body. In the medial geniculate body, the posterior lesion of the temporal lobe resulted in degeneration of the rostro-lateral portion of this nucleus, while the anterior lesion caused degeneration in the caudomedial portion. Degeneration in the pulvinar was caused by lesions both anteriorly and posteriorly, although with the posterior lesion there was greater degeneration in the medial part of the pulvinar.

Tract degenerations were also studied in the tapetum, and Dr. Van Buren has outlined these in his project. Degeneration was also found in the anterior commissure, the uncinate fasciculus, the ventral thalamic peduncle, and the fimbria-fornix after anterior temporal lesions. Five other cases with focal destructive lesions have been accumulated by Dr. Van Buren, and are undergoing serial sectioning at this time. These anatomical studies, after a prolonged followup from focal lesions, are of utmost importance in understanding the anatomical ramifications in man.

Dr. Van Buren is also continuing his studies on the functional anatomy and pathology of the human visual system, as described in the previous Annual Report, and he has now arrived at a satisfactory method for two-dimensional retinal reconstruction. By projection of his photographic enlargement, Dr. Van Buren now has stretched the circumference of the closed segment of the retina to over a meter, and tracings of lesions at this magnification can be measured with relatively small percentage of error.

Finally, Dr. Van Buren is continuing his studies on involuntary movements, in which he is still analyzing, in postmortem material, the accuracy of Horsley-Clark device which he has himself constructed. He has completed a visit to clinics abroad and in this country to compare the technique he anticipates using here with those used elsewhere. The proposed course of this project will be to study those diseases in which stereotaxic intervention is indicated on therapeutic grounds. Such patients will be done by photographic records and multiple flash stroboscopic photographs. It is hoped that with the use of accelerometers a means may be found for simple graphic recording of the involuntary movements, which may be correlated at the same time-base as other features, such as EEG, and autonomic recording. These accelerometers are now being constructed by the central instrument facilities of the National Institutes of Health.

Dr. Choh-luh Li, with the aid of Dr. Shelley N. Chou and Dr. Joseph Miller, is continuing his studies on cortical intracellular potentials and on cells grown in tissue culture. In studying afterdischarge produced by continued stimulation, Dr. Li arrived at what appears to be the identical conclusions as Dr. Ajmone Marsan, using the tungsten electrode. In Dr. Li's study also, during the so-called epileptic afterdischarge, depolarization occurred and restoration of the resting potential periodically took place. If the depolarization continued to grow, then the spike action potential disappeared. There was also an accentuation of oscillation of potentials when spike activity was absent. Thus, this appears to be an intracellular confirmation of Dr. Ajmone Marsan's thesis from extracellular recordings using the tungsten electrode.

Dr. Li also has made an attempt to determine the response in a single cortical neuron from a closely approximate cortical stimulus. In this the somatosensory cortex of the cat was chosen. The micropipette was introduced into the neuron, and single shocks were applied to the suface of the cortex not more than 5 mm away from the recording electrode tip. It has been stated in the past that the initial surface-negative potential in response to a weak stimulus was the result of activation of superficial elements, probably dendrites, and that the surface-positive potential in response to strong

stimulus was produced by deep neurons in the cortex. Dr. Li's investigation shows that this is not necessarily so, and that the refractory period of the cells under direct stimulation and the synaptic activation of these cells enters into the response. Dr. Li plans to pursue this further with conditioned volleys initiated from other structures of the central nervous system.

Drs. Li, Chou and Ortiz also carried on a study of the inhibitory interneurons of the cerebral cortex in the somatosensory and visual areas. The method employed was that of stimulation of peripheral nerve, the lateral geniculate body or a corresponding point to the opposite hemisphere, while cells in the somatosensory and visual cortex were impaled with micropipette electrodes. Hyperpolarization of many cells was recorded, most often from the visual cortex and the size and duration were similar. When the lateral geniculate body was "poisoned" with gamma-aminobutyric acid, and the responses recorded from the surface of the visual cortex, it was found that such responses were enlarged, suggesting that this chemical agent might have suppressed the inhibitory interneurons. This particular work will appear in a book titled "Conference on Inhibition of Central Nervous System and Gamma-aminobutyric Acid", published by the U.S. Air Force.

In a study of the synaptic activation of cortical nerve cells, Drs. Li and Chou introduced microelectrodes into cells of the somatosensory cortex, while a contralateral sensory nerve was stimulated. They found that about 1 msec. after the onset of the initial surface, positive potential was recorded from the cortical surface, and cortical neurones would respond with growing depolarization and spike responses were initiated from a level of about 10 mV. Subthreshold depolarization was found to be composed of small potentials, and these small potentials could probably be considered to be units of postsynaptic potentials. A synchronous discharge of such units gave rise to a depolarization of critical size from which the spike potential could be generated. Oscillating potentials were found to be rhythmic and graded, and probably responsible for the rhythmicity of the spike activity. The oscillating potentials did not require a continuous presynaptic activity, and could be considered as an intrinsic factor with which the excitability of the nerve cell is maintained. Finally, they found that tubocurarine does not impede the synaptic transmission in some elements of the nervous system.

In their work of recording from single cells of tissue culture, Drs. Li, Klatzo, Chou and Miller, report that the resting DC potential of the cultured nerve cell was found to be comparable to that obtained from nerve cells *in vivo*, i.e. in the level of 70–80 mV. The only difference was perhaps the duration of the action potentials, which were found to be relatively prolonged. A study of individual glia cells in tissue culture is now being undertaken in an attempt to confirm and continue some of the work of Tasaki, et al.

Dr. Dekaban is continuing his investigations of the etiology, pathology, and clinical manifestations of cerebral palsy and epilepsy during childhood. In the latter disease, he is doing a combined survey of the electroencephalographic abnormalities and clinical findings in seizures during infancy, in combination with the Mayo Clinic. At the present time 160 patients have been under study. Dr. Dekaban has just completed a book, *The Neurology of Infancy*, published by Williams and Wilkins, which summarizes his basic studies in the embryology and developmental neurology of the perinatal period. This is the eighth book or research monograph to be completed by the Clinical Unit, since its activation.

In cooperation with the National Naval Medical Center and Walter Reed Army Hospital, Dr. Dekaban is continuing his studies which now cover 4,156 pregnancies, in relation to the course of birth in relation to neurological abnormalities in infants, and pathologic lesions in products of abortion. This has also been evaluated against the maternal age. Of these 4,156 pregnancies, there were 312 abortions, 22 still-births, 59 neonatal deaths, and 10 infantile deaths. Surviving full-term infants with complicated gestation and delivery, or abnormal clinical state at birth, were 921, as against 2,567 normal infants with normal gestation and delivery. Surviving premature infants with complications at gestation and/or abnormal clinical state at birth, were 103; surviving premature infants with normal gestation and delivery, and normal clinical state, were 153. It can be seen from these figures the complications of gestation and delivery, or abnormal clinical states, exist in one out of three surviving full-term infants, and one

out of two premature infants. A complete study of this large amount of patient material is obviously a long-term one, but from the preliminary statistics listed by Dr. Dekaban, it should be rewarding.

Dr. Dekaban has now accumulated 23 brains from children who suffered acute birth injury or who were in chronic phase of cerebral palsy, and a careful evaluation of the pathology and pathogenesis of acute birth injury is made on the material consisting of 15 of these brains. It appears that correlation of some of these acute hemorrhagic lesions with clinical findings of other patients may form a basis for clarification of certain instances of cortical blindness and cerebral diplegia.

Dr. Dekaban and Dr. Baird are continuing their long-term study also on the offspring born to diabetic mothers, and now have the outcome of 234 pregnancies in 48 diabetic women, and 249 pregnancies in 48 normal controls. The percentage of abortions and previable deaths in the diabetic sample was 29.9, in the prediabetic sample, 20.5, while in the normal controls, 12.4. Stillbirths were similarly high in the diabetic and prediabetics as compared to the normal controls. Neonatal deaths accounted for 8.3 percent of all pregnancies in diabetic women, as against 3.6 percent in the sample of normal controls. Of the infants surviving in the sample of 157 diabetic pregnancies, there were six abnormal surviving offspring. In the normal control sample, of 249 pregnancies, there was only one abnormal surviving offspring; a difference of 7.6 percent in the diabetic as contrasted to 0.4 percent in the control. Three of these six abnormal surviving children had severe congenital malformations; two had mental deficiency, and one epilepsy. Of 41 offspring who were stillborn or died during the neonatal stage, of diabetic women, 16 had comprehensive postmortem examination. Cerebral birth injury was found in two; pulmonary hyaline membrane disease in eight; congenital malformations in three. Three publications have resulted from this study; Dr. Dekaban's measurements of external and internal orbital distance in males and females from birth to adulthood are included in these.

In his preparation of the horizons of the normal development of the central nervous system in mice, and in experimental production of congenital malformations of the central nervous system in these animals, Dr. Dekaban completed an atlas which is now in current use in this laboratory. Of the irradiated mice used, 98 litters were obtained, and approximately 10 percent of these had major abnormalities, and about 25 percent minor abnormalities.

Dr. Lansdell, in the Section on Psychology, is continuing his psychological evaluation of temporal lobe disease, and he suggests from his studies a complex verbal facility is maintained by the integrity of the dominant temporal lobe. The number of cases to date, however, in some comparisons is rather small, and results may be due to chance variations until this series is enlarged.

The Section of Neuropathology has carried, as one of its main interests in the past year, the investigation into some of the basic features of permeability and metabolism of various cell types grown in tissue culture. For this purpose, the uptake of nontoxic neutral red dye and several proteins, such as albumin, globulin and fibrinogen, labeled with fluorescein isothiocyanate according to the Coons' technique, has been studied under various experimental conditions. The study is utilizing both nerve and muscle tissue, and cultures of living cells are incubated with varying solutions of neutral red or labeled proteins. The observations are made at time intervals, the cultures being subjected to various experimental conditions. The cells are observed in the brightfield, phase and fluorescence microscope, as well as photographed by time-lapse cine camera. The uptake of neutral red appears to occur only in living cells in good physiological condition, and disappears from cells which are dying, and is obviously not taken up by dead cells. There appears to be a marked difference in the ratio of uptake in the various cell types. Macrophages thus are filled with granular inclusions of neutral red in an extremely short amount of time. Astrocytes show conspicuous granules of the dye in the processes and in the cell body after an interval of time. Nerve cells and muscle fibers, however, take up neutral red in a limited way, and only at the terminal expansions of such cells which are provided with undulating membranes.

The uptake of the various proteins resembles in many ways that of neutral red. Again there is a similarly different ratio of uptake among the various cell types. The striking difference is the

disappearance of such proteins within one hour from the macrophages, whereas the astrocytes preserve the proteins throughout the time of observation, i.e., 24 hours. The ratio of uptake of the various proteins is correlated generally with the size of molecule, being highest for albumin and lowest for fibrinogen. The effect of low temperature on this has also been studied, and under low temperature there is conspicuous reduction in the uptake both of neutral red and of proteins. It is of interest that under hypothermic conditions the macrophages hold the fluorescent proteins and there are only a few protein inclusions in the astrocytes, after 6 hours. This picture corresponds to the normothermic picture at 30 minutes. It is suggested by these observers the initial uptake of neutral red and proteins takes place by pinocytosis, "drinking by the cells", and that apparently macrophages can metabolize such proteins and extrude them within a comparatively short amount of time.

The project concerning a quantitative study of the precipitin reaction, by Dr. Miquel, reported in last year's Annual Report, and also discussed in the Branch of Medical Neurology, has now been completed. The labile proteins move rapidly on the electrophoresed paper, but the precipitin stays at the point of application, and subsequent eluting allows a semiquantitative analysis. This will be reported in the *Journal of Immunology*.

Dr. Klatzo and Dr. Laskowski have also finished a project on the effects of hypothermia on injured and normal brain tissue. They find a striking difference between the normothermic and hypothermic animal, as revealed in the behavior of the blood-brain-barrier. Thus, at 24 hours, when edema is maximal in the normothermic animal, the area of altered blood-brain-barrier in the hypothermic animal is diminished. The response of glia cells at comparable periods in the hypothermic group, as compared to the normothermic, shows a marked diminution. These studies carry implications in the use of hypothermia in the treatment of severe head trauma with brain edema, and have resulted in three publications in the past year.

Studies of hypothermia in neuroanesthesiology, however, have been continued by Dr. Pritchard, Dr. Bucknam, and Dr. Chou, in that these investigators have now successfully worked out a technique for selective brain cooling so that the brain could be lowered to temperatures of 12 to 15° C., while the esophageal temperature was maintained at 25–30° C. Since the cardiac effects of hypothermia are the main limiting controls on this technique, it would appear that an isolated cooling of the cerebral circulation would obviate this undesired reaction and still allow the neurosurgeon the benefit of selective hyperthermia to the head. Similar studies are being carried out, in Oslo, by Dr. Christianson, and Dr. Baldwin and Dr. Christianson are now cooperating on this problem.

Dr. Pritchard, during his tenure of the past year with Neurosurgery, has continued his studies on the hypertonic urea solution on intracranial pressure, in which the desired effect of reduction of cerebral edema and intracranial pressure has been uniformly observed, both in the operating room and on the ward. No serious side effects have been noted, with the exception of two cases in which there was a transient ischemic syndrome in the extremities in which the urea was administered by vein. The cause of this latter peculiar syndrome is still not understood, but the value of urea is now becoming generally accepted in most neurosurgical clinics. Dr. Pritchard has also carried out a combined anesthetic study with the utilization of succinylcholine in patients who must cooperate with the surgeon while under local anesthesia.

Dr. Norris, Dr. Klatzo, and Dr. Baldwin are continuing the studies initiated 4 to 5 years ago by Dr. Charles Wood and Dr. Lawrence Frost on some of the inner connections between the amygdala of the two sides of the brain. Chronically implanted electrodes in cats and monkeys were utilized in this study. Such depth electrodes were placed stereotactically under moderate to deep hypothermia, and as many as nineteen electrodes were placed in a single preparation. Stimulation of one amygdala resulted in a stereotyped behavioral automatism, with a characteristic spike-and-wave afterdischarge, which is recorded both ipsilaterally and (after a few msec. delay) contralaterally. Both the automatism and afterdischarge were very sensitive to anesthesia. Bilateral sequential or simultaneous amygdala stimulation sometimes enhanced or, in some cases, depressed the effect which would have been explained on the basis of algebraic summation. This study gives some insight to the fact that the amygdaloid com-

plex may be one of the sites of automatism. The precise nucleus in such a complex remains yet to be discretely localized.

The **Branch of Ophthalmology** reports, during the current year, the admission of 177 new inpatients, and 75 new outpatients, with a total of 7,491 inpatient days. This Branch has continued to be the largest consulting Branch of the Institute, with 838 consultations seen during the past year. Fifty-nine surgical operations were performed during the current year.

Dr. von Sallmann and his associates report the following specific projects:

Dr. van Alphen has continued his study as to the basic factors underlying refraction abnormalities. The theory most often quoted, and/or defended, is that of Steiger on the origin of refraction anomalies. This theory gives no adequate explanation for the mechanism of emmetropia or ametropia. Dr. van Alphen has approached this by a factor analysis of the five optical elements based on the data of Stenström. Dr. van Alphen's approach was based upon the fact that the degree of tension in the choroid must counteract the intraocular pressure, and is determined by measuring the pressure in the suprachoroidal space, as described in the past year; secondly, the relative tension in the choroid by direct strain gauge measurement at various sites before and after sympathetic and parasympathetic stimulation; and third, the effects of the relationship of intraocular pressure as against scleral elasticity.

Pertinent to Dr. van Alphen's theory is his consideration that the choroid acts in essence as a sheet muscle, and the results he has obtained on correlative pressure changes in the subscleral space and the anterior chamber examined with a variety of techniques on the exposed choroid, strongly suggests that the assumption of this function of the choroid is correct. The interrelation of these five factors ultimately combines to form three oblique correlations between the five optical elements in the human eye. Dr. van Alphen proposes to continue this study into the basic abnormalities of refraction by further investigations into the tension of the choroid and its effect on the sclera by strain gauge measurements on the eye *in situ* and isolated. From this he hopes to complete a concept as to the possible mechanisms of emmetropia and its aberrations.

The Unit is continuing its basic studies as to how light is perceived at a retinal level and subsequently transmitted as a nerve impulse. Such studies in electrophysiology of the eye are also being applied in the utilization of the electroretinogram and parametric light sense studies, both in animals and man.

Each year the Clinical Director, in his report, makes an attempt to point out what seem to be outstanding contributions made during the past year by given Sections within the Clinical Unit. The work previously described in the Section of Neurochemistry, by Dr. Tower and his associates, and the work described now, by Dr. Fuortes, Dr. Tasaki, and Dr. Rushton, are both outstanding in their approach, their logic, and the probability of increased understanding of basic mechanisms in their respective fields, during the current year.

Dr. Fuortes is continuing his study of changes evoked by light in the eyes of invertebrates, using as his model the Limulus. Part of this work was done at Woods Hole, where fresh animals could be obtained daily; part was confirmed at spinal cord level in cooperation with Dr. Karl Frank, in the Laboratory of Neurophysiology, in order to determine whether certain properties seen in the Limulus are also common to other structures. The techniques involved, again, were the utilization of micropipettes, and in cases of work done in Dr. Frank's laboratory, two pipettes were introduced into the same cell, and various responses studied with the voltage-clamp method.

Three major aspects have been investigated during the past year: (1) the site of the impulse initiation; (2) the subliminal responses to light; (3) the study of hyperpolarization potentials elicited by light in the fish retina.

Dr. Fuortes and his colleagues have found, in the past year, that the resistance of the nerve cell membrane in the Limulus eye decreases during illumination. Spikes recorded during illumination are smaller than those recorded in comparable conditions in the darkness. Dr. Fuortes feels that this could be explained, assuming that the resistance of the soma membrane does not decrease very drastically during impulse activity. A direct measurement of the membrane resistance of the soma in the Limulus could not be obtained, but results obtained on spinal motor neurons with the voltage clamp do support this assumption. To explain this apparent discrepancy, it may be

556044—60——18

thought that the mechanism of impulse generation in the soma is different from that in the axon where membrane resistance decreases by a factor of 500 or so, or, as an alternative, that this impulse activity does not involve the cell soma or involves only a part of the cell soma.

Experimental findings obtained so far, in this Section, are in agreement with the second hypothesis. The latter hypothesis, in particular, is supported in the Limulus by experiments on inhibition. Thus, following excitatory illuminations, the frequency of firing bears a strict relation with the membrane potential of the soma (note here same findings by Drs. Ajmone Marsan and Li in the cerebral neuron), but inhibitory illumination decreases the frequency of firing without perceptually changing the recorded membrane potential. This is what one would expect if both the place of origin of the rhythmical impulse and the site of inhibitory action were at a distance from the soma. Dr. Fuortes believes, in fact, that the inhibitory synapses are not on the soma but on the axons, at 100–200μ from the soma. He concludes, in the eye of the Limulus and motoneurons of the cat, that impulses originate in the axon.

As may be recalled from previous Annual Reports, Dr. Fuortes' investigations led him to the conclusion that a chemical substance liberated by the photoreceptor is responsible for evoking the nerve cell depolarization. He has continued this work now on the study of the properties of transmission from photoreceptive structures to nerve cells in Limulus by means of an analysis of subliminal responses to dim lights, and has found that, following dark adaptation, a steady dim illumination produces an irregular series of transient depolarizing pulses. He relates this to much the same type of information found at the motor endplate, and feels it reasonable to think that transient depolarizations recorded during dark adaptations are, in fact, due to discrete liberation of "droplets" of transmitter substance. The possibility that each "droplet" is liberated following absorption of a single quantum of light is now being investigated, and Dr. William Rushton, from Cambridge, England, has taken over this extremely fascinating facet of visual physiology.

Dr. Tasaki completed his work on the fish retina, and confirmed Svaetichin's findings that hyperpolarizing potentials are elicited by light in the fish retina, and that such potentials could be obtained in the absence of a negative DC shift. Electrical currents through the microelectrode do not affect the size and properties of these potential changes, and from these results he concluded that the hyperpolarizing potentials originate in a large space which could be either a large cell or an enclosed extracellular space.

Dr. Rushton also is carrying on his studies on regeneration of visual pigments, using the technique he developed in Cambridge, in which light is received from the fundus oculi into an optical arrangement and analyzed by photoelectric equipment. This study is being done on normal human controls.

Using the pure-cone retinae of the American tree squirrel, Drs. Tansley, Copenhaver, and Gunkel have studied the spectral sensitivity, the dark adaptation, and the flicker fusion frequencies of various members of the squirrel family. They have obtained evidence that certain species of squirrel have only cones but not rods, by demonstrating the absence of a shift in the peak luminosity of the spectral sensitivity curve from light to dark adapted states. These high critical fusion frequencies and the absence of a type of spectral sensitivity curve would suggest the presence of rhodopsin. Two "humps" were, however, found and this was thought to represent at least two photo-sensitive cone pigments. The maximum of these peaks were 490 mμ and 535 mμ. In a further study, after destruction of the retinal blood vessels so that a retinal degeneration was obtained (in some animals limited to the entire inner retinal layers down to and including part of the bipolar layer) there was noted a marked reduction of the electroretinogram which, to these investigators, confirmed the hypothesis that the positive potentials originate on the bipolar cell layer while the negative retinal potentials arise on the deeper retina.

The use of the tree squirrel should be most helpful in providing information on the responses of the purely photopic mechanism uncontaminated by any scotopic mechanisms. Dr. Tansley's previous investigations have already demonstrated the reactions of the retina of the tree squirrel are in many ways unlike those of the more usual mixed rod and cone retina of which the human is

an example. Thus, the tree squirrel retinal spectral sensitivity curve is much narrower and appears to reflect the activity of only one of the three postulated mechanisms for color vision, i.e. the "green" mechanism. The ground squirrel, on the other hand, apparently has two, a "blue" and a "green". It is believed, from these studies, that the photopic and scotopic responses in man can be separated by means of their reactions to flickering stimuli. The squirrel responses to flicker have not as yet been systematically studied, but it is hoped that this will be done in the near future.

In the clinical counterpart of this work, Drs. Copenhaver, Gunkel, Goodman, and Dodt have continued their studies of the physiology of the cone and rod vision of patients with various forms of retinal degeneration and color defects, by means of elaborate psycho-physical testing and spectral sensitivity curves, in addition to the electroretinogram.

During the past year, for example, these investigators have isolated a cone monochromat patient who exhibited normal visual function except for a complete absence of color discrimination. Scotopic function, as tested by the adaptometry and perimetric light sense studies, appeared normal. Photopic function, at least that mediated by red sensitive cones, was significantly diminished as demonstrated by the investigation of the electroretinal spectral sensitivity and perimetric light sense studies. This information suggested to these investigators that part of the color loss was due to a retinal defect but did not contribute to the question as to the site of the other abnormalities which must have been present to give so severe a color deficiency.

Dr. Dodt, while with the group, continued his studies on pigmentation of the retina as related to the spectral sensitivity curve, and chose albinos, Caucasians, and Negroes as his subjects. It was found that where the retinal pigment is very light, there is an increase in red sensitivity which reflects the absorption spectrum of blood. Stimulation of the retina by scleral illumination also altered the spectral sensitivity according to the absorption spectrum of blood. These studies indicated that the pigment in the coats of eyes does not itself alter the electroretinal spectral sensitivity curve, but when the pigment is nearly absent or reduced the unmasking of the choroidal blood vessels results in a pronounced increase in red sensitivity. Thus, the pigmentation of the retina must be considered in future studies utilizing the method of spectral electroretinography.

Two children with infantile amaurotic family idiocy, and others with late infantile and juvenile stages of amaurotic idiocy, completed the clinical evaluations of this disorder, in that the electroretinographic potentials were found to be abnormal in the late infantile and juvenile form, but normal in the infantile form. This confirms the pathology described in the retina by the late Dr. J. Godwin Greenfield, and, together with the previous studies of Copenhaver and Goodman, completes the clinical evaluation of this disorder. It appears that in the late infantile juvenile form of amaurotic idiocy, the electroretinogram could be a sensitive indicator for the progression of the disorder.

In all of these studies, Dr. Gunkel has been primarily responsible in designing and constructing devices as needed. In particular during the past year he has worked on the modified adaptometer, and the various optical devices necessary for Dr. Tansley's work listed above, including sector discs and shutters for providing photopic stimuli of different duration; flicker rates and on/off ratios; and the apparatus needed for an intense bleaching light which could be changed in color and introduced into the pupil without interfering with the entry of the stimulating beam, for Dr. Rushton's studies on the regeneration of rhodopsin. Dr. Gunkel has also made frequent measurements of the spectral emission of the high-pressure Xenon lamp and measurements of brightness of the stimulus beam.

The Branch lists three projects, correlating various aspects of knowledge, concerning function of the cornea both in health and disease. Dr. von Sallmann reports his studies for demonstrating possible injuries to the corneal endothelium, in which it was necessary to develop a technique in which an entire cell population of this tissue could be studied. This is of great importance in clinical ophthalmology, and particularly in the so-called Fuchs' dystrophy. It was of even more importance, however, with the recent demonstration that chymotrypsin, when injected into the posterior chamber of the eye, has greatly facilitated the

removal of the lens at operation. This was found accidentally in other Centers in which chymotrypsin had been injected into the vitrous chamber and subsequent subluxation of the lens was found to occur. Its use now into the posterior chamber of the eye, for the subsequent removal of cataract, promises to be a possible great advance in the removal of cataracts. Accordingly, the Academy of Ophthalmology and Otolaryngology asked that certain Centers undertake a study as to the effects of this substance on other structures of the eye. In this study on corneal endothelium, chymotrypsin was utilized by Dr. von Sallmann, and the report to the Academy may be found in the *Trans. Am. Acad. Ophth. Otol.*, now in press.

Dr. von Sallmann has found, by this method, than in contrast to the generally held opinion that karyokinetic cell division does not occur in the corneal endothelium of the adult animal, mitosis does take place, and the mitotic index of an average of 15 dividing cells per population is similar to that determined in the pre-equatorial zone of the lens epithelium. He found that the injection of 0.9 percent sodium chloride solution into the anterior chamber leads to endothelial damage indicated by gaps in the regular cell mosaic, and by cell degeneration and a marked increase of cell division in the area of the lesion. This injury is even more extensive when the enzyme alpha-chymotrypsin (which, as has been pointed out above, has been recommended for zonulolysis) was infused for several minutes. The process of wound healing is initiated by the formation of a fine and dense fibrin net covering the defect in the endothelium and by an ingrowth of cell elements from the margin of the wound. In his report to the Academy, Dr. von Sallmann indicates, however, that such lesions may be transitory, and do not of necessity contraindicate the use of chymotrypsin for cataract removal.

The enzymatic systems of the cornea continued to occupy Dr. Kuhlman up to the time of his departure. Dr. Kuhlman has turned to other connective tissue substances to find if they vary from his previous report in 1958 on enzymatic studies of the cornea. In this respect he used the collagen of the developing epiphyseal plate. He found that the primary spongiosa and cartilage cells contained more total phosphorous, solids, and acid soluble material than unorganized cartilage cells; that the activity of both lactic dehydrogenase and phosphoglucoisomerase was higher in organized cartilage area and lower in unorganized cartilage area; and that malic dehydrogenase activity parallels calcification. Glucose-6-phosphate dehydrogenase doubled in activity as the primary spongiosa developed. Alkaline phosphatase activity increased with each advancing stage of calcification. There was, in all, a general increase in enzyme activity with the maturation of the animals.

This is, in essence, in agreement with what was previously found in the cornea by Dr. Kuhlman, in the last Annual Report.

Dr. von Sallmann and Dr. Paton also have described a clinical abnormality, of a familial type, of the conjunctiva and the oral mucosa, which is that of a dyskeratosis, which occurs in a triracial isolated population in Halifax County, North Carolina. This was done in combination with Dr. Witkop, of the Dental Institute, and Dr. Graham of the Department of Pathology at the University of North Carolina. It appears that the disorder is inherited as a simple Mendelian dominant, and the eye changes occupy the perilimbal conjunctiva, and consist of a firm granular semitranslucent proliferation, which is raised above the surface of the surrounding tissue. The shape of the lesion varied, but most frequently it assumed a triangular form or horseshoe-like configuration. The surrounding part of the conjunctiva showed a rather characteristic vascularization. Serious corneal complications were rare, but sometimes dense membranes of proliferated tissue covered the cornea for periods of time. There was, on microscopic examination, epithelial hyperplasia, which was accompanied by degenerative changes of the dyskeratotic type and signs of acanthosis. Degenerative changes of the cell nuclei were common, and with the Giemsa stain irregular light blue structures could be demonstrated near the nucleus or its remnants. The tunica propria was usually free of major pathology, but was sometimes the site of extensive round cell infiltration.

Such patients, also, during physical examination, were noted to have a change in the buccal mucosa, coexisting with the eye lesions. This was demonstrated by the Dental Department to also be a dyskeratosis.

Six projects of the Branch were concerned with

either basic or clinical aspects of intraocular pressure and the possible ramifications of these observations in regard to glaucoma. Three such studies were reported by Dr. Macri, of the Pharmacological Section, one of which was done in combination with Dr. von Sallmann. Such studies were centered on the interrelationship of venous and intraocular pressure, and attempts to modify either or both of these by pharmaceutical agents. Thus, Dr. Macri made casts by injection into the anterior chamber, showing filling of the ciliary body of three species: cats, rabbits, and monkeys. In all monkeys (12), the material was also found in the suprachoroidal space. This filling was found in addition to that of the aqueous veins, and raises the question of whether or not this represents a second outflow pathway or anatomic damage due to perfusion of the cast material. This observation, however, together with the knowledge that the outflow of the eye could be altered in a biphasic pattern in which the proportionality of outflow to pressure was lost, indicated the possibility of a second outflow system. Dr. Macri now has started preliminary experiments on monkey eyes in which fluorescein was injected and again demonstrated in the suprachoroidal state after injection into the anterior chambers, lending further support to this study. In view of the late Dr. Friedenwald's studies, it would appear more investigation will be necessary to determine how much such a secondary outflow system contributes to clinical disorders.

Further investigation into this, so that true outflow pressures may be calculated, was centered around the technique of making a small incision, 2–3 mm, in the sclera and separating the choroid and the sclera circularly for a distance of 5 mm. A piece of thin Saran Wrap is inserted between the two layers of tissue, and a piece of No. 10 polyethylene tubing, bent to coincide with the curvature of the eye, is placed in the suprachoroidal space, through this incision. This Saran insert prevents the tearing of the choroid by the tubing during its placement. The tubing is then tied in place and the incision tightly closed. Dr. Macri feels that if the flow to the suprachoroidal space does exist, this procedure may allow its quantitation, and total flow from the anterior chamber will also be determined so that, by difference, flow through the aqueous veins may be calculated.

Dr. Cohan is carrying on a somewhat similar study with intraocular venography, in which Hypaque is placed into the anterior chamber of the eye. Using intricate radiological techniques, Dr. Cohan has succeeded in demonstrating the venous channels of small calibre. He has also shown that introduction of such Hypaque into the anterior chambers is well tolerated and can be used clinically to establish the presence of some clinical forms of choroidal detachment.

Dr. Macri has utilized the cat eye extensively to elucidate mechanisms which affect or control the intraocular pressure, and, once again using casts, in the cat he found anastomotic vessels connecting the anterior ciliary vein and the vortex veins. This cast technique also showed filling of the intraocular aqueous veins in the cat. Although the cat has a large number of episcleral veins, it has but one large anterior ciliary vein, which is prominent just anterior to the superior rectus muscle, and this vein joins the circle of Hovius by 3–5 branches. At the area of the four vortex veins, large superficial, intrascleral vessels lead from the circle backwards to the ampulla of the vortex. Vessels of the iris, ciliary body, and choroid were also well seen, and their interconnections noted.

Continuing a study of previous years on the facility of aqueous outflow (flow expressed as cmm/min/mm.Hg), Dr. Macri recorded venous pressure in different veins of the eye, elasticity and aqueous inflow, in addition to measurements of intraocular pressure and local venous pressures, and the systemic arterial blood pressures, to determine the correlation between these various factors. It was reported last year, by Dr. Macri, that two patterns of aqueous outflow could be discriminated by such methods; the first pattern he called "monophasic", in which the outflow was proportional to the outflow pressure through the range of pressures studied. The second pattern he called "biphasic", and was one in which this proportionality was upset, and in which the outflow measured as cmm/min/mm.Hg decreased progressively as the intraocular pressure was elevated. The values of this latter outflow and the biphasic outflows were always greater than those of the monophasic at pressures close to the normal for the animal studied.

Utilizing these findings, Dr. Macri has attempted to demonstrate the effects of Diamox on

both such pressures, and has arrived at a new concept which implies that the carbonic anhydrase inhibitor, such as Diamox, lowers the venous pressure of the intraocular vasculature selectively without interfering with the general blood pressure. He has also noted the change between the two types of patterns, i.e. monophasic or biphasic, may occur by utilization of pharmacological agents such as epinephrine, or sectioning of preganglionic sympathetic fibers. He presents, as a working hypothesis, that the two outflow patterns are dependent upon the volume in the vitreous compartment, and that this volume, probably through vasculature, is under the control of the autonomic nervous system.

Studies on the central nervous system control of the intraocular pressure have continued from the anatomical viewpoint, by Dr. von Sallmann and Miss Grimes, and from the physiological correlations by Dr. Lele and Miss Grimes. In Dr. von Sallmann's study, the orbital contents were removed completely, including the nerve supply as far back as the fifth nerve ganglion. External ocular muscles were carefully dissected away before fixation. For staining, the fixed material was washed in water for 1 hour, and then placed in silver nitrate, and subsequently in formalin and sodium thiosulfate. In the resulting preparation, the nerves are stained a dark brown, while the ganglionic tissue remains white. Other tissues were unstained. The dissection is accomplished under water, using the Zeiss stereomicroscope.

Dr. von Sallmann and Miss Grimes find that the posterior ciliary branches of the human orbit are similar to those observed in the monkey. Occasionally a fifth nerve branch was seen to travel upon the optic nerve to the posterior pole of the globe, without undergoing anastomosis in the orbit. Such branches not fusing with the postganglionic branches of the ciliary ganglion have been demonstrated in the cat but not in monkey. On the other hand, the human ciliary ganglion receives a heavy branch from the fifth nerve, corresponding to the fine branches in the ganglion in the monkey. Such understanding is necessary to select a proper pathway for studies of afferent and efferent impulses, and to stimulate the nerves which could be so classified. Such knowledge has been used by Dr. Lele and Miss Grimes in their study on the neural mechanisms in the regulation of intraocular pressure.

These investigators studied the afferent nerve discharges of the long ciliary nerve in response to intraocular pressure increases and obtained, in almost all instances, impulses (in contrast to many failures when the technique usually used previously was applied in this laboratory). Also, in contrast to previous findings, no spontaneous activity signalling resting pressure was observed in such preparations or *in vivo*. Dr. von Sallmann does not feel that the site of origin of the pressure induced after an activity was conclusive, but did suggest that such nerve structures are contained in the outer coats of the eye. Stimulation of the retrobulbar nerves did produce changes in the eye pressure, and these effects were most readily observed in the isolated perfused eye where the complicating factor of simultaneous stimulation of extraocular structures is eliminated. Stimulation of the long ciliary nerve produced pupillary dilatation in all cases, but there was no pressure change without circulation of fluid in the vessels.

These investigators conclude that a relatively small amount of *afferent* activity elicited by intraocular pressure changes, and the absence of spontaneous activity signalling resting pressures, makes it doubtful that this sensory path carries enough information to participate normally in the regulation of intraocular pressure; there are pressure effects resulting from third and fifth nerve stimulation, although these do not appear to effect long-term control of the intraocular pressure.

The clinical program, dealing with intraocular pressure pathology, i.e. glaucoma, is being carried out by Dr. Okun and Dr. von Sallmann, in which the particular objectives are to evaluate suspected and known cases of glaucoma in an effort to find earlier means of diagnosis and control; secondly, to find the mechanism of diurnal changes in the intraocular pressure, as investigated by tonography; third, the mechanism of action of various drugs and operative procedures, as studied by tonography; and fourth, the effect of instantaneous blood pressure changes on intraocular pressure by tonography.

These investigators also wish to study the disturbed intraocular fluid dynamics in glaucoma, the inflow mechanisms of which will be subject to the

greatest study. Just as tonography gives an indication of the ease with which fluid leaves the eye, the inflow studies will give an indication of the ease with which fluid enters the eye. These investigators feel, from the limited material so far studied, though they draw no conclusions, that there are some extremely provocative cases illustrating the broad spectrum of glaucoma forms. They expect, also, to undertake measurements to estimate the rate of aqueous formation in health in patients with borderline glaucoma, and those with frank glaucoma of various types.

The uveitis study has again accounted for the greatest number of patients admitted to the ward, i.e., 78, and this clinical study was backed by a study of the thyroid/hormone turnover in uveitis by Dr. O'Rourke; a study of ocular toxoplasmosis and its therapy, by Dr. Kaufman; and a study of the immunological relations in ocular tissues by Dr. van Alphen. In the basic study of Dr. van Alphen, guinea pigs are immunized to their own lens, their own corneal epithelium, and their own vitreous, and the tissues are homogenized with Freund's adjuvant to enhance antibody production. Dr. van Alphen finds that skin, conjunctiva, corneal epithelium, and corneal stroma are related immunologically. The lens, corneal epithelium and vitreous are also related; however, no relation was shown between skin and lens. Of the various eye tissues so tested, the organ specificity was most confined to the lens. Corneal epithelium appeared to be a relatively strong antigen as compared to corneal stroma. Dr. van Alphen plans to study the immune responses to the cornea and lens in certain dermatoses, and might expand his work to the uveal problem in the future.

The records of the large group of patients with uveitis and toxoplasma dye tests, admitted during the past five years of the Institute, have now been studied by Dr. Kaufman, with emphasis on certain diagnostic and therapeutic aspects. Dr. Kaufman appears to feel that the readily available toxoplasmine skin test seems to be reliable in that it was, in the majority of cases, in agreement with the positive results of the toxoplasma dye tests. A false positive skin test was extremely rare. It is of interest that 50 percent of patients with a positive serological skin test and uveitis reacted satisfactorily to antitoxoplasmic chemotherapy; Daraprim and sulfa were the main drugs of choice. The addition of corticosteroids to the therapy with Daraprim and sulfa improved only the occasional patient. Dr. Kaufman also writes the conclusion that the early onset of the uveitis under the age of 20 with an acute or subacute course, can be considered favorable for a therapeutic success, whereas in uveitis with a chronic course the prognosis was less good. Satisfactory results with Daraprim and sulfa therapy were usually not convincing before the 10th day of treatment. There was no correlation between the level of the dye test and the therapeutic responses.

In evaluation of hypometabolism as a possible coexisting accelerator of uveitis, Dr. O'Rourke has tabulated the results of his thyroid hormone turnover studies in uveitis. He has used 30 patients with uveitis and 10 controls. He found that hypometabolism coexisted with many chronic diseases, including uveitis, but that no definite conclusions could be drawn from the data at the present time. The percent of the iodine pool, utilized daily, was lower in the uveitis patient than the normal, and the percent of iodine utilized daily in uveitis patients did not vary statistically from the norm, either calculated per kilogram or per square meter, but both set on the low side of the norm. The results of this study did not show anything definite about uveitis' etiology or its responsiveness to treatment. It is suggested that as a followup a demonstration of thyroid function or dysfunction, and its influence on immune response in patients with uveitis, should be checked.

Basic studies in the chemistry and anatomy of the normal lens, as well as in the cataract, have continued. In the normal lens, Dr. Resnik has now demonstrated the great complexity of the soluble lens proteins, of which he has now separated eight fractions. Fractions A and E have been referred in the past reports as beta-crystallin, while F-H have been designated as alpha-crystallin. In collaboration with Dr. Wanko, electron microscopic observation of electrophoretically isolated samples of fractions of lens proteins have been carried out. It was observed that the fraction denoted as alpha-crystallin contains compound, elongated structures. Samples from the remainder of the soluble proteins contain spheres of different sizes. It is now recognized that the low density elements, observed by the electron mi-

croscopist in sections of osmium-fixed lens, are the lens proteins.

Dr. von Sallmann lists, in his review, that his estimation of the outstanding contribution to the cataract project was the demonstration of the ultrafine structures of the epithelium and fibers in the normal and cataractous lens, by Dr. Wanko and Miss Gavin. In this study it was noted, in the visualization of the X-ray-induced changes of the lens, that the cytoplasmic components are seen at an early stage after irradiation. In the past such changes were considered as relatively late effects. These cytological changes were displacement of the nucleoli, conical tapering and elongation of mitochondria, and partial disintegration of the nuclei. The Golgi complex alone remained unchanged during the time studied. Studies also continued on the myleran cataracts, and the changes observed were somewhat similar to those seen in the later phases after X-irradiation. In the mimosine cataracts, in addition to the data reported in last year's Annual Report, these investigators now have demonstrated that the initial changes in the fine structure in these lens epithelia involve the endoplasmic reticulum and nucleoli. Both structures have a high RNA content. Later, mitochondria and nuclei also undergo changes.

These investigators now have under study seven human lenses with senile cataracts. In the human lens one striking difference from the animal cataracts is the presence of an opaque conglomerate, usually located near the nuclei, and of approximately 200 mμ in diameter. This complex bears resemblance to the cytoplasmic inclusions previously observed in adult human skeletal muscle fibers, and is tentatively identified as a lipofuscin-type of pigment.

Studies of the similar type body in skeletal muscle are also continuing in Dr. Wanko's laboratory. Dr. Wanko and Dr. Shy are shortly initiating the electronmocroscopic findings in the mitochondria of familial periodic paralysis in which there is a high cationic transport.

Of great interest is the combined study of Dr. von Sallmann and Dr. Reid of the Laboratory of Nutrition and Endocrinology, NIAD, in nutritional cataracts. Thus Dr. Reid, during nutritional studies on the guinea pig, observed a frequent occurrence of cataracts when only tryptophane was deficient in the diet. The growth curves of these animals appeared normal, but the cataracts developed regularly and early. It was shown that animals on a moderately supplemented diet showed normal growth, but lens changes developed in what were apparently healthy animals. The histological features of this cataract differed from other types of experimental lenticular opacities as they did not not involve the equatorial zone where new fibers formed. The clinical similarity of these tryptophane-deficiency cataracts, and of various forms of paranuclear cataracts in the human, was felt to be striking by Dr. von Sallmann. Thus, it has been shown for the first time, the requirement of the lens for one essential amino acid, i.e. L-tryptophane, was greater than that for maximal growth of the body as a whole. This leads to the intriguing possibility there are, or may be, early or congenital cataracts in the human associated with transient deficiency of essential amino acids.

The studies of ocular tumors by isotope tracer methods, by Dr. O'Rourke, has now been concluded. Dr. O'Rourke found that the total activity detected by the posterior counting technique for phosphorus^{32} exceeded the anterior value by 74 percent in the six patients studied. He found, in addition, that the highest anterior quadrant count failed to correspond to the tumor bed in six of eight cases studied, but the highest posterior quadrant count was localized correctly in the quadrant containing the tumor in six consecutive studies. However, the point of highest radioactivity during posterior counting did not always lie directly over the center of the tumor mass. Thus, P^{32} uptake has definite limitations for localization of ocular tumors, particularly in the anterior quadrant, as has been anticipated in the utilization of a pure beta emitter. Future work directed to such ocular localization will have to await further examination of tumor uptake by gamma-emitting sources. Of interest in ocular tumors is the report of Dr. Peton and Dr. Thomas on simultaneous occurrence of primary malignant melanoma of the eye and skin, in which these investigators feel that the choroidal tumor and the cutaneous melanoma were considered as independent primary neoplasms which occurred simultaneously in this patient. It is also of interest that the patient's brother was subject to enucleation of the right eye for malignant choroid melanoma 9 years previously.

Dr. Paton and Dr. von Sallman have pointed out a clinical correlation of angioid streaks and sickle cell anemia in three patients. Such angioid streaks of the fundus have previously been correlated with two other diseases, pseudoxanthoma elasticum and Paget's disease. Skin biopsies were taken from these patients to rule out pseudoxanthoma elasticum. The vascular calcifications frequently seen in patients of angioid streaks could not be demonstrated, but the presence, now, in these three disorders, indicates that possibly anemia, retinal hemorrhages, or occlusive vascular disease play a role in the pathogenesis of such fundus changes. This study may be compared with the study of the previous year, reported as an addendum herein, of the vitreous opacities diagnostic of familial primary amyloidosis. Here again thorough opthalmoscopic studies may lead to a diagnosis of a generalized systemic disorder.

CLINICAL DIRECTOR'S SUMMARY

In closing it can be seen that the past year has been a productive one. After 7 years of operation, it can be fairly stated that the Clinical Investigative Unit has been flexible and constantly changing in its approach to various areas of development which may shed further light in our knowledge of both normal and abnormal functions of muscle, central nervous system, nerve, and organs of special senses. However, one of the main problems an Institute must constantly face is that of diminishing returns, as research projects are continued over the number of years this Institute has now been in operation. In the first part of 1960 it is anticipated that the Clinical Director will discuss with each Branch and Section chief this problem, to make certain that the reward of such continued research is such as to justify the energy of the investigator.

Once again the Clinical Investigative Unit acknowledges its debt to the various other Institutes which have collaborated in part on much of the research listed above. In each case, a sincere effort has been made to point out the collaborating unit. Much interchange has occurred and much knowledge gained by a close cooperation with the basic unit of the Institute, and in particular is this true in the Laboratory of Neurophysiology. Much of the data reported herein would have been impossible without the cooperation of the Clinical X-ray Department, Clinical Pathology, and the Instrument Section of the Central Services. We would once again like to acknowledge the cooperation of the National Naval Medical Center, Walter Reed Army Hospital, and the Atomic Energy Commission, with whom many of these projects were undertaken. Each year the cooperation and smooth relations between the Clinical Unit and the Nursing Service have been a pleasure to observe, and the last year has not been an exception to this. The Clinical Unit once again acknowledges its debt to Miss Hulburt, and her three head nurses: Miss Saltow, Mrs. Thompson, and Miss Maccia.

Not infrequently, problems have arisen in patient care which have necessitated the cooperation of the staff of the Director of the Clinical Center. The cooperation and advice from this office have been indeed extremely helpful during the past year, and the Clinical Investigative Unit acknowledges its debt to Drs. Masur, Chapman, and Farrier.

NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

INTRODUCTION

The pursuit of new knowledge and the employment of new methods for studying infectious and allergic diseases have accelerated during the year 1959. This has been evident not only in the etiologic, epidemiologic, and clinical aspects of disease, but also in the basic understanding of how cells react to infection. Exploration of infectious processes has always contributed to an understanding of human diseases, whether actually caused by microorganisms or not. Host-parasite relationships frequently have been models for the reaction of the living cell and tissue to noxious agents. Many techniques of investigating human diseases have originated in studies of parasitism. For instance, the methods of immunology now applied to noninfectious processes were derived in part from the study of immunity to infectious agents. Conversely, it is becoming increasingly apparent that infectious agents may have latent or heretofore hidden influences on human diseases not thought to be infectious. For example, new knowledge of viruses has led to the supposition that some or possibly all abnormal growth of cells may in a direct or remote way be related to infectious viruses which enter the cell and take control of it. These and other concepts are emerging as new advances demonstrate the extensive influences of microbial agents on complex forms of life such as the mammalian host.

NIAID's research aim is to conduct selective and influential investigations within its mission. The resources available to scientists here in Bethesda carry with them the responsibility to direct studies toward the most important and significant questions. These are not always evident and require experienced insight to recognize. The ability of the researcher to ask the right questions, to devise proper experimental methods, evaluate results properly, and report clearly, determines the difference between investigations of high quality and those of little importance. The development of effective research projects in large part derives from the imagination and persistence of the individual scientist who develops his own approach and research objectives. If such an investigator works in a favorable environment and atmosphere with adequate support, influential new knowledge usually results. It is essential, therefore, that the institutional organization be so structured that opportunities for original thought and unconventional methods are not only possible, but are encouraged.

The last few years have seen increased emphasis upon selecting staff members who will bring originality to their investigations. One way is to select, from leading research centers, persons with special skills in allied medical sciences; another—which will be followed more vigorously in the future—is to permit certain Institute scientists to study in other research centers for a period of at least a year. This opportunity is aimed particularly at the younger scientist who has given evidence of research competence, but has not yet become involved in a large comprehensive program.

Another important development has been the experience offered to young medical graduates who serve their National Selective Service obligation in the Public Health Service. These men spend 2 and sometimes 3 years in clinical studies or in full-time laboratory investigations with a senior experienced scientist. They now form, and will continue to form, a valuable group from which to recruit permanent staff members for NIAID, or for positions in teaching institutions.

In addition, there are a number of guest workers from domestic and foreign institutions working with NIAID scientists for periods of several months to a year. In effect, a significant segment of Institute activities is involved in advanced research experience by carefully selected young scientists.

In line with the concept that NIAID organization should fit its reseach mission and the individ-

uals conducting it, a new administrative structure of the Institute was effected in 1959. This recognized the development of several new programs around key researchers and broadened objectives of several project areas. The Laboratories of this Institute now number eleven, including two with major activities located outside Bethesda, the Rocky Mountain Laboratory at Hamilton, Mont., and the Middle America Research Unit in the Panama Canal Zone. Studies in tropical disease formerly unified under the direction of Dr. Willard Wright were separated into the Laboratory of Parasite Chemotherapy, Laboratory of Parasitic Diseases, the Laboratory of Germfree Animal Research, and a portion of the Laboratory of Tropical Virology. The Laboratory of Cell Biology and the Laboratory of Biology of Viruses, both with an outstanding senior investigator as chief, are now separate from their former organizational place in the Laboratory of Infectious Diseases. The Laboratory of Clinical Investigation, under a newly appointed clinical director, has continued its studies of clinical diseases in patients and efforts made to strengthen its contacts with the nonclinical laboratories. The continuance of former investigations and initiation of new ones promise well for the results of clinical research in the future.

The Board of Scientific Counselors met in July at the Rocky Mountain Laboratory to consider the research programs of that center. In the fall the Board met in Bethesda to review the activities of the Laboratory of Clinical Investigations and their relationship to the total research effort of the Institute. The members of the Board have been quick to comprehend Institute resources and potential for future investigations, as well as the special problems involved. Their advice has been constructive and pertinent. Individual members of the Board have been helpful in advising on special research projects within their experience and in recruiting both junior and senior staff members.

A review of last year's accomplishments indicates that Institute research is moving into new areas both scientifically and geographically. The investigations at MARU have not only permitted new projects in Central America, such as those on the encephalitides, poliomyelitis, and influenza in tropical areas, but also have stimulated interest in other special problems of warm climates. This has resulted in plans for clinical studies of schistosomiasis in Puerto Rico, and the possibility of importation to the Clinical Center of patients with tropical diseases.

Advancement has occurred in studies of tumor viruses and viruses causing tumors both in laboratory-reared animals and also in animals in their natural state. Studies, in association with the National Institute of Neurological Diseases and Blindness, of the occurrence of human infections during the perinatal period have extended greatly the opportunity for investigation of viral infections in adults and children. This is consistent with past efforts of this Institute to study disease as it actually occurs in nature, rather than to depend chiefly upon laboratory models of natural phenomena. The future will see a continuation of this approach, a strengthening of the clinical investigation program using the unparalleled facilities of the Clinical Center, and an emphasis on basic phenomena occurring in mammalian cells either infected or uninfected with microorganisms.

The broad interests of Institute scientists in infectious and allergic diseases, and fundamental cellular and subcellular processes underlying the disease state, are illustrated in the following summary of our research activities.

During 1959, investigations of the role of viruses, bacteria and fungi as causes of disease continued to be the primary interest of the Laboratory of Infectious Diseases. Thus, the research programs of the viral and epidemiological sections are almost wholly integrated and designed to define the behavior and importance of viruses (respiratory viruses, enteroviruses, and cancer viruses) in their natural hosts, observed in certain natural environments. Thus, studies of respiratory diseases and other undifferentiated illnesses were continued in two pediatric groups—at Junior Village and at Children's Hospital—and in groups of adults, including several installations of the U.S. Navy, and at the University of Maryland. The role of viruses in respiratory and intestinal diseases of cattle was observed on farms, feedlots, and in experimental pens at the Universities of Maryland and Illinois. The potencies and possible effectiveness of respiratory virus vaccines were examined in a preliminary manner in relation to both human and animal respiratory illness, and broad

seroepidemiologic studies of the prevalances of many viruses in various age and economic groups in various parts of the world were initiated.

During 1959, viruses as a cause of tumors and cancer in animals developed into a major research interest. At first, studies were focused on the development and evaluation of new techniques for demonstrating and detecting tumor viruses in experimental mouse colonies and chicken flocks. In July 1959 attention turned to the occurrence of mouse and avian tumor viruses in natural populations.

Respiratory Virus and Enterovirus Studies

The National Health Survey, 1957–58, revealed once again and with greater force than ever the magnitude of acute respiratory diseases in the U.S.A. The Survey estimated that several hundred million such illnesses had occurred and confirmed previous impressions developed in several independent studies about their greater prevalence in children. Consequently, studies of this essentially unsolved problem were pursued with undiminished vigor.

The Junior Village orphanage study, now in its fifth year, continued to serve magnificently in its dual role as (1) a source of basic and essentially new information on the total viral and bacterial experiences of the urban infant, and (2) a focal point for a number of more specific studies on the behavior and possible prevention of certain of the apparently more important agents.

A 3-year analysis of the overall experiences, now completed, continued to show that an average of 25 percent of infants suffered a new acute febrile illness (exclusive of measles and chickenpox) each week; and that approximately 30 percent of them acquired a new identifiable viral or bacterial infection during the same interval. A total of over 1933 separate infections with viruses belonging to 53 different serotypes and 718 infections with presumed bacterial pathogens were observed. Crude estimates indicate that 40 to 45 percent of the more severe illnesses observed in the orphanage infants could be attributed to known agents, most of them viral. Statistical analysis showed certain agents were significantly and repeatedly associated with febrile illnesses. These included adenovirus types 1, 3, and 5; influenza A; parainfluenza 1 and 3; poliovirus 2; Coxsackie B5; Group A streptococci 4, 12, 23; *Shigella sonnei;* and, of course, measles and chickenpox viruses. Others, including many respiratory and enteric viruses, and bacteria presumed to cause illness, failed to produce sufficient illnesses in excess of the high "normal" rates to provide significant associations. Although these latter observations do not exclude the likelihood that under other circumstances they might well produce clearcut illnesses, repetitive occurrences of viruses and bacteria over several years in this carefully studied population provide valuable information on the relative pathogenicities of many common agents.

Definition of Specific Viral Diseases

Perhaps of even more importance at this time are the specific and original contributions to our growing fund of information about the existence and behavior of new human viruses, first discovered or delineated in our Junior Village studies.

Investigators found Junior Village valuable in developing much new information on the natural occurrence of salivary-gland virus, particularly its continuous presence in the oral secretions of many infants. They discovered the first hemadsorption viruses (parainfluenza 1 and 3) in Junior Village specimens, and the first outbreak defining the clinical importance of parainfluenza 3 was reported in this population. In 1959, three large outbreaks of parainfluenza 3 showed that, although reinfection of infants with serum antibodies occurred, increasing levels of antibody provided increasing protection against both infection and illness. Ten percent of 120 cases had pneumonia—all in infants without antibodies or other evidence of previous infection. Recently a medical virologist was able to confirm his discovery of a fourth parainfluenza virus during an outbreak of infection with this virus in Junior Village. Others, using the Junior Village study system, finally developed conclusive evidence that most of the adenoviruses that commonly infect children also cause significant amounts of illness—nearly 50 percent of the infections with types 1, 3, and 5 showing clearcut evidence of association with febrile ill-

ness. Similarly, a number of new serological prototypes of adenoviruses, including a group of them that can be demonstrated only in the intestinal contents, were discovered. The natural occurrences of ECHO-10-like viruses observed in this population provided the necessary information employed in 1959 to construct the three separate prototypes, the new reovirus group. The new H1 techniques used for diagnosis of infections with these agents were also developed using serums from patients in Junior Village with demonstrated reovirus infections. This procedure was used to determine much of what is known about the epidemiology of these agents.

Respiratory Viral Vaccines

In 1959, preliminary controlled tests of commercially produced experimental respiratory virus vaccines were initiated in Junior Village. Adenovirus, influenza, Coxsackie B, parainfluenza, and measles vaccines, all of them inactivated by formalin, were placed under study in the orphanage. Recently a multivalent vaccine, including 12 different antigens, was also introduced. Except for suggestive evidence of some protection from measles vaccine, the Junior Village vaccine studies have served chiefly to reemphasize that antibody response of young infants to inactivated viral antigens is remarkably less than that of adults and that inactivated vaccines of much greater antigenic potency will be required to support any reasonable hopes for producing adequate protection in young children.

Seroepidemiologic Studies

The development and evaluation of suitable methods for acquiring large amounts of data on viral infections in large general and selected populations represent perhaps a major current activity in the field of viral technology. Thus, virologists have now developed complement fixation and H1 procedures for measuring type specific serum antibodies to more than 40 viruses and with the use of group specific antigens it is now technically possible to test for antibody responses to 75 or 80 viruses.

This represents a major advance which can now be exploited to carry out really comprehensive seroepidemiological studies of human viral experiences in any special or general frame of reference. Thus, differences between the prevalence of many agents can now be determined for different times, different geographic areas, different economic or social groups, and different ages. The occurrence of common viral infections of both wild and domestic animals and the likelihood of some of them being shared between species, can now be studied. Well-controlled seroepidemiologic studies will be of particular value in developing leads concerning needs for viral vaccines and the roles of viruses in neonatal, chronic, and neoplastic diseases.

The seroepidemiologic studies were much advanced in 1959 through the collaborative study undertaken with NINDB, particularly through a contract with Microbiological Associates, Inc., which has successfully developed and produced in large amounts the majority of viral antigens needed.

Specific Respiratory Disease Studies

The role of the "Eaton" agent in primary atypical pneumonia (PAP) has been a controversial one for many years. Furthermore, to those who accepted it as one of the causes of nonbacterial pneumonia the importance of the agent as a cause of disease and its natural history remained unknown. LID scientists with the Bureau of Medicine and Surgery, USN, studied longitudinally continuous endemic occurrences of primary atypical pneumonia at a Marine recruit training center in the South. The Coons fluorescent antibody technique adapted in LID for large-scale routine diagnosis of PAP made the study possible. Approximately 70 percent of all pneumonia observed was associated with Eaton virus infection, whereas 23 percent of persons with febrile respiratory illness and 6 percent of persons with no respiratory illness showed rises to PAP. The ratio of illness to infection was approximately 1 in 12. The existence of this rather large clinically inapparent segment of PAP was unexpected. The data obtained from military recruits was supplemented by information on the occurrence of PAP in chil-

dren observed in the cooperation study at Children's Hospital. Here it was found over a 16-month period that 10 percent of lower respiratory illnesses was associated with infection with the Eaton agent.

Respiratory Viruses Possibly Shared By Man and Domestic Animals

The demonstration in 1958 that parainfluenza 3 virus could be isolated from young cattle with respiratory disease and/or shipping fever led to further studies of the epidemiology of this and other viruses in the bovine species. Cooperating with the University of Maryland Division of Veterinary Science, and the University of Illinois School of Veterinary Medicine, LID scientists found parainfluenza 3 to be one of the most common virus infections of cattle—often producing little or no clinical disease. As in man, reinfections with the virus were also demonstrated. Despite occasional illnesses produced in calves by artificial inoculation and the recovery of the agent from pneumonic lungs, the importance of the virus in producing shipping fever was not determined. Experimental vaccines, with and without adjuvants, produced antibody levels capable of modifying the virus propagation on subsequent challenge. However, field studies designed to determine the ability of the vaccine to protect against shipping fever were vitiated by the failure of shipping fever to occur even in controls in the one study that could be completed.

Studies of Tumor (or Cancer) Viruses

Now a major research effort in the Virus Section, studies of animal tumor and cancer viruses have been aimed at three unsolved problems that appear to be interfering most with maximum progress in tumor-virus research.

The first problem is the lack of simple viral techniques for studying most tumor viruses in the laboratory and for recognizing their occurrence in their natural hosts. Although signal advances have been made in the laboratory study on polyoma, Rous sarcoma, avian leukosis, and Friend leukemia viruses, most tumor-virus activity must still be measured entirely by the production of tumors—a very clumsy, slow, and uncertain method.

In 1959, LID scientists applied new viral techniques to studies of pathogenesis, modes of infection, excretion, laboratory spread, and natural occurrence of a number of viruses thought to be tumor agents—most notably polyoma virus, Friend leukemia, and the presumed avian lymphomatosis tissue-culture agent.

Polyoma virus was shown to grow to extremely high titers in certain organs, 10 to 20 days after infection of the newborn mouse. Hemagglutinins, complement-fixing antigens, inhibitors, and specific antibodies, in addition to infectivity, were compared in a number of different tissues and excretions at regular intervals from 3 days to 3 months after infection. Remarkable differences in titers of these various attributes were noted between various tissues. The most sensitive method for recovering virus from polyoma-tumor tissues proved to be cultivation of the tumor tissue itself when infection was produced in infancy. Virus could often be demonstrated regularly beyond 3 months and in some mice appeared to persist throughout life.

Infection of weanlings led to significantly less multiplication of virus and excretion was minimal. Our serological group furnished information to other cancer research laboratories on the prevalence of spontaneous polyoma and extrinsic virus infections in their passage materials and breeding colonies.

The second problem, a very complex one, is produced by the numerous "extrinsic" viruses that sooner or later contaminate laboratory tissue-culture study systems and also (not unexpectedly) the experimental animals used for studying tumor viruses. During recent months it was demonstrated that a number of viruses occur as "fellow travelers" in many mouse-tumor, virus-study systems. Passage materials and host animals used for passage were often found to contain not only one but multiple extrinsic viruses. Some of these viruses, such as K virus, mouse salivary gland virus, LCM, and mouse hepatitis, had been recognized previously. Others were new agents related to human adenoviruses, reoviruses, and even polyoma. The latter virus, perhaps now the best studied tumor virus available, was found in many different laboratories also to be a notorious con-

taminant of other tumor-virus study systems. It has been found in passage material of Gross leukemias, Schwartz leukemia, Bittner mammary cancer, and even human tumors, but its association with the characteristic manifestations of those tumor systems appears to be wholly spurious. Thus, tumor viruses are found complicating the study of other tumor viruses, which really is not so surprising in that latent viruses are more likely than "acute" viruses to persist in tumor tissues. Interestingly enough, virtually all the viruses so far found in the tumor-study systems appear to be latent agents—and perhaps for this reason themselves are possibly suspect as tumorigenic agents. Recently, we studied the tissue-culture-grown RPL–12 virus, widely used in model study systems, as an example of visceral lymphomatosis virus—an avian leukosis tumor virus. An extensive seroepidemiologic study using a tissue culture neutralization test showed almost conclusively that the presumed tumor agent being propagated in tissue culture was not the virus which causes avian lymphomatosis but a biologically similar latent agent, undoubtedly picked up, as would be expected, during numerous passages of lymphomatosis tumor virus through chickens. This tissue-culture virus apparently occurs in as many as 90 percent of the breeding animals in chicken colonies in various parts of the country, thus assuring a high rate of association of antibodies to the tissue-culture agent with lymphomatous chickens. All chickens involved in several clearcut outbreaks of classical lymphomatosis were negative.

The third critical need is information on the natural history of tumor viruses. Almost without exception, contemporary tumor-virus studies are confined to artificial laboratory study systems utilizing chiefly special categories of mice, chicken, or tissue cultures. These various and quite valuable modes of approach to tumor virus study are meant to be "models" of what (it is hoped) happens in nature. This in part is frustrated by the "extrinsic" viruses. However, until the natural occurrences and biology of these agents are known, at least in part, their real importance as biologic entities and as causes of cancer must remain obscure.

Studies of Tumor Viruses in Nature

To answer these and other questions about the natural history of polyoma virus we decided in July 1959, to do now what had to be done eventually, namely, study the occurrence and behavior of polyoma in *Mus musculus*, and in its natural environments, both rural and urban. Without exception, *Mus musculus* and other rodents in rural areas have so far failed to reveal any evidence of infection with polyoma. When the first 500 mice trapped in 43 different areas in lower Manhattan, Queens, Brooklyn, and the Bronx showed widespread infections with certain other viruses but absolutely no evidence of polyoma, serious questions were raised about the origins and natural hosts of polyoma. This question was quickly resolved when we turned to systematic surveys of mice in the more densely populated areas of Harlem. Here varying percentages of individual mice in more than half of the mouse populations surveyed were serologically positive. A field laboratory was immediately established in Harlem with the assistance of the New York City Department of Health. Although the story is quite incomplete, the following was determined about the natural history of polyoma.

All newly developed techniques for assaying polyoma (HI, CF, MAP, tissue culture isolation and neutralization techniques) confirmed the widespread presence of polyoma in the extremely dense mouse populations of certain tenement areas in central Harlem. In this area nearly 20 percent of approximately 1,000 mice of all ages and sizes showed serological evidence of polyoma; polyoma virus was isolated from tissues and urine of 25 percent of the serologically positive mice, and at least two of the wild strains were shown to be highly tumorigenic.

Although arthropods (*A. sanguineous*, *L. bacoti*, and hair mites), and many insects (particularly roaches) were observed, often in great profusion, the studies failed to suggest that they played an important role either as reservoirs or vectors.

Polyoma virus was readily demonstrated in mouse nesting materials. This was consistent

with our previous experimental observations that polyoma is excreted in large amounts from newborn mice, but inconsistent with our data on spontaneously infected adult laboratory mice which apparently did not. However, certain naturally infected adult wild mice excreted considerable amounts of polyoma. This information provided the basis for a hypothesis which could account for the natural spread of polyoma. This hypothesis recognizes the fact that certain mice, including adults, become chronic carriers and shedders of polyoma virus, an agent well equipped to survive outside the body. Also postulated were mouse populations large enough and dense enough to furnish a constant supply of susceptible mice, and an environment in which mice shared community nests contaminated with polyoma virus; all circumstances observed in the Harlem tenements. Since data previously reported showed that mice could be infected easily by oral and respiratory exposures to polyoma virus, the hypothesis seems to satisfactorily explain much of the natural spread of polyoma. So that even in nature polyoma virus behaves much like an ordinary highly resistant respiratory or entero-virus. A number of rats and 60 humans living in the apartments containing heavy mouse and environmental infections with polyoma showed no definite evidence of specific polyoma antibodies. A number of mouse viruses were also encountered in the wild mice, including several related to common human viruses; and, as continues to be the case in nearly all laboratories studying tumor viruses, these agents created many problems of a technical nature as well as difficulties in interpreting the data.

Studies in Medical Mycology

NIAID mycologists have continued to find ecologic associations of pathogenic fungi with certain specific types of soil or organic material. *Cryptococcus neoformans* has been isolated from many additional sites where pigeons roost or nest. Virulent strains of the fungus have been isolated from attics of old open buildings and window ledges outside office buildings. Detailed studies of microflora of *Histoplasma*-positive soil samples taken adjacent to a building housing bats and comparative studies of *Histoplasma*-negative soil samples taken a few feet away have not as yet revealed a recognized difference in fungal flora, except for presence or absence of *Histoplasma.*

NIAID scientists have continued to test antifungal drugs in a standardized *in vivo* test using mice. Others have collaborated by making and supplying drugs of one series which was systematically examined and by testing promising drugs in clinical trials. One antibiotic, found 3 years ago to surpass other antibiotics in experimental therapy studies, has now had encouraging clinical application.

In collaboration with the Bureau of Medicine and Surgery of the Navy, the etiology of a febrile illness occurring in underwater swimmers was investigated at Key West, Fla. Evidence has been obtained which indicates that this may be an allergic manifestation of exposure to a fungus in persons already hypersensitive to this fungus. Several fungi were found growing in the masks used and three of these are still under investigation in experimentally hypersensitized animals and by field use of an antigen prepared from one of them. Experimental studies of two fungi and standardization of antigens prepared from them are incomplete. As a result of these unfinished studies better sanitation of the masks has been enforced and apparently has controlled the febrile disease.

Biochemistry of Antibiotics

A study is being made of staphylococcal penicillinase both in the whole cell and in cell-free extracts. Emphasis is placed on the uncovering of an inhibitor for staphylococcal penicillinase. Since the penicillin nucleus is composed of cysteine and D-valine, it was thought possible that certain dipeptides could inhibit the activity of the enzyme. This has proved to be the case. Numerous dipeptides have been found to exert an inhibitory effect and, in particular, D-valyl D-valine has been found to inhibit strongly both cell-free and whole-cell staphylococcal penicillinase. An offshoot of this work on the inhibition of penicillinase has yielded the surprising observation that relatively simple alcohols strongly stimulate the rate of penicillinase activity. Indeed, stimulations in rate up to 35-fold have been obtained in the presence of n-propanol. As an operational

hypothesis, it has been postulated that propanol is active by virtue of the removal of a naturally occurring inhibitor present in both whole cells and cell-free penicillinase.

Streptococci and Other Human Bacterial Pathogens

A relatively new method for the isolation of M protein from Group A streptococcal cells is now used. A streptococcal cell-wall enzyme, lysin, is prepared from fresh phage lysates of Group C streptococci and is reacted with Group A streptococcal cell walls. The resulting digest, when fractionated by various means, including ion-exchange column chromatography, yields an M protein which compares favorably in respect to antigenicity to the M protein extracted by conventional means. Type-specific antibodies, as demonstrated by various tests, developed in rabbits after intracutaneous injection of 1.5 mg. per animal. Following such injections, no toxicity was noted. In the course of these studies, it was noted that lysin M as well as purified acid M and crude acid extracts from Group A streptococci contained a factor which precipitated mammalian plasmas and purified fibrinogens. This so-called FPF (fibrinogen precipitating factor) is under investigation to determine its biological significance.

Fractionation of Hydrogen Isotopes by a Marine Pseudomonad

A marine pseudomonad isolated from the Bahama Banks has been found to enrich with respect to deuterium as compared to light hydrogen to the extent of 20-fold. Similar results have been obtained using tritium. In both cases, the enrichment exceeds by far that which can be expected from exchange-reaction equilibria. Warburg studies and biochemical analyses have shown that the initial fermentative degradation of glucose is by the Embden-Meyerhof scheme. Hydrogen arises presumably from glucose via formate. Formate is decomposed to CO_2 and H_2. One or perhaps two separate sites for deuterium discrimination have been fairly well delineated. One is the glucose-pyruvate step and the second occurs between formate and CO_2 and H_2. Practical implications of this study include the possibility of devising a cheaper, more efficient fractionation of the hydrogen isotopes.

Q Fever Studies at Rocky Mountain Laboratory

Possibly the most significant expansion of the research program at the Rocky Mountain Laboratory has resulted from renewed interest in Q fever occasioned by our findings that this disease is present and spreading among dairy cattle in the United States and that a large number of cases of severe illness have occurred in persons residing in Twin Falls County, Idaho. Concomitant with discovery of human cases, serologic surveys of certain population groups in Idaho were performed, revealing the presence of a large number of persons who had specific antibodies against *Coxiella burnetii* even though it was not possible to elicit evidence of clinical illness. Epidemiologic investigation of persons engaged in the dairy trade in the Twin Falls area revealed that those associated with infected herds had more evidence of illness than those whose herds were free of Q fever. Similar studies are now being done in Ada County, Idaho, an area where only serologic evidence of disease has been encountered.

The situation in Montana can be illustrated best by quoting figures collected by RML investigators. Among 8,000 dairy herds in the State there are 119 containing infected animals. Most infections are concentrated in the milk sheds and only near Kalispell are herds found to be free. In Ravalli County, the herd infection rate is 4.2 percent; in Gallatin County and Teton County, the rate is 7.0 percent and 10.0 percent. Among 11,277 human serums examined, 162 or 1.4 percent were found to have antibodies against *C. burnetii*. While these figures indicate that Q fever is a problem in Montana, data accumulated on the spread of infection in dairy cattle are more enlightening. Dairy cattle from selected dairy sheds were surveyed for the presence of infection in February 1959 and 45 of 1,741 herds were found to be infected. Similar surveys conducted in October 1959 showed 119 of these herds to be infected. In 8 months the herd infection rate had increased from 2.6 percent to 6.8 percent.

The discrepancy between the number of clinical human cases, serologically positive persons, and the presence of a vast animal reservoir of infection is of interest. Although only a very few clinical cases have occurred in Montana, it has been found that these persons have been associated with cattle from which it has been possible to isolate organisms of about the same degree of virulence encountered previously in California. The questions of whether or not relatively avirulent strains exist in cattle, whether or not antibodies present in infected milk influence the course of human disease, or whether some other animal such as sheep is a factor in determining human reaction, are being studied. In Ravalli County, studies have been initiated to determine if and how human disease may develop during a period when the disease is spreading among dairy cattle.

Laboratory investigations have been directed largely toward production of *C. burnetii* on modified Zinsser tissue cultures, purification of these organisms, and extraction of rickettsiae to obtain antigens. *C. burnetii* can be extracted with dimethyl sulfoxide, and a component acting as a haptene in the CF system has been obtained quantitatively. When *C. burnetii* is grown on tissue cultures, there is an increase in the number of organisms and in the neighborhood of 1 to 2.5 mg of *Rickettsiae* can be obtained from each gram of tissue. By judicious application of continuous sucrose gradients, it is possible to purify the organisms so that no contaminating material can be observed by electron microscopy. Recent results show glycerol-water gradients to be equally valuable in this purification process. Since the entire procedure is one which has been adapted to relatively large-scale experimental methods and is one in which chick tissue need not be employed, it is obvious that it has application to many problems dealing with immunity and sensitivity in Q fever. The problem of sensitivity will be dealt with in the very near future.

Colorado Tick Fever

One of the very interesting observations coming from field investigations on Colorado tick fever is that the incidence of ticks infected with *Rickettsiae* in areas where many ticks are found to harbor CTF virus is practically nil. Results of preliminary experimental studies indicate that ticks which have been infected as larvae or nymphs with CTF virus prior to or after infection with *Rickettsiae* yield only CTF virus when tested for both organisms after they have moulted to the adult stage.

In further studies of the ecology, RML scientists have been able to reemphasize the fact that microecological conditions greatly influence the incidence of infection of rodents and ticks with CTF virus. In Montana, at least the presence of golden-mantled ground squirrels greatly facilitates the maintenance and spread of virus. Studies were made of the duration and height of viremia in native rodents, and it was found that viremia in this rodent is both prolonged and of sufficient severity to account for infection of ticks feeding upon them.

Laboratory studies have produced an antigen from brains of mice infected with CTF virus which is effectively killed and still is capable of producing neutralizing antibodies in recipient animals. Present preparations are inactivated at 4° C. for periods as long as 60 days with 1:4,000 formaldehyde before all virus is killed. It is interesting to note that with the old vaccines there was evidence in human beings that while the occurrence of disease was not prevented, the clinical illness appeared to be considerably less severe than in nonimmunized persons. That this disease is by no means an uncommon one in the West is demonstrated by isolation of virus from 553 patients to date and by the observation that 8 percent and 3 percent of serums from persons living in rural areas of Wyoming and Idaho possess neutralizing antibodies against CTF virus.

Considerable interest has been manifested in the strain of virus isolated in Powassan, Ontario. A field investigation was most valuable in deemphasizing the idea that this virus might be transmitted by ticks, since few ticks are present in the area and the periods during which cases occur do not coincide with periods when ticks are active. Laboratory studies likewise have shown this virus to have, at best, only a remote serologic relation to the viruses of Russian spring-summer encephalitis and of louping ill. It is of interest that serums from residents of rural areas of the West and Alaska were all negative when tested for antibodies against this virus.

Bat Rabies

The presence of rabies in bats has continued to be a problem. This year isolations were made from eleven bats, representative of three genera and four species, including *Eptesicus fuscus*, *Myotis lucifugus*, *M. evotis*, and *Lasionycteris noctivagans*. Three isolations were made directly in mice following bat bites.

Fractionation of Cell Walls

A great deal of interest has been manifested by a number of investigators in the techniques for the physical isolation of cell walls and protoplasmic fractions of various organisms. Isolation of these fractions makes it much simpler to collect and purify antigenic materials useful for diagnostic or immunological purposes. In conjunction with the University of Montana, investigations were made of the role of cell walls and protoplasm of *Bordetella pertussis* in producing certain effects in mice. It had been postulated previously that cell walls of this organism might confer protective immunity on mice, but no definite proof of this had been obtained. Our experiments showed that protoplasm contained a heat-labile toxin which was toxic both for mice and for the skin of rabbits and that the cell walls contained the histamine-sensitizing factor and the protective antigen.

In conjunction with a scientist from the University of Georgia, studies were made of the components of *Brucella abortus* to determine their toxicity and their potentialities as protective antigens. Cell-wall and protoplasmic fractions were obtained and a water-soluble material was obtained by the extraction of whole cells with ether. When studied in mice, it was found that the protective index conferred by these various materials was as follows: controls=0, protoplasm=1.0, acetone-dried whole cells=1.9, residue ether extract=2.7, cell walls=3.7, and aqueous ether extract=>4.6. The cell walls and the aqueous ether extract are potent endotoxins for mice. The antigen extracted from *Br. abortus* by ether is soluble in water and is toxic. It resembles similar antigens obtained from *Salmonella* in these respects, but differs from that of *Bacterium tularense* since in the latter organism this treatment results in the isolation of a particulate nontoxic fraction.

Thus, the studies carried out this year serve to extend observations made in the past which emphasize the importance of cell-wall fractions and of the cell wall itself in producing protective immunity in experimental animals. This upholds the theory that protective antigens of bacteria should be located upon the surface of the organisms rather than in the interior. Studies with *Salmonella* have been directed mainly toward determining the effect of lipid which is contained in the so-called complete antigens. According to Westphal, the lipid contained in this protein-carbohydrate-lipid complex is both toxic and immunogenic. This idea is difficult to accept in view of the evidence accumulated that carbohydrates and proteins are in general responsible for immunogenic reactions.

Studies carried out at the Rocky Mountain Laboratory and in conjunction with the National Cancer Institute show that the lipid fraction of complete antigens is not responsible for many of the biological activities of complete antigens of *Salmonella*. By reducing the lipid content of antigens from approximately 19 percent to 1 or 2 percent and testing the original and the delipified antigen by various methods, it was found that the delipified material reacted, by and large, in the same way as the normal antigen. Thus, they were toxic, they produced pyrogenic reactions in rabbits, they produced skin lesions in rabbits, they had a tumor-damaging factor, they were toxic for thorotrast-treated rabbits, and both conferred specific and nonspecific protection upon mice which were immunized with them. It was found that lipid did not produce specific protection in mice, but was capable of inducing nonspecific resistance.

Antigens From Tubercle Bacillus

The original enthusiasm for concentrating efforts to obtain cell-wall fractions of tubercle bacilli to immunize animals against tuberculous infection has been dampened considerably by the consistently negative results obtained in immunized mice challenged by the pulmonary route. While it has been possible to show that killed antigens are capable of protecting mice against infections produced by intravenous inoculation of virulent organisms, it has not been possible to demonstrate that cell walls, protoplasm, or phenol-killed antigens are capable of producing significant immunity in mice which are subsequently challenged with a rela-

tively small dose of virulent organisms given by aerosol. On the other hand, preparations of living BCG or H37Ra will effectively immunize mice against aerosol infection, and the dose of avirulent organisms given as vaccine may be considerably diluted and still confer protective immunity. It has also been demonstrated that significant immunity persists for at least 26 weeks after administration of vaccine. One of the interesting aspects of this study has been that the results in mice are similar to those obtained in human populations where it is stated that the incidence of pulmonary tuberculosis is not greatly affected by vaccination with BCG, but the incidence of tuberculous meningitis and miliary tuberculosis and other secondary syndromes is greatly decreased. In our experimental system it can be shown that bacteremia is uncommon following infection of immunized mice, whereas organisms are found consistently in the lungs even in the absence of tubercles. As a basis for a potency test for living vaccines to be used in man, this system appears to be quantitative and to result in a graded immunity response in mice.

With cell walls obtained from *Mycobacterium butyricum* and *M. tuberculosis*, it has been possible to demonstrate the specificity of the hypersensitivity produced in rabbits by injection of one or the other of these fractions. There is considerable need for this type of test and, in conjunction with serological studies, it may be possible to demonstrate chemical and antigenic differences in various species and strains of mycobacteria.

Studies of Delayed Hypersensitivity

Continued investigations serve to add emphasis to the theory that delayed hypersensitivity is a preliminary and essential step in production of immediate sensitivity and circulating antibodies. This relation of delayed to immediate hypersensitivity was strengthened by demonstration of the anamnestic response displayed by animals receiving a primary dose of antigen so small that they develop only delayed hypersensitivity but develop circulating antibodies very rapidly if a secondary stimulus is applied at the time delayed hypersensitivity is at its height. Since delayed hypersensitivity to a protein-haptene complex is directed toward the broad protein portion of the moiety and immediate hypersensitivity toward the spesific haptene portion, it is evident that specificity of the immune reaction in animals changes as the process matures.

Antigens of *Trichophyton rubrum* are being employed to study the role of hypersensitivity in skin infections due to this organism or to *T. mentagrophytes*. Patients suffering from infections with the latter organism display delayed reactions to the antigen, but patients infected with *T. rubrum* fail to do so, suggesting that the reason for the development of chronic infections with *T. rubrum* is due to its lack of antigens which are responsible for production of immunity.

Purification of Viruses

From a practical point of view, the discovery of methods whereby large quantities of tissue-culture fluid infected with poliovirus can be concentrated and virus purified makes it feasible to produce purified virus for immunogenic studies. Routine harvests of 5×10^{11} PFU of virus are obtained. Based on 10^6 PFU as a suitable immunizing dose for man, about 500,000 doses of vaccine are produced with limited experimental facilities. From a theoretical viewpoint, data indicating that the RNA of poliovirus is made up of a single strand which can be inactivated by disintegration of a single bond are fundamental to studies of proliferation of animal viruses. Stress is placed upon observations of the failure of S^{35} and P^{32} of virus used to infect cells to be incorporated in significant amounts into new virus. These observations differ from those made regarding tobacco mosaic virus and bacteriophage. It should be noted that labeling of poliovirus with S^{35} contained in methionine serves to mark the protein fraction of the virus in contrast to the marking of nucleic acid which is accomplished with P^{32}. Labeling of protein of poliovirus by this method has not been reported previously.

Tick Paralysis

Studies of tick paralysis have continued and have established that hamsters, marmots, dogs, foxes, and monkeys develop disease when female ticks are allowed to feed upon them. An interest-

ing observation which may have some relation to the transmission of tick paralysis is the appearance of a previously undescribed fat-like body developing in female ticks during engorgement. In conjunction with scientists at the University of Utah, investigations of the pharmacological action of the toxin have been expanded, and it is now definite that the central as well as the peripheral nervous system is involved in this disease.

Germfree Animal Research

Significant program development occurred along two major lines during the year. First, the germfree mouse colony, initiated at the end of last year, increased to the point where these animals became available for experimental use. In the past, the Institute's research program in this field had been limited to the use of germfree guinea pigs. Secondly, and undoubtedly associated with the first, there has been an increase in the variety and number of cooperative projects either planned or underway with other Laboratories.

A study of the lesions produced by *Entamoeba histolytica* associated with a variety of individual species of bacteria, has shown that only mild to moderate infections are usually produced by some species, e.g., *Escherichia coli* and *Streptococcus faecalis*. On the other hand, a more acute ulcerative amoebiasis occurs when either *Bacillus subtilis* or *Lactobacillus acidophilus* is present. These findings may explain, in part, the varied picture that infection with this parasite can present.

Germfree animals, especially guinea pigs, have notoriously enlarged ceca. On the "natural product" type of diet ordinarily used to raise this species germfree, the cecum and its contents can comprise up to 40 percent of the animal's body weight. With the use of semisynthetic diets sterilized by irradiation, a scientist has obtained germfree animals with an average cecal weight of less than 10 percent of the body weight, a figure which approaches that of the conventional animal. These studies may point out ways in which the lack of an intestinal flora affects the physiology or histology of the intestine.

In another type of study, we had shown earlier that the protozoan parasite, *Trichomonas vaginalis*, multiplied vigorously and produced large lesions when inoculated into the loose subcutaneous tissues on the back of germfree pigs. However, for the most part, the organisms disappeared in conventional animals. Usually, a tiny fibrous nodule was all that remained, if anything, two weeks after inoculation. Recently, we have observed that if the germfree animals are orally contaminated with a single species of nonpathogenic, slowly-growing *Micrococcus* isolated from conventional guinea-pig feces, and then are inoculated two weeks later, results resemble those obtained in the conventional animals. The presence of even a single "mild" species of bacterium appeared to stimulate seemingly nonspecific defensive mechanisms that were lacking or inadequate in the germfree animal. This animal-parasite system may provide a good device for the study of the role ordinary contamination may play in maintaining "natural" defense mechanisms.

As an example of the extra-Institute collaboration we attempt on a limited scale, we have just completed a study with NIDR in which it was demonstrated that bacteria are not essential for the development of periodontal disease in mice. This appears to be true, also, for the development of calculus on the teeth. It is to be recalled that bacteria were required to produce dental caries in the rat.

In collaboration with the Laboratory of Tropical Virology, we have initiated studies on the behavior of viruses in the germfree host. It was noted that germfree mice survived somewhat better than did conventional mice following inoculation with either dengue type 2 virus or Japanese encephalitis virus. The differences, though not great, were significant. This finding would be of special biological interest if noted with other viruses, particularly in view of the fact that germfree animals have tended to be more susceptible than the conventional ones when challenged with other types of organisms.

Germfree Mouse Kidney Tissue Culture

One of the most intriguing things noted in this collaborative project has come from the use of germfree mouse-kidney tissue culture. It was thought that tissue cultures from cells of animals which, for many generations, had not had to contend with a variety of infectious agents might possibly provide an especially favorable growth medium for viruses. If true, this might be more

easily demonstrable with viruses that do poorly in conventional mouse cell lines. In parallel tests with dengue type 1 virus (the Mochizuki strain), marked differences in virus multiplication and production of cytopathogenic effects were noted between the germfree mouse-kidney tissue culture and those prepared from mice of the NIH colony. In the latter CPE was usually equivocal and titers ranged from 2.5 to 4.5 log $TCID_{50}$. In the germfree animal cell cultures, CPE was marked and titers as high as 7.5 log $TCID_{50}$ have been obtained. We are now using mouse kidney cells from the LGAR conventional colony, derived originally from the germfree mouse colony, to assess the possibility that a genetic or strain difference is involved. In our first simultaneous trial of cells from the three groups of mice, both conventional lines gave about the same result, but viral growth and CPE were much greater in the germfree mouse kidney. Thus, it would appear that the latter provides a good system for detecting dengue (M) virus.

As yet, of course, we do not know whether or not this is a general phenomenon or merely peculiar to this strain of virus. It is likely, however, that additional viral agents will be tried on germfree animal cells. If such cultures prove, in general, to be an especially sensitive system, their use for the detection of viral agents in suspect clinical material may develop. In anticipation of this we plan to increase our supply of germfree mice.

Middle America Research Unit

During the year the Virus Section of MARU has grown from a field station into a research laboratory engaged in investigations which range from emergency workup of influenza epidemics to controlled laboratory studies of pathogenesis and experimental transmission of encephalomyocarditis virus in swine; nevertheless, the primary emphasis, as planned, has remained on the arthropod-borne virus diseases. The staff has applied, modified, or developed techniques utilizing most of the diagnostic armamentarium of a modern virus-research laboratory, including extensive use of several tissue-culture systems; animal and egg inoculation; immune-serum production; and serological techniques of neutralization, hemagglutination-inhibition, hemadsorption-neutralization and inhibition, and complement-fixation. To our knowledge the range of methods and techniques employed is not duplicated in any other laboratory facility in the Americas, outside of the United States and Canada.

Probably the most important activity now in full progress is the full-scale inquiry into the ecology of arthropod-borne viruses in Panama conducted in collaboration with the Gorgas Memorial Laboratory. Collections of mosquitoes and *Phlebotomus* flies have been made daily since November 1959. Of the approximately 200 arthropod pools processed and routinely inoculated into suckling mice (inoculations performed in duplicate at MARU and GML) and into hamster kidney-tissue culture tubes, several isolates have been made. Most significantly, two isolates were made from *Phlebotomus* pools, one of them of a virus quite unfamiliar in its biological characteristics. The 18-month study of the eastern equine encephalomyelitis virus in Panama resulted in elucidation of the role of this virus infection in horses and human beings in Panama and disclosed the presence of antibodies evidencing infection not only in certain birds and mammals, but also in reptiles. The encephalomyocarditis virus has been found to be the cause of a fatal disease in swine; its possible pathogenesis and transmission are being investigated. This serves as the first conclusive demonstration of this virus in Central America; the finding may lead to the establishment of a new important zoonosis. Interesting results are being accumulated by examination of the biweekly collections of rectal swabs from Panamanian infants under 3 years of age. This longitudinal study has disclosed a periodic fluctuation of certain enteroviruses related and unrelated to community illness. Epidemic influenza outbreaks in Panama (Type B), in British Guiana (Type A_2), and in Costa Rica (Both A_2 and B) were investigated and laboratory support of the Costa Rica national program of live polio vaccine administration was provided.

Histoplasmosis in Panama

During the year, approximately 50 cases of clinical histoplasmosis were confirmed by serological techniques and two fatal cases of disseminated histoplasmosis were confirmed by recovery of the organism from clinical materials (these

were in native Panamanians and represented the second reported fatality in a child and the first in an adult). Epidemological investigations of all confirmed clinical cases of histoplasmosis resulted in the first isolation of *H. capsulatum* from Panamanian soil around a dwelling in which two consecutive occupants developed clinical disease. The ecology and microecology of *H. capsulatum* remain unsolved; however, the organism has been repeatedly isolated from fresh soil during both dry and wet seasons. Naturally occurring histoplasmosis has been demonstrated in spiney rats and opposums by isolation of the organism from liver and spleen. These infections were accompanied by diagnostic serum complement-fixation titers. The approaching completion of the histoplasmin and tuberculin skin-testing program in the Canal Zone school system will furnish data on approximately 8,000 children between the ages of 6 and 19 years. The current pre-school-age program will supply data on the younger age groups.

Arthropod Borne Virus Studies

Certain significant advances in laboratory methods merit individual mention: (1) the development of a practical and specific flocculation test for the demonstration of arthropod-borne viruses and antibodies, (2) demonstration of significant differences in rate of reaction of viruses with homologous and heterologous antibodies which has resulted in practical application of the phenomenon to the heretofore uninterpretable tests of group reactive sera against various ARBOR viruses, (3) evaluation of several available tissue-culture systems as practical tools for propagation, quantitation, and isolation of certain anthropod-borne viruses, (4) a newly observed phenomenon of "hemonuclear-adsorption reaction" which may be useful as an index of the presence of virus in infected tissue-culture systems, (5) studies on the pathogenesis of Ilheus virus infection in adult and suckling mice and of its transmission using mice as hosts and *Aeges aegypti* mosquitoes as vectors.

Bacterial Diseases

The staphylococcal studies have been directed almost entirely toward development and standardization of tests to determine relative pathogenicity of staphylococcal strains, since such baseline measurements are essential to further basic studies on the several factors determining pathogenicity of a particular strain. With such a complex organism no single test will serve to characterize the potentials of a strain. It appears that tests determining minimal killing dose (for the mouse), minimal infective dose, and toxic activity, employed in conjunction with the others, may serve this purpose. While both bovine and human brucellosis in the United States are decreasing, worldwide brucellosis is one of the major infectious diseases with tremendous impact on agricultural economics as well as a significant cause of human illness. We continue to collaborate with other brucellosis centers in problems relative to diagnostic tests and taxonomy.

Malaria—Human

This country's commitment to the worldwide program of malaria eradication and the long-term interest in malaria contributed to a research effort, during the past year, largely directed toward problems identified with that disease. Special emphasis was given to malaria chemotherapy as applied to eradication. Inasmuch as regular facilities provide for only a minimum of clinical material, which continues to become less each year, facilities at the Federal Penitentiary at Atlanta, Ga., were again made available. Studies have been in progress there since April, and resulted in more specific knowledge of the action of drugs in man. For instance, single doses of chloroquine (Aralen) as low as 75 mg. (base) per week will result in complete suppression of Chesson strain vivax malaria even under conditions of repeated infection via mosquito bite.

Primaquine given as a single 30 mg. dose, weekly, or as a single 3.0 mg. dose, daily, beginning the day before infection by mosquito bite, did not prevent or suppress infection. The drug is sporontocidal at a dosage as low as 1.5 mg., single dose, daily, against either *Plasmodium vivax* or *P. falciparum*.

A strain of *P. vivax* obtained from the State of Merida in eastern Venezuela, where the population has been under mass suppression with pyrimethamine, was found to have a prepatent period of 13

to 17 days; the incubation period was also 13 to 17 days (data from 10 patients). The parasites of this strain were found to be resistant to a dose of 50 mg. and of 100 mg. of pyrimethamine. This was true whether the infection was induced by blood inoculation or by sporozoites.

Malaria—in Monkeys

Morphological studies of the exoerythrostrains of *P. cynomolgi* have been made which will be useful as guidelines in chemotherapy studies and in studies of *in vitro* growth. Growth has been seen from the fourth through the ninth day and mature parasites have been observed as late as the 120th day. Reaction of the host was lacking in most monkeys, but was found in one animal. Size changes through the cycle have been studied, and it was found that on a given day the size was consistent. Distribution of parasites was found to be uniform throughout the liver and fit the Poisson distribution well.

Cell lines have been established from monkey liver and other sources. Considerable success has been achieved in maintaining organized liver tissue *in vitro*. Good survival of organized fragments of adult monkey tissue has been obtained for as long as 10 days which is sufficient time for *P. cynomolgi* and other malaria parasites to complete an exoerythrocytic cycle. The ability to maintain liver is a major step and is basic to studies of the parasites *in vitro*.

Exoerythrocytic stages of *P. cynomolgi* have been grown *in vitro* for 2 and 4 days from fragments of tissue taken from heavily infected monkeys. The parasite increased greatly in size during the period of *in vitro* maintenance. The cytology of the parasites compares well with normal *in vivo* parasites. This may represent the first *in vitro* cultivation of mammalian exoerythrocytic parasites.

Monkeys treated with pyrimethamine prior to infection did not develop detectable exoerythrocytic parasites in the liver. Since parasitemia occurred eventually, in some of the monkeys, parasites must have survived at subpatent levels, in a form difficult to detect, or in tissues other than the liver.

When pyrimethamine treatment was initiated on the sixth day after injection of sporozoites, an effect was evident within 24 hours and marked after 48 hours. Subsequent to 48 hours, the parasites became vacuolated and disorganized. They showed signs of swelling and by 120 hours after treatment were so altered as to be hardly distinguishable. Very little cellular reaction developed, although occasionally mononuclear cells accumulated around the dying parasites.

When primaquine was given prior to infection, no parasites could be found in the livers. When primaquine was initiated on the sixth day of treatment, an effect was not discernible for 72 to 96 hours after treatment. At 120 hours, after treatment, many parasites appeared as dead, amorphous, dark-staining, shrunken masses without cellular reaction. Studies are now in progress to follow the "dying" parasites for longer periods.

Both primaquine and pyrimethamine retarded, then stopped the growth of the parasites. This resulted in keeping parasites in the liver at a time the majority would have ruptured and initiated blood infections.

Drugs—Mechanism of Action

The results using *P. gallinaceum* indicate that the drugs fall into groups on the basis of their mode of action. Thus, pyrimethamine and chloroguanide specifically inhibit DNA synthesis, the former under all conditions studied, and the latter only *in vivo*. The drug effect is reversible only by thymidine, indicating that the drug inhibits the enzymatic conversion of deoxyuridine to thymidine.

Drugs with a quinoline or acridine nucleus (quinine, chloroquine, quinacrine) inhibit respiration, RNA, and DNA metabolism apparently nonspecifically through interference with energy conservation by the parasite. Studies with quinine in greater detail revealed that the drug action is dependent on a high potassium-ion concentration surrounding the parasite, such as occurs inside the red cell. Thus, the action of quinine and related drugs on the malarial parasite and heart muscle may be similarly connected with potassium-ion metabolism or transport.

In studies involving *Pseudomonas sp.*, pyrimethamine inhibited growth, glucose utilization, and pyruvate utilization to approximately the same extent. At the levels tested, formate utilization was not inhibited. Of many substances

556044—60——19

tested, pyruvate itself was the only material capable of reversing pyrimethamine inhibition.

Preliminary experiments have shown that in the case of *P. gallinaceum*, the DNA content of infected cells appears to be no different from that of uninfected cells. On the other hand, the RNA content of infected red cells is five times that of uninfected chicken erythrocytes.

Drugs—Pharmacology and Lachesiology (Fate)

An *in vitro* system for chloroquine metabolism was found in rabbit liver homogenates. Chloroquine was metabolized at a rate of about 45 percent per hour per gram of tissue at pH 7.4 in an atmosphere of 95 percent oxygen. In the presence of SKF 525–A, Lilly 18947, and pyrimethamine at 1 x 10^{-4}M, chloroquine metabolism was inhibited 80, 90, and 100 percent, respectively. The metabolic activity of liver homogenate was virtually lost after dialysis but could be restored by the addition of a TPN–H generating system. One of the metabolic products is acetaldehyde and evidence suggests that both alkyl groups of the parent compound are oxidized *in vitro*.

Administration of metabolic inhibitors to rabbits in combination with chloroquine gave rise to higher blood levels and a prolongation of the half-life of the antimalarial drug. However, in most cases, metabolic inhibition beyond 48 hours could not be achieved because of the rapid turnover of the prolonging agent.

Drugs—Trials Against Intestinal Parasites

A total of over 300 cases of hookworm have been treated with bephenium (four different salts) employing a number of dosages and regimens. The more effective regimens, using the granular hydroxynaphthoate salt (Alcopara (R)) at 5 grams base per day, have produced worm reductions of 74 percent (5 grams total base), 93 percent (15 grams base), and 98 percent (25 grams base). The drug is almost completely curative against *Ascaris lumbricoides*, and higher dosages appear also to have some effect against *Trichuris trichiura*. Trials of the newer drug dithiazanine iodide (Delvex (R)) against *T. trichiura* and other helminths continue to confirm the effectiveness of this drug; results against other helminths (hookworm and *Ascaris*) have not been encouraging. Use of this drug, alone and in combination with the antihookworm drug tetrachlorethylene, in the long-term mass treatment of the population of an entire building has been carried out. The results are as yet incomplete. After the first 10 months of use of regularly repeated treatments with small doses, it appears likely that elimination of existing worm infections or elimination of transmission has not been achieved.

Drugs and Nutrition as Related to Schistosomiasis

Oral and parenteral regimens of 165 compounds were given to infected mice. Several compounds proved to be weakly schistosomacidal at tonic levels. Two of the compounds were more promising and were studied intensively. The phenylpiperazine (Hoechst S 688) previously found to be curative in mice and monkeys was found to have some prophylactic effect in dogs. An oral dose of the compound given on each of the 3 days following exposure prevented some of the *S. japonicum* from developing in dogs but this prophylactic effect was not observed in monkeys exposed to *S. mansoni*. The therapeutic index of S 688 was low in monkeys and dogs.

The second compound selected for trial was an antibiotic, S 1629. A regimen of several oral doses prevented one-half to three-fourths of the worms from developing in mice. In a prophylactic test, no eggs have been found in the feces of a treated monkey while they have been found in the nonmedicated control.

Helminthic Infections in Mice

The importance of nutrition in relation to self-cure and chemotherapy of helminthic infections has been shown in studies on mice infected with schistosomes and various nematodes and cestodes. Nicotinic acid deficiency enhances the susceptibility of mice to *Schistosoma mansoni* infections, but the worms are stunted and incapable of normal egg-production. Folic-acid deficiency, with or

without B_{12} deficiency, does not alter susceptibility of the host, although infection aggravates the resultant anemia, but decreases egg-production of the worms. Mice on a semisynthetic diet alone or supplemented with vitamin B_{12} apparently completely lost their oxyurid and tapeworm infections, or harbored stunted *Nematospiroides dubius*, as compared to controls on a Purina pellet diet. Of great significance is the finding that treatment with stibophen produced much higher cure rates of *S. mansoni* infections in mice on an enriched semisynthetic diet as compared to mice on a commercial diet. It is because of these very promising leads that we have initiated the Puerto Rican study on human beings infected with *S. mansoni.*

The importance of liver disease in parasitic infections has led to a series of studies of general medical interest on the mechanism of ammonia toxicity (121–M). It has been demonstrated that hypoxia markedly increases the toxicity of ammonia in mice. A large number of other factors have been studied but have had only slight effect.

Molluscicides

The development of a method for testing molluscicides against snail eggs rather than adult snails is an important technical improvement in this field. Also, the finding that ultraviolet light rapidly inactivates sodium pentachlorophenate is of great importance in explaining failures in the effectiveness of this compound under certain field conditions. Since NaPCP has been demonstrated to be about 10X as effective against snail eggs compared to adult snails, this suggests that low concentrations of the chemical may be useful in maintaining bodies of water snail-free.

Metabolic Changes in Parasitic Infections

Studies of the pathologic physiology of various protozoan infections, as revealed by histochemical techniques, have revealed that infections with *Trypanosoma congolense*, *T. equinum*, *T. equiperdum*, and *Bartonella muris* show individual peculiarities with respect to liver glycogen and lipid, and that such nonspecific factors as anoxia and adrenal involvement cannot be the only pathogenetic mechanisms. They indicate the possibility that the parasites produce specific substances that influence the observed changes. This has an important bearing in relation to research on chemotherapy and immunology.

The basic studies of the biochemistry of mitochondria which developed from earlier work on the effect of sodium pentachlorophenate on snails have been continued, with the demonstration that isolated mitochondria have an appreciable endogenous respiration in the absence of added substrate, and that this is linked with their stability. Only certain reactions of isolated mitochondria have been shown to be affected by the age of the animal; the oxidation of beta-hydroxy-butyrate is impaired with age, but not the subsequent oxidation of aceto-acetate. These studies now have a parasitologic orientation as well; it has been shown that the mitochondria isolated from rats parasitized with *T. equinum* are deficient in oxidative phosphorylation.

Toxoplasmosis

The work on toxoplasmosis has shifted to emphasis on the chronic stage of infection and on the cyst. The nature of the cyst wall, which is of considerable importance in relation to exacerbations during chronicity, has been shown by the electron microscope to be a complicated structure rather than a simple membrane. The occurrence of congenital transmission during chronicity has occurred most frequently with parasite strains that produce abundant cysts and also late parasitemias. Such parasitemias are seen in mice, rabbits, and guinea pigs, but not in rats; in the rat, cortisone occasionally induces parasitemia. The injection of hyperimmune serum into chronically infected mice results in some increase in the number showing parasitemia. This may be of clinical importance in relation to the effect of reexposures to the parasite on already existing chronic infections. Additional progress in evaluating the usefulness of the hemagglutination test in various animals has shown that the sera of monkeys, pigeons, and sheep give good results as compared with the dye test, while sera of rats and dogs do not. Of various fractions prepared from the hemagglutination antigen, one appears to be stable on storage.

Axenic Cultivation

The axenic cultivation of *Entamoeba terrapinae* for the first time, and of *E. invadens*, in an improved medium without particulates, represents an important step towards the goal of cultivating *E. histolytica* without associated microorganisms. Some work on *E. histolytica* has indicated that the mammalian embryo extracts useful for *E. terrapinae* and *E. invadens* may be of value as substitutes for living cells. The usefulness of fluorochrome stains for identifying DNA and RNA in protozoan cells has also been exploited in identifying DNA in the endosome of amebae and differentially staining the parabasal body of a trichomonal. One of the most promising developments in this project is the development of cryogenic techniques for storing parasitic protozoa. This will eventually reduce the burden of strain maintenance and also preserve the characteristics of strains.

Immunology*

The Chief of the Laboratory of Immunology was appointed on January 2, 1957, and the fiscal existence of the Laboratory began on April 5, 1957.

It seems appropriate at this time to look back and take a view of the activities of the Laboratory of Immunology since this may disclose its aims and policies as well as how close we came to fulfilling them. Although the Laboratory of Immunology is one of the newest units of the NIH, its activities represent the oldest and one of the most significant basic and applied subjects in which the NIH has been engaged. Mention may be made of the international standardization of biologics that received many theoretical and practical contributions from the NIH. The basic studies of many aspects of experimental anaphylaxis—conducted half a century ago by Rosenau and Anderson—still exert influence on our thinking on problems of anaphylaxis and certain aspects of allergy not recognized during their scientific epoch.

In starting to organize a laboratory, obviously the main directive is the aim of the Laboratory as expressed in its title although this is broad and has many aspects. The nonclinical part of the Laboratory of Immunology started its activity in April 1957. Our numerous attempts to initiate a section of clinical immunology until lately were not successful. However, it now appears probable that in July 1960 this section will begin to operate.

Selection of personnel (10 professional) was guided by the nature of the problem—availability of space, salaries, and facilities. Workers with experience, who had proved their ability in immunology or in allied fields, and promising beginners were sought. The latter were included with the hope that they would benefit by working on problems suggested to them or on their own problems. It was hoped that these investigators would soon reach some independence, while benefiting through contact with experienced investigators. Other workers, depending on interests and experience, would work on problems alone or in small groups. This approach was favored because, in general, it is unlikely that persons with independent minds would always be satisfied to be part of a research group. The situation is strikingly different, of course, when the members of the groups represent different disciplines.

Most of the problems currently studied represent continuation of work begun some years ago. There are a few new problems or subjects which are being attacked by a conspicuously new method. The most important of these is the introduction of genetically distinct, highly inbred guinea pigs into immunologic research work. The initiation of such studies is of particular importance in delayed type of hypersensitivity studies, for which the guinea pig is uniquely suitable. In the guinea pig, high degrees of local (skin, cornea, snout) and systematic (anaphylaxis—protracted type and delayed type, i.e., tuberculin type) reactions can be produced. In the other experimental animals, these types of reactions may occur only in low degree or not at all. Furthermore, some of these reactions may be difficult to distinguish. For example, the delayed type of skin reaction may be exquisitely intense in the guinea pig and mild in the rabbit and hardly demonstrable in the mouse or rat, using the same preparatory injections.

It appears now that without the use of highly inbred mice certain problems of transplantable tumors could not be profitably studied. Similarly,

*Report prepared by Dr. Jules Freund, Chief.

the use of two highly inbred families of guinea pigs, No. 13 and No. 2, were demonstrated to be essential for homotransplantation and isotransplantation studies of the skin. The transplantation studies of the skin are only one of very many immunologic subjects to which inbred guinea pigs may be applied. It is very likely that transfer of many kinds of cell-born, passive sensitization can be achieved using inbred guinea pigs. The chief of the Laboratory suggested experiments to determine whether isotransplantation of the skin within the inbred families and crossing on another would yield the results one would expect. These families have been inbred for about fifty years. The chief of the Laboratory's hopes rested on the gross morphologic specimens of tuberculous guinea pigs of families No. 2 and No. 3 which he examined in 1926 at the Henry Phipps Institute of the University of Pennsylvania. These guinea pigs were infected with virulent human tubercle bacilli. When they were killed or died of tuberculosis, the lesions were found to be very similar in the guinea pigs of the same families, while very dissimilar in the animals of family 13 to the lesions found in family 2. There was an interesting relationship found in these guinea pigs with regard to resistance to tuberculosis and antibody formation. Lewis and Loomis observed that tuberculous guinea pigs of family 13 produced antibodies more abundantly to sheep red cells and killed typhoid bacilli than those of family 2.

The experiments on isotransplantation of mouse skin with the aid of inbred families have been successful. Other problems on the transfer of hypersensitiveness to organs are being investigated.

Treatment of Fungus Infections

With the increasing interest in infections due to fungi, it is noteworthy that important advances in treatment are being made by the clinical group. The number of patients (40) at the Clinical Center now treated with amphotericin permits the conclusion that this drug is the first effective treatment for some cases of cryptococcal meningitis and is a superior therapeutic agent in blastomycosis and histoplasmosis. A still newer antibiotic drug, known only by code number, under exclusive study at the NIH has been demonstrated in the laboratory to be even more effective than amphotericin in experimental infections in mice due to *Histoplasma* and *Blastomyces*. The use of this drug in 12 patients with a number of infections suggests that it may be superior in human infections due to these same fungi.

Clinical Studies of Viral Diseases

In studies of viral diseases, our interest has more and more focused on possible involvement of the kidneys in infection. In addition to clinical and laboratory studies, appropriate to the diseases (respiratory, aseptic meningitis, etc.), special efforts have been devoted to search for virus in urine. In the past 2 years, six enteroviruses have been found in urine of patients and five of these urinary isolates have been studied in animals. Viral content of mouse kidney at certain times after inoculation is of sufficient titer ($10^{2.5}$ to $10^{4.0}$/mg.) to be poorly, if at all, explained by possible concurrent viremia. On the other hand, recent herpes and vaccinia isolates from man have not been found in rabbit kidney following intravenous inoculation. Virologic studies of urine are similarly being performed to evaluate the significance of urinary cells containing inclusion bodies. Studies of 57 hospitalized patients with ECHO type 9 seen during the summer of 1958 have correlated virologic and clinical aspects of this disease.

Staphylococcal Problem

One aspect of the alarming staphylococcal problem is under intensive study here. Investigations into the production and inhibition of penicillinase, encountered invariably in resistant staphylococci isolated from man, have revealed a wide variety of factors which influence both the basal level of this enzyme in *Staphylococcus aureus* and the greatly increased formation of enzyme after treatment of the organism with penicillin. Production of the enzyme in a chemically defined medium containing relatively few amino acids has been shown to be independent of bacterial multiplication and to be inhibited by analogues of some of these essential amino acids. Since penicillinase is considered to be the principal basis for the present ineffective-

ness of penicillin in staphylococcal infection, it is clear that a knowledge of factors controlling its production might yield practical benefits in therapy of this infection.

Studies of another important bacterial infection in man, *Salmonella*, have centered around the carrier state, which complication continues to be a problem despite otherwise effective antibiotic treatment. Stones recovered at cholecystectomy in 11 patients have been found infected in every instance, whereas gallbladder wall, liver, and bile were less frequently culturally positive. As a result of these studies, five stones from three carriers were implanted into the gallbladders of five rabbits. *Salmonella* was subsequently and repeatedly isolated from stools for periods now exceeding 7, 6, 6, 4, and 2 months.

Antibodies in Ascitic Fluid

Extension of the technique devised in this Laboratory for the production of potent antibodies in ascitic fluid of mice has shown difference in response of some strains of inbred mice. In addition, plasma-cell tumors have been induced by this method in three BALB/CAnN mice. This method has proved suitable for the production of viral antibodies at a great saving in time and number of animals necessary. Continuing studies of patients with lupus erythematosus and agammaglobulinemia have revealed that with a much more sensitive technique of measurement patients with the latter disease do have detectable antibody to a number of enteroviruses. This provides a partial answer to the intriguing observation that patients with this disease seem to handle most viral illnesses well and without recurrence—in contrast to their experience with bacterial disease.

Filariasis

Parasitic disease research this past year has been most productive in the problem of filariasis. Transmission of the disease has been shown to occur at low levels of microfilarial density (e.g., 1 microfilaria/20 mm^3 of blood). *Mansonella ozzardi* infections have been shown to respond less well than *Wuchereria bancrofti* to diethylcarbaniazine treatment, and tetracycline has been shown to concentrate in the adult *Loa loa* worm as well as in *Dirofilaria immitis* and *Ascaris lumbricoides*. The avidity of filarial tissue for tetracycline is quite remarkable to observe. The phenomenon is distinguishable *in vivo*, which would suggest a method of diagnosis of the disease. Further, by chemically combining tetracycline with an active antiparasite compound, effective therapy might be achieved.

Lupus Erythematosus

In association with the Laboratory of Immunology, members of the staff of LCI have devised and evaluated a test for systemic lupus erythematosus. The test is based on a reaction of lupus serum with purified animal deoxyribonucleic acid and it uses the volcanic ash, bentonite, as a vehicle for precipitating the reactants. The results of this test are not identical with those of the conventional lupus-cell preparation since they do not so far show significant cross-reaction with patients with rheumatoid arthritis. An extensive survey among nonlupus patients revealed almost no false positives. Thus, a positive DNA-bentonite test strongly indicates the diagnosis of systemic lupus erythematosus. This development will have wide application because of its high specificity and ease of performance. At a basic level, it appears to reveal a hitherto unidentified reaction of lupus serum. Many consider that this and the other reactions of lupus serum are indicative of a complex autosensitization to the hosts' own nuclear components.

Biology of Viruses

Significant scientific findings in the biology of viruses represent the beginning of systematic elucidation of individual facets of larger problems and the development of technical and procedural tools for future use. Under the former come the studies indicating two mechanisms of virus-cell association after adsorption, the mode of action of certain virus inhibitors, the intracellular carrier infection with typhoid organisms and their susceptibility to antibiotics, the localization of increased metabolic activity in tumor virus infected cells to perinuclear microsomes, and the resistance of ICM virus carried in tumor trans-

plants to the immune mechanisms *in vivo*. Technical developments include development of methods for continuous observation of cells in tissue culture by phase-contrast microscopy and the better preservation of metabolic activities of subcellular elements.

Cell Biology

The work of the Laboratory of Cell Biology is developing along several lines. The seven "nonessential" amino acids, which the cell can synthesize in amounts sufficient for optimal growth, have been found to fall into three sharply defined groups. Aspartic acid, asparagine, glutamic acid, and proline derive their carbon almost wholly from the glutamine of the medium. Of the remaining three nonessential amino acids, serine and glycine are metabolically quite distinct from alanine. When cells are grown in a glucose-free medium on ribose and pyruvate, the serine and glycine derive almost wholly from ribose, and the alanine almost wholly from pyruvate. The α-amino group of all the amino acids derives directly from that of glutamic acid. A large number of other transaminases known to be present in these cells are therefore physiologically inactive in these cell cultures.

A paradoxical finding of major interest is the fact that certain growth factors are rigorously essential for growth, despite the fact that they can be synthesized by the cell in amounts which should suffice for survival and sustained multiplication. One is inositol, which the cells can make from glucose; a second is cystine, which the cells make from methionine and serine; and certain mouse cell lines require pyruvate, despite the fact that the cells carry out active glycolysis, and are thus continuously making large amounts of pyruvate.

One cell line has been found which does not require added inositol, and which actually liberates small but significant amounts into the medium. This cell, growing on one side of a cellophane membrane, release sufficient inositol into the medium to permit the growth of a second inositol-dependent strain, growing on the other side of the membrane. This example of parabiosis may serve as a useful model for the complex metabolic inter-relationships of different cells and different organs *in vivo*.

The puzzling nutritional requirement for serum protein by almost all cell lines, despite the fact that this protein is not used to a significant degree for the synthesis of cell protein, is now nearing solution. Human and animal cells are being grown in a protein-free medium separated by a cellophane membrane from the same medium supplemented with serum protein and proteolytic enzymes. It is obvious that essential growth factors are being provided which are either initially bound to the serum protein, or are formed from it on proteolysis. The identification of the specific enzymes concerned and of the types of protein which can release the active growth factor(s) is under continuing study.

Biosynthesis of Virus

The second major line of investigation concerns the biosynthesis of the virus macromolecules, with particular reference to the precursors used by the cell, and the time course of their synthesis relative to the appearance of mature virus. These studies have been made possible by the use of highly radioactive precursors and the development of techniques for the rapid purification of large amounts of poliovirus, grown in suspension cultures of Hela cells. The results to date indicate that most of the viral protein and nucleic acid are synthesized at about the same time, simultaneous with the appearance of mature infectious virus. Both the viral protein and nucleic acid are synthesized from the small molecular-weight components of the acid-soluble pool of the cell, rather than by the breakdown of the preformed cellular protein and nucleic acid. In the case of vaccinia virus, however, most of the nucleic acid is synthesized within six hours after infection, at a time when only small amounts of mature virus have appeared, and approximately six hours before virus formation is completed. The significance of these findings is under continuing study.

NATIONAL INSTITUTE OF DENTAL RESEARCH

INTRODUCTION

In the simplest terms of definition and viewed in a broad sense, the mission of the intramural program of the National Institute of Dental Research is to contribute with all the means at its command to an ever-expanding knowledge of oral health and disease; this to the end of insuring ever-improving oral health for all the people. To pursue this goal, a variety of resources, including high-caliber scientific manpower and technological equipment, are available, in striking contrast to the early days of dental research when responsibility was primarily in the hands of individual practicing dentists and manufacturers of dental materials.

It has been said that dental research, as well as other areas of biological research, probably begins with such questions as how to treat and how to prevent departures from normal in certain structures and functions. Although this concept encompasses structural components and related physiologic and pathologic processes, it should be emphasized that the broadening scope of responsibility of dental research also counts heavily on the epidemiological, sociological, and physical sciences. Thus, in its total intramural activity, the Dental Institute is concerned not only with the cause and control of dental caries and periodontal disease, but also with a variety of other conditions and malformations of the mouth and adjacent structures which include oral cancer, cleft palate and lip, oral manifestations of systemic disease, and the influences of oral diseases on other organ systems of the body.

This program is a far cry from the modest beginning of dental research in the U.S. Public Health Service. Initiated in 1931 as a Dental Hygiene Unit, consisting of a single dental officer, its primary function was to apply principles of epidemiology to a series of community studies on mottled enamel. Very shortly, this activity was expanded into a full-scale epidemiological program designed to document the effectiveness of fluoridation as a caries-control measure. Beginning in 1936, the dental research program was broadened to include animal studies on the physiology and toxicology of fluoride compounds. Understandably, many of these investigations led away from fluoride and into basic research on the causes and prevention of a variety of diseases involving the teeth and surrounding structures.

Staffed today by 50 principal investigators and approximately 70 supporting personnel (excluding administration and the grants program), the various organizational units of the Dental Institute cover a wide range of research activity, constituting today one of the world's largest centers devoted to the study of oral health and disease. Augmenting this professional staff of biochemists, histologists, anatomists, microbiologists, pathologists, epidemiologists, geneticists, and clinical investigators—all with varied backgrounds of educational and research experience—is a small but effective group of visiting scientists from Japan, Denmark, England, and Germany. It is not too optimistic to anticipate that the continued encouragement and support of such collaborative research ventures with outstanding scientists from both the United States and abroad, and the parallel arrangement for assignment of NIDR scientists to other research centers (currently limited to assignment of one scientist at the National Institute for Research, University of Reading, England, and another at the California Institute of Technology) will bring mutual benefit to all participants, and possibly contribute to earlier major breakthroughs in the oral-health field. Exemplifying this international exchange of high-level investigators in key areas of scientific endeavor is the recent temporary appointment of Professor Albert E. W. Miles to our professional staff. Having served many years as Professor of Dental Histology and Pathology at the London Hospital Medical College, Professor Miles brought to our Institute a wide experience on the subject of calcification and an expert knowledge in the field of forensic medicine which are currently being applied to important research on the identification of age changes in dentition.

The Visiting Scientist Program demonstrates an additional responsibility to provide preceptorship training of a fellowship type to the fledgling researcher who will be the mature scientist of tomorrow. Several such research associates have received temporary appointments in various sections of the Dental Institute to acquire skills in new research techniques and to profit from association with experienced and eminent scientists. Hopefully, the benefits of these experiences will be carried back to the respective home institutions and thereby contribute to a more effective program of interinstitutional and international cooperation in oral-health research. In the fellowship category of the Visiting Scientist Program may be listed Dr. Shosaburo Takuma, from the Tokyo Dental College, who, during his preceptorship tenure with Dr. D. B. Scott, pursued an electron-microscopic study of the early stages of bone development that led to significant observations of the stages of osteogenesis from earliest beginning to the point at which mineralization begins. Another research associate, Dr. Ellinor Weiss, from the Institute for Physical Chemistry, University of Basel, Switzerland, completed a joint assignment with Dr. F. J. McClure, NIDR, and Dr. H. Eagle, NIAMD, that culminated in the preparation of many new and unusual compounds, including the first unsymmetrical peptide of cystine to be synthesized.

In the overall program activity of the Dental Institute, a compromise in research philosophy or methodology is necessarily reached as the respective advantages and disadvantages of so-called directed team research are weighed against the independent worker whose direction and pursuit of leads are limited only by his own imagination and competence. In this atmosphere of scientific freedom, it is essential that periodic review and assessment of progress be made in order to provide a judgment for expansion or curtailment of certain research activities. Because our research to date has demonstrated that the major oral diseases, namely, dental caries and periodontal disorders, are probably related etiologically to a complex multiplicity of factors, a principal effort has been directed toward the isolation of each of the various suspected causes. In anticipation of the vigorous and broad frontal attack that would be required to accomplish even a small part of this objective, a number of scientists with varied and specialized skills joined forces. It was in such a manner that, in order to insure the greatest flexibility of research project administration, a new section (Gnotobiotic) was established in the Laboratory of Microbiology. The early demonstration by this study group of the caries-initiating role of various strains of streptococci in germfree rats is testimony to the effectiveness with which its members utilized their collective imaginations, perseverance, and technical skills.

Somewhat different criteria were considered in establishing another new section on Medical Investigations in the Clinical Investigations Branch. Recognizing the responsibility of the Dental Institute for covering the broad aspects of dental research with emphasis in the area of oral medicine, competent medical personnel were made available to evaluate correlations between oral and medical illnesses; to collaborate in studies of the general pharmacologic activities of drugs useful in the treatment of dental disease; to make observations of the physiologic actions of preanesthetic and anesthetic agents; and to provide consultation to the Institute's genetics program in correlating pedigree data with physical findings so as to determine modes and linkage of inheritance.

Although, for purposes of effective administration, staffing, and budgeting, the Dental Institute operates within an organizational framework of laboratories, branches, and sections, its principal objective of encouraging and supporting meaningful research can only be met by a day-to-day conduct of activities governed by program areas rather than departmental lines. An analysis of the overall Institute organization makes it possible to present an outline prospectus of the major program areas constituting this research effort.

A. Genetic Studies
   (1) The investigation of hereditary dental and oral defects in humans and their relation to other systemic diseases of hereditary character.
   (2) Experimental animal studies to gather additional information on the relation of hereditary factors to caries and periodontal disease.

B. Epidemiological Studies
   (1) Field investigations to amplify our knowl-

edge of the relationship between oral diseases and nutrition, aging, sex, and other factors.

(2) Continued studies of fluoridation.

C. Studies in the Area of Clinical and Experimental Pathology

(1) Histochemical and morphological studies of diseases of the soft and calcified tissues of the mouth.

(2) Microbiological studies of dental caries, periodontal disease, and other oral conditions.

(3) Investigation of local-systemic factors in oral diseases (periodontal disorders, aphthous stomatitis, chronic diseases, and aging).

D. Germfree Studies

The utilization of germfree technics for determination of the specific microbial relationships to dental caries and periodontal disease.

E. Nutrional and Biochemical Studies of Dental Caries and Periodontal Diseases

F. Growth and Development Studies

(1) Clinical observations of the normal pattern of oral and facial development.

(2) Experimental studies on the pathogenesis of cleft palate and other congenital malformations.

G. Non-disease-oriented Basic Research Programs

(1) Biophysical, biochemical, and physiological studies on calcification, collagen, saliva, and blood clotting.

(2) Electron and X-ray microscopic studies on the structure and physical properties of bone and teeth.

(3) Studies of fluoride metabolism.

Anticipating the expanded facilities that will be provided in the new dental research building in fiscal year 1961, considerable attention has necessarily been given during the past year to the formulation of plans for the best utilization of scientific manpower and equipment. Thus, a continuing series of weekly planning sessions has been held with the laboratory, branch, and section chiefs of the Institute in order to discuss and define research goals, and outline methods for the fruitful pursuit of these objectives. Obviously, this undertaking has required a careful appraisal of current programs of research so as to establish a baseline upon which to make reasonable assumptions of future trends, and to construct and reconstruct areas of promising productivity.

In parallel fashion, the NIDR Board of Scientific Counselors, charged with responsibility for advising on intramural programs in matters of general policy, particularly from a long-range viewpoint, has given consideration to several important areas. In so doing, the following actions and recommendations have been made.

A. The Board recommended that consideration be given to NIDR's interest in establishing a low-level, radioisotope-counting facility at NIH suitable for strontium-90 assays in foods, excreta, and calcified tissues, and permitting surveys on intake, retention, and elimination of radioisotopes derived from the "fallout". Since the health problem, especially with respect to strontium isotopes, appears to be mainly related to the calcified tissues, the Board recommended further that the low-level counting facility be established as a service function for biological studies.

B. The Board hoped that plans for expansion of the Clinical Investigations Branch will come to fruition in order to provide an increased fund of knowledge on the interrelationships of dental and systemic diseases. This hope was optimistically expressed insofar as the patients in the Clinical Center, and the facilities of the Center, furnish a unique opportunity for human studies in the dental field.

C. The Board observed that NIDR has an outstanding opportunity to work in the field of growth and development. The facilities and multiple disciplines available at the National Institutes of Health suggest that the Dental Institute might devote a major share of attention to basic approaches to the many problems inherent in studies of growth and development. While also endorsing the pursuit of clinical activities, the Board believed that treatment aspects should continue to be deemphasized.

D. The Board suggested that a fruitful area of research might be initiated through the purchase of cine-fluoroscopic equipment for the conduct of such studies as temporomandibular joint function. The success of this activity would necessarily depend upon the recruitment of a well-qualified scientist to supervise the program.

E. With the expansion of facilities that will become available upon the completion of the new building for the NIDR, the Board suggested that consideration be given to revision of the Institute's administrative organization to allow better integration of existing programs of investigation with new programs soon to be undertaken. Activities in biophysical studies of dental tissues having given important results, there is need to enlarge their scope through newer technical methods, now available, and through additional specialists in the examination of calcified tissues with physical methods. On such grounds, the Board advised on the need to establish, as soon as feasible, a Laboratory of Physical Biology.

Laboratory Research Activities

During the past year, major attention in the Laboratory of Biochemistry was given to the analytical and structural aspects of collagen, the biochemistry of saliva, mineral metabolism, proteolytic enzymes, and the broad field of experimental caries.

Inasmuch as collagen is the major protein of teeth (dentin), as well as skin, tendon, and bone, it may well play a role in the function of these tissues and in their pathological states. Although the data assembled, to date, are basic and fundamental, there is the ultimate objective of application of such acquired knowledge to the problems of dental caries and periodontal disease. Thus, amino-acid analyses by Dr. K. A. Piez on a number of collagens from invertebrate phyla have shown them to resemble vertebrate collagen in that they contain hydroxyproline and hydroxylysine, and one third of the residues are glycine. While in other respects they are quite different (the differences possibly being related to function or structure at the tissue level), similarities are in accord with the requirements of the structure of the molecule. Other significant findings suggest that thermal denaturation is associated with the total content of pyrrolidine rings rather than with the hydrogen bonding by the hydroxy group of hydroxyproline.

In studies on the physiology of the salivary glands and the biochemistry of their secretions, it has been shown by Doctors E. F. Geever, I. Zipkin, F. J. McClure, and M. S. Lewis that a lysine deficiency in the diet of experimental rats, aside from growth influences, produced no fundamental changes in the glands and no alterations in certain protein constituents of saliva. Thus, the promotion of experimental dental caries by lysine-deficient diets does not appear to involve either the salivary glands or their secretions. Other biochemical studies of saliva have evolved a reliable method for the analysis of two essential amino acids, i.e., tyrosine and tryptophan, which are currently being evaluated in terms of possible relationship to disease within the oral cavity. In a correlative approach designed to study the metabolism of the oral flora as related to the salivary gland, it has been found by Doctors H. Blumenthal and T. Shiota that the hexosamines, which are constituents of salivary polysaccharides, are metabolized by lactobacilli casei with the liberation of ammonia and the formation of lactic acid. Such release of ammonia by the submaxillary gland of the rat is indicative of the presence of adenosine triphosphate, a pyrophosphate, or adenosine deaminase within the metabolic turnover of the gland.

A much-favored hypothesis for the cause of dental caries is abnormal mineralization of dentin and enamel. Repeated studies by H. G. McCann, involving extreme variations in dietary calcium, phosphorus, and magnesium, have shown a significant influence on the proportions of these elements, as well as of carbon dioxide, in the teeth of rats. Of considerable significance is that these variations in the inorganic chemistry of dentition did not coincide with the incidence or severity of the caries experience. On the other hand, additional data obtained by Dr. F. J. McClure continued to verify the cariostatic effect of certain phosphate compounds, whereas a calcium mineral supplement to the diet has given inconsistent results. The presumed implication of these studies is that some fundamental inhibitory reaction occurs locally, perhaps on the exposed surfaces of the teeth.

Dr. R. H. Larson has demonstrated that the disposition of an experimental animal (the white rat) to develop dental caries is dramatically affected by treatment during gestation, or during a part of lactation, with a broad spectrum antibiotic, tetracycline. The mechanism of this action appears referable to the oral flora, although its

significance extends to the influence of prenatal and developmental environment on oral disease.

Collaborating with the National Bureau of Standards in studies of mineral metabolism, Dr. R. C. Likins showed that the comparative renal clearance of calcium 45 and strontium 89 is presumably affected by the nutritional status of the animal; and that the skeletal metabolism of these tracer elements materially affected their excretion in the feces and urine. While the relative amounts of free and protein-bound Ca^{45} or Sr^{89} appear to be unaffected by inanition, possible differences in the binding of these ions by smaller molecules are being investigated by membrane-dialysis techniques.

Demonstrating the essential component of non-disease-oriented research in the total institutional activity are the basic studies on protein and enzyme chemistry by Dr. J. E. Folk in collaboration with Doctors K. Laki, J. A. Gladner, Y. Levin, and W. R. Carroll of NIAMD. Nearing completion during the past year was the final purification and further elucidation of the chemical structure and essential kinetics of the new pancreatic enzyme carboxypeptidase B. Employing this enzyme as a valuable tool in both natural and chemically modified protein structure research, there has been gained a better understanding of the biochemistry of blood clotting. This information should open the way to a more systematic pharmacological and clinical approach for the correction of certain abnormal blood-clotting reactions.

While this year marked the termination of a 15-year study of dental caries as influenced by water fluoridation in Grand Rapids, Michigan, investigation continues concerning the non-dental aspects of water fluoridation. Inasmuch as the content of fluoride in skeletal tissues may be increased with an increased fluoride intake, the stability and variability of this bone fluoride deposition was the subject of study by Dr. I. Zipkin. The several different bones in the body of the experimental white rat varied significantly in their capacity to retain fluoride. Likewise, following withdrawal of fluoride from the drinking water, an average of 7.5 percent of the bone fluoride deposit in the case of the growing animal, and 11.5 percent in the mature animal, was eliminated in a relatively short time.

The research program in oral microbiology continued in 1959 to center on dental caries, periodontal disease, and associated studies in systematic microbiology, microbial physiology, and germfree techniques.

Based on a series of highly significant studies by Dr. P. H. Keyes, Laboratory of Histology and Pathology, in which so-called "resistance" to dental caries was shown to be partly due to the absence of a necessary microflora, a concerted effort was directed toward further understanding of microbial factors in caries etiology and transmissibility of this disease. In a collaborative study, Dr. R. J. Fitzgerald (Gnotobiotic Section) and Dr. P. H. Keyes demonstrated conclusively for the first time a specific bacterial factor in dental caries. Utilizing various refined techniques, including a mouth prop developed by Dr. A. A. Rizzo, it was found that certain kinds of streptococci isolated from various lesions in hamsters could be implanted so as to cause caries in previously "caries-resistant" hamsters. Prior to the experiments, the infecting microorganisms were made streptomycin-resistant; it was therefore possible to isolate and positively identify them during and at the end of the test periods. Thus the fundamental Koch postulates, which require uncomplicated infection, demonstrable disease, and final recovery of the infectious agent, have been shown unquestionably to apply in the case of caries. It should be noted at this point that the infecting microorganisms are very similar to those previously shown by Dr. Fitzgerald to produce caries in otherwise germfree rats. When "caries-resistant" hamsters were experimentally infected with a variety of other streptococci, lactobacilli, and diphtheroids, no carious lesions developed. The results of these important investigations clearly establish caries in hamsters as a transmissible, infectious disease and encourage renewed search for specific microbial agents in human caries. In the latter connection, timely new data obtained by Dr. A. Homell, Jr. substantiates the predominance of actinomycetes in certain human carious lesions.

In related investigations on dental caries, it has been found by Dr. H. V. Jordan, Jr., that incorporation of an aldehyde- and ketone-binding compound, sodium metabisulfite, in a cariogenic diet, reduces the incidence of caries in rats by from 33 to 77 percent, probably by alteration of

the metabolism of caries-producing bacteria. Thus, subbacteriostatic concentrations of sodium metabisulfite were noted to strongly reduce acid production by streptococci growing *in vitro*, whereas carbon dioxide and nonacid products such as ethanol increased correspondingly.

Definition of the role of microorganisms in periodontal disease should be facilitated by study of experimental infections with oral microorganisms, as developed recently by Doctors E. G. Hampp, S. E. Mergenhagen, H. W. Scherp, and R. R. Omata. Based on these investigations, methods have been evolved to follow the pathogenicity of each of the major kinds of bacteria implicated in periodontal disease, alone and in combination with each other and with ordinarily nonpathogenic oral symbionts. For example, the long-suspected but never conclusively demonstrated mutual enhancement (syngergism) of oral fusobacteria and spirochetes was substantiated. Ancillary studies revealed oral microbial products that might mediate periodontitis: (a) classic glucolipid endotoxins were isolated from fusobacteria, spirochetes, veillonellae, and selenomonads but not from anaerobic and viridans streptococci or diphtheroids; (b) spirochetes exhibited protease and peptidase but not mucopolysaccharase activity; and (c) cultures of gingival accumulations were shown for the first time to digest an undenatured collagen.

Since deposition of dental calculus on a microbial matrix is generally regarded as a critical determinant of periodontal disease, it is significant that Dr. P. N. Baer in collaboration with Dr. W. Newton (NIAID) reported the presence of tartar-like deposits on the teeth of germfree mice accompanied by periodontal disease. Subsequently, Dr. R. J. Fitzgerald and E. G. McDaniel (NIAMD) found that calcified deposits on the molar teeth of germfree rats contained the same inorganic component, hydroxyapatite, as human calculus. This experimental situation affords opportunity to evaluate the influence on calculus formation of such factors as diet and host physiology without interference from the as yet uncontrolled oral flora.

Viral studies directed by Dr. H. W. Scherp, with material from the Gnotobiotic Section, accumulated evidence of the occurrence in salivary glands but not other tissues of germfree rats of a virus cytopathic for certain kinds of cultured mammalian cells. An apparently similar agent was demonstrated in the salivary glands of conventional rats. Other studies established the basis for a serological grouping of strains of herpes simplex virus.

Studies of the nutritional interactions of the oral flora progressed significantly. Dr. T. Shiota demonstrated the synthesis of a phosphorylated folic-acid derivative by oral lactobacilli. Dr. T. A. Nevin showed that the symbiotic stimulation of growth of a spirochete by a diphtheroid depends on synthesis by the latter of the unstable growth factors, thiamine pyrophosphate and acetyl phosphate. Dr. Nevin also determined the pathway of arginine metabolism, which seems to be focal for this spirochete.

During the past year, studies with the electron microscope and related physical tools continued to be directed toward bringing forth further basic information on the formation and calcification of bones and teeth, and on their ultimate structure in the fully mature state. Prominent among the significant accomplishments of Doctors D. B. Scott and M. U. Nylen may be mentioned observations on the manner in which enamel formation takes place, and the collection of increasingly precise data on the exact size, shape and distribution of the crystals in both mature and developing calcified tissues. Based on these studies, it now appears that some of the concepts of amelogenesis, established earlier by optical microscopy, will be subject to revision. For example, there is evidence that (a) the first inorganic crystals appear immediately following matrix deposition, with organic material actually found within the crystals themselves; (b) the enamel matrix may be formed in a complex fashion with interrod matrix appearing between the distal ends of the ameloblasts and with the proximal ends subsequently splitting away as parent cells, leaving the isolated distal portions to change into rod matrix; and (c) the so-called distal terminal bar system is not an artifact, as previously suggested by some investigators, nor is it a true terminal bar system although it may appear as such in the optical microscope. Instead the substance responsible for the optical image is actually identical with the initial interrod matrix mentioned above. A true terminal bar system, on the other hand, is

found in the proximal ends of the cells and comprises a morphological structure that makes unlikely any direct contribution of stratum intermedium cells to enamel matrix formation.

Another important facet of the laboratory activity employing biophysical methods has been the wide scope of collaborative studies with investigators from other categorical institutes and from outside laboratories and institutions. Some that might be cited are: (a) studies of calcareous corpuscles in tapeworms (with Dr. T. von Brand, NIAID); (b) development of technics for microradiography of ectopic calcifications and tumor formation (University of Texas); and (c) studies on calculogenesis (Eastman Dental Dispensary, Rochester, N.Y.). These interdisciplinary and interlaboratory activities have been fruitful not only because of the new information yielded but also because of the broadened experience of the workers involved.

As alluded to in a previous section of this report, the training of research associates in the fellowship category of the Visiting Scientist Program, as well as the training of guest workers, is a most important segment of the Institute's responsibiilty. With some trainees terminating their assignments at the close of this year, arrangements have been made to accept three new young professionals to gain experience in electronmicroscopy.

In other phases of laboratory activity, Dr. M. S. Burstone has made considerable progress in the development of new histochemical techniques which broaden the scope of investigations into the composition and reactivity of various tissues. In collaboration with Doctors G. G. Glenner and D. B. Meyer (NIAMD), a most comprehensive study of neoplastic tissue was conducted which provided significant new information about enzymatic and other changes associated with pathological processes. In addition, Dr. Burstone devised methods which led to (a) the finding of high succinic dehydrogenase activity in osteoclasts associated with active bone resorption; (b) the first demonstration of alkaline phosphatase activity by fluorescence microscopy; and (c) the visualization of mitochondrial activity in tissues.

Other continuing histochemical investigations have focused on the composition and characteristics of normal and diseased connective tissues. Under the direction of Dr. H. M. Fullmer, particular emphasis has been placed on the fibrillar components and other noncellular constituents of connective tissues which have hitherto not been thoroughly investigated. Thus, the properties of a new type of connective fiber, found last year, have been studied further, and the evidence suggests that the partial resemblance of these fibers to elastic fibers might be explained either on the basis of incomplete maturation or specialized function. Although these activities have been basically planned as an initial step in research on periodontal disease, a most important byproduct has come from the application of originally devised methods in a collaborative study (with Doctor L. T. Kurland, H. D. Siedler and R. S. Krooth, NINDB) dealing with amyotrophic lateral sclerosis. In this project it has been found that a high percentage of patients (60 percent) with ALS also demonstrate a connective-tissue disorder which has been defined histochemically. This abnormality, found in the skin, is characterized by (a) a distinctive elastosis; (b) an increase of mucopolysaccharide; (c) a degeneration and elastosis of arrector pili muscles; (d) focal areas of apparently regenerated connective tissues; and (e) several characteristic alterations of collagen. Thus, additional information on this little-understood disease has been provided, as well as a possible new diagnostic criterion.

Research during the past year in the field of epidemiology continued its efforts to amplify the descriptive and determinative characteristics of oral-disease processes.

Recognizing that the occurrence of periodontal disease is widespread and that it contributes significantly to tooth loss, special emphasis was placed on obtaining information on prevalence and severity in various population groups. In collaboration with the Interdepartmental Committee on Nutrition for National Defense, the Dental Institute initiated a number of studies to evaluate the nutritional status of selected populations in various participating countries. Working closely with the biochemists, nutritionists, clinical pathologists, and physicians comprising the survey teams, Doctors A. L. Russell, N. W. Littleton, E. C. Leatherwood, J. C. Greene, and G. E. Sydow completed large numbers of oral examinations in Alaska, Ethiopia, Peru, Ecuador, and Viet Nam. Although tabulation and analysis of the consider-

able data assembled has not progressed sufficiently to allow for general conclusions, a few examples of promising leads and findings may be given. In the Alaska study, dental caries was virtually nonexistent in individuals from remote villages, and many of the primitive groups living in these areas were found to be essentially free of periodontal diseases. Although other so-called "primitives" exhibited a uniformly prevalent and severe gingivitis, there was little tendency toward progression into destructive periodontal diseases even in the absence of personal oral hygiene or professional dental care. In contrast to these findings, Eskimos who had lived for some time under relatively "civilized" conditions demonstrated a prevalence and severity of oral diseases quite comparable to those seen in average male population groups within the United States. In the Ethiopian study, a most significant finding was that natives subsisting on a high "natural" carbohydrate diet showed a very low prevalence of dental caries. On the other hand, the prevalence and severity of periodontal diseases were high, with no significant differences as related to sex, ethnic origin, or geographic area. Nutritional factors appeared to exert only a minor influence on the periodontal tissues in individuals from this sample, whereas positive associations existed between severity of periodontal disease and oral cleanliness. Presumably, the oral and dental findings in the Alaska and Ethiopia studies, as well as those still to be reported in the other surveys, will be appreciably enhanced in significance when correlations are made with the biochemical, nutritional, and medical data.

In another approach to the epidemiologic study of periodontal disease and dental caries, Dr. C. J. Donnelly undertook to evaluate the etiologic significance of familial factors and geographic location. Preliminary findings in this survey, conducted on Adventist families, show a consistently low caries rate among children, regardless of geographic differences. Although a similar absence of association was apparent in the case of periodontal disease, there was a tendency for offspring to reflect the periodontal status of their parents.

Clinical Research Activities

As indicated earlier in this report, most oral diseases are controlled by a variety of complex factors which include not only local conditions in the oral cavity, but also the general metabolic pattern for the individual and the environment in which he lives. Thus, the range of clinical studies pursued during the past year has been necessarily broad.

In the general area of human genetics, a significant accomplishment was the description of a heretofore unrecognized hereditary disease transmitted as an autosomal dominant trait and characterized by a defect of cellular maturation affecting the oral tissues and conjunctiva. The related, detailed recording of clinical and laboratory findings by Dr. C. J. Witkop, Jr., and his collaborators illustrates well the fertile areas of research on hereditary diseases which may be opened by broad-base field studies. In another study designed to define the oral aspects of the results of consanguineous marriage in Japan, a unique opportunity exists to observe the genetic effects of a specific type of mating pattern which complements the continuous type of inbreeding being studied in certain small triracial groups in the eastern United States. It is reasonable to expect that in the former population, certain recessive characteristics determining normal growth and development, as well as pathological traits, will show up in the homozygous state with increased frequency.

Sponsored by the National Academy of Sciences and utilizing facilities of the Atomic Bomb Casualty Commission in Nagasaki and Hiroshima, a team consisting of specialists in such fields as pediatrics, anthropology, hematology, and dentistry (Dr. J. D. Niswander, NIDR) has been studying approximately 5,000 offspring of first-cousin marriages. Although, to date, no striking difference in the prevalence of known hereditary defects has been found when compared with a control group, the narrative data indicate that between 1951 and 1959 there were significant increases in stature, weight, and number of permanent teeth erupted for 6-, 8-, and 10-year-old children. It is apparent that this type of infor-

mation on growth and development is vital to a realistic quantitative appraisal of such problems as are posed by the increasing exposure of the human species to ionizing radiation.

Another promising area of clinical investigation has been concerned with the identification of various external and environmental etiologic factors involved in the pathogenesis of recurrent oral mucous-membrane ulcerations. Although a considerable body of evidence is available to support a viral etiology of recurrent aphthous-like lesions of the lips, no such evidence applies in the case of recurrent intra-oral canker sores. In collaboration with the University of Pennsylvania, a student population of 1,039 males and 749 females has been under study by Dr. I. I. Ship to determine the pattern of patient experiences with the two types of recurrent ulcerations. At the end of the first year of study, several interesting observations may be noted: (1) recurrent aphthous lesions occurred in 52–57 percent of the study group with a statistical difference between the sexes, (2) recurrent herpes labialis occurred in 38 percent of the group with no notable sex difference, and (3) based on expected and observed proportions of students having both diseases, there appears to be a common etiology in the case of males. This correlation has not yet been established in the female group.

Increasing activity in clinical investigations during 1959, related principally to expansion in the genetics program and greater attention to the general field of stomatitis, provided justification for the establishment of a Medical Investigations Section. The functions of this professional group of two physicians and supporting personnel, under the direction of Dr. A. D. Merritt, were described in a previous section of this report. As indicated, a major responsibility has been to give consultation in studies of the general pharmacologic action of drugs useful in the treatment of oral disease, particularly related to physiologic response to preanesthetic and anesthetic agents. In this regard, Dr. E. J. Driscoll, in collaboration with Dr. G. R. Christenson, Anesthesiology Department, Clinical Center, has been engaged in a study of alterations in physiological mechanisms following use of various barbiturates including sodium methahexital, neraval, and pentothal. Sufficient data is now available to determine objectively from brain-wave activity the precise anesthetic levels at which various operative precedures are carried out. In general the anesthetic plane is extremely light for all three drugs. With baseline data such as this, the profession is in a better position to improve and refine present methods of anesthesia and lay a sound foundation for further anesthesiology research.

In another activity having practical application for the dental profession, Dr. H. R. Stanley, Jr. has demonstrated a favorable pulp-tissue reaction to the newly introduced techniques of high-rotary-speed instrumentation for surgical removal of normal and diseased enamel and dentin. Emphasizing the important interrelationships of rotary speed, adequate cooling at the site of cutting, and light load of operating pressure not to exceed eight ounces, this clinical study has furnished a basis for the practitioner to better understand the uses and abuses of a new tool in operative dentistry.

With the rather diverse approach to clinical research exemplified by the foregoing activities, and the demonstration by Dr. R. M. Stephan and his collaborators that the development of caries and periodontal disease results from the interaction of many different factors including hereditary predisposition, physical properties and nutritional value of the diet, and retention of food and growth of micro-organisms on the teeth, it is apparent that a broad base of knowledge is being assembled upon which to build an increasingly productive program.

While it has often been said that denstistry and dental research are part and parcel of the total broad field of biology and medicine, relatively few examples may be cited where this unity of purpose exists to the virtual exclusion of all barriers of traditionally vested interests and conflicting philosophies of purpose. In a research institution devoted to a common objective of learning, there prevail a sense of skepticism, spirit of inquiry, and dedicated responsibility to seek for new knowledge that makes for a most unique environment. With the further asset of a multiplicity of disciplines and scientific talents which encourage intellectual cross-fertilization, the National Institute of Dental Research faces the challenge of the future with optimism.

556044—60——20

DIVISION OF BIOLOGICS STANDARDS

INTRODUCTION

The Division of Biologics Standards is primarily responsible for administering the provisions of the Public Health Service Act as they pertain to the control of biological products and for the development of regulations within the provisions of this Act. The legal description of the products covered by the Act is complex, but in effect they include vaccines, serums, toxins, antitoxins, and related products, including human blood and its derivatives, which are offered for sale, barter, and exchange in interstate commerce or for export.

Development of realistic standards for these products and the exercise of proper control over their safety, purity, and potency are the main statutory responsibilities of the Division, but the exercise of these responsibilities in such a complex field is only possible with the support of an active research program of sufficient flexibility to provide the information needed to meet emerging problems. A main objective has been to develop a research program based on the premise that control of biological products can be successful only when the program is able to anticipate the Division's requirements. In addition to providing any information needed in meeting the control responsibilities of the Division, the research program also provides a background of ideas for all workers in this field be they in Government service, private industry, or elsewhere, and serves as a reservoir of trained and alert scientists who can be called upon when problems arise. Since it is obvious that such calls, by their very nature, must be unpredictable and fluctuating, research in cognate fields is encouraged so that the professional staff of the Division may follow some of the leads suggested by their work. This is difficult to keep in balance, but all our scientists, except those with continuing and full-time administrative responsibilities, are encouraged to devote up to one-half of their time on research projects of their own devising. Most of the latter are related to the basic mission of the Division, but many examples of lateral activities can be seen in individual activity reports. Many worthwhile leads actually develop in the course of considering the problems of manufacture and control of biological products.

A major administrative activity of the Division is maintaining proper balance between control activties and the research programs, the latter being supported to the degree that such investigations are required to meet Division control responsibilities.

The Division conducts a continuing review of existing regulations for control of biological products, making revisions as necessary and developing additional regulations required for new products.

During the past year the regulations concerning sterility, pyrogenicity, as well as establishment standards, have been carefully reviewed and reworked through the mechanisms for the establishment of Federal regulations. This is laborious, detailed, and time-consuming, but will have to be continued through the entire roster of biological products.

Close and continuous liaison is maintained with technical representatives of industry. Frequent meetings are held between members of the Division's professional and administrative staff and groups of manufacturers concerned with common problems. In addition, as many as 200 conferences are held each year with technical representatives of manufacturers who desire to discuss production and testing programs peculiar to their own organization.

The Division services the Technical Committee on Poliomyelitis Vaccine, the Public Health Service Committee on Live Poliovirus Vaccines, as well as numerous ad hoc committees appointed from time to time to consider matters relating to control of biological products.

As reported last year there were encouraging signs of increasing overall potency of the poliomyelitis vaccine reaching the market. These have continued through 1959. A consideration of these findings has led to increased activity in an attempt to develop a more satisfactory method of evaluating potency. A new reference vaccine has

been prepared and at the year's end data were being assembled which would help fix its value with respect to antibody response in monkeys, chicks, guinea pigs, as well as in children. A portion of this reference vaccine was provided for the use of the Biological Standardization Section of the World Health Organization and is being used along with other selected vaccines obtained from other countries in a rather extensive evaluation of potency methods involving a number of countries, including the U.S.A.

During the latter part of 1958 the Division became interested in the problems of live poliovirus vaccine. The Committee on Live Poliovirus Vaccines, appointed by the Surgeon General in 1958, served as a focal point for this work. During 1959 the Committee held seven meetings—some with potential manufacturers and others with staff members of the Laboratory of Viral Products. The Division has become active in examining the factors involved in the concept of a safe and potent live poliovirus vaccine. Working closely with the Committee on Live Poliovirus Vaccines, the Division has turned its attention to such matters as the virulence of candidate strains and the conditions for the conduct of the various tissue-culture and serological tests needed to assure safety and potency.

A continuing problem for the Division is the acquisition of a balanced professional staff. A number of members have been lost to industry and there continues to be a rapid turnover of the younger, medically qualified people who leave after the required two-year term of duty. The following additions to the senior staff should be mentioned: Dr. H. M. Meyer, Jr. (Virology), Dr. Harold Baer (Bacteriology-Immunology), Dr. J. N. Ashworth (Biochemistry of Blood), Dr. E. B. Seligmann (Bacteriology), and Dr. J. A. Morris (Rickettsiology). In order to broaden the capabilities and experience of existing staff members, arrangements have been made for one year of training and experience away from the Division as follows:

1959–60 Dr. G. L. Van Hoosier, Jr.—California State Health Department Laboratories in Berkeley (Dr. E. H. Lennette).

1960–61 Dr. Samuel Baron—Medical Research Council Laboratories, London (Dr. Christopher Andrewes).

In the same general effort to broaden the experience and contacts of the scientific staff, Dr. Frank Perkins, a visiting scientist from the Medical Research Council Laboratories, London, who is engaged in virus research related to control of biological products in his home laboratories, is spending a year working in the Division's laboratories.

Staff members attend meetings and work with committees and other advisory groups arranged by such diverse organizations having international activities in this general area as the World Health Organization, and on a national scale, the National Research Council, the Armed Forces Epidemiological Board, the Council on Drugs of the American Medical Association, the General Committee of Revision of the United States Pharmacopoeia, and others. During 1959, Division scientists took an active part in the World Health Organization's program for development of recommendations for the international uniformity of biological products.

During 1959, considerable time continued to be devoted to matters concerned with constructing, equipping, and financing the new DBS building. As the year ends this Division looks forward to occupying its new quarters sometime during 1960.

The Board of Scientific Counselors, established in 1957, meets twice per year and reviews the scientific program of the Division. Such meetings give the senior staff members of the Division an opportunity for describing their projects and particularly for discussing the way in which these integrate with the control responsibilities of the Division as a whole.

The operating functions of the Division are implemented at the present time through the activities of its four laboratories; the Laboratory of Viral Products, the Laboratory of Blood and Blood Products, the Laboratory of Bacterial Products, and the Laboratory of Control Activities. The more important of the activities of these individual laboratories may be summarized as follows:

Laboratory of Viral Products

The Laboratory of Viral Products (LVP), the largest of the present four laboratories, in keep-

ing with Division policy, devotes itself to both control and research projects. Although it is run as two sections, the research section and the testing section, control and research activities are carried on by both.

The main single activity continues to be the control of poliovirus vaccine. One new license was issued during the past year, the first since 1955, and the necessary conferences, examinations, discussions, etc., represented a considerable effort on the part of the Division. Two new products containing poliomyelitis antigens were licensed early in 1959; to Merck Sharp & Dohme and to Parke, Davis & Company; these were multiple antigens containing pertussis, diphtheria, tetanus, and poliomyelitis components. Other establishments are interested in similar products.

Although poliomyelitis vaccine has now been licensed for about 5 years, day-to-day problems continue to arise. With the passage of time there is a tendency on the part of manufacturers to regard the manufacture of poliomyelitis vaccine as a complex, but now routine, procedure. The main problem in this regard is to remain alert and ever watchful, despite the excellent record of the past 4½ years, in order to assure the safety and potency of the vaccine which reaches the market and to avoid anything untoward which might arise from complacency.

The total program of the laboratory cannot be summarized briefly, but mention of the more significant projects and accomplishments included in the following brief summary arranged according to general subjects will indicate the nature and scope of the program:

POLIOMYELITIS VACCINE

During the year the following tests were carried out on poliomyelitis vaccine:

Tissue culture safety tests	74
Monkey safety tests	188
Monkey potency tests	47

In addition 55 lots were tested for potency using chicks instead of monkeys.

Studies on the antibody response to poliomyelitis vaccine by man and various laboratory animals such as monkeys, chicks, and guinea pigs have been continued with a view to adopting a more satisfactory potency test. A test using chicks can be made to work, but collaborative studies carried out in various manufacturing establishments and by the DBS indicate it is subject to variation from laboratory to laboratory. An important need has been a really satisfactory reference vaccine having known characteristics which correlate with past experience in man and animals. Such a vaccine is now available and recent results suggest a workable test design should shortly be available.

A large single inoculation (10 ml.) of poliomyelitis vaccine of average or better-than-average potency was capable of evoking detectable antibody responses within 1–2 weeks following inoculation.

Further investigation on the neutralizing antibody-combining test as a method of determining the antigenic properties of inactivated poliomyelitis vaccine has been continued. While the test appears reproducible in the case of individual vaccines, the results do not correlate very well with those of animal antigenicity tests. For this reason, before the procedure can be recommended for general use in manufacture, it is necessary to carry out further evaluation studies despite the attractive simplicity of the method.

Concentration of poliomyelitis vaccine in the ultracentrifuge was studied with a view to increasing the sensitivity of the monkey safety test. Results with added virus and vaccine containing residual virus indicate the feasibility of the methods and suggest that this procedure could be used for increasing the sensitivity of the test.

LIVE POLIOVIRUS VACCINE

The ability of attenuated polioviruses to revert to increased virulence is being studied in serial stools from persons given such vaccine in field studies.

Comparison of the neurovirulence for monkeys of the strains of attenuated polioviruses that had been used in field trials done under comparative conditions indicates that while all are capable of producing lesions of poliomyelitis when inoculated intraspinally, definite differences can be demonstrated on intrathalamic inoculation. Under these conditions the Lederle strains show a greater tendency for the spread of histological lesions down the spinal cord. The Sabin strains show this property to a lesser degree, while the

Koprowski strains are intermediate in character. These differences in neurovirulence demonstrated by intrathalamic inoculation of monkeys have been reported at the meeting on Live Poliovirus Vaccines held by the Pan American Health Organization last June and have been stressed in the reports of the PHS Committee on Live Poliovirus Vaccines.

Among the markers which may be utilized in the identification of attenuated strains, the K marker has been selected for close study. Attempts are being made to find a test system in which poliovirus strains will be effectively neutralized by homologous sera and heterologous strains will be incompletely neutralized. Two approaches are being made: in the one, the rate of virus neutralization is compared for both the homologous and heterologous systems; in the other, dilutions of virus and serum are made in an attempt to achieve complete neutralization in the homologous system and incomplete neutralization in the heterologous system.

MEASLES VACCINE

Programs with measles virus and measles vaccine were early initiated in anticipation that an immunizing agent against measles would soon be available; however, total progress in this area in the country as a whole has been much slower than originally expected. Experience with killed measles vaccine has been discouraging because of low-titer virus harvests and poor stability after inactivation, while development of a useful live vaccine has been hampered by the fact that all strains so far tested have caused illness in some of those vaccinated. This illness, although not severe, does represent a roadblock in the path to the early availability of a "live" measles vaccine. The Division's main efforts have been in the development of standard reagents and methods. The former pursuit has thus far been rather unprofitable because most of the virus materials obtained from pharmaceutical laboratories—even on a contract basis—have been unsatisfactory due to low antigenicity or instability. Other studies are directed towards development of standardized serological methods, preparation of reference sera, and identification of possible "markers of virulence."

RICKETTSIAL VACCINES

During the past year it has been possible to initiate a limited effort in the area of rickettsial vaccines. This effort, limited by present lack of space, will become more effective with occupancy of the new building. This program has as its first objectives the devising of more precise measures of potency for inactivated epidemic typhus vaccine, the evaluation of attenuated E-strain live typhus vaccine, and the development of requirements for Q-fever vaccine.

RESPIRATORY VIRUSES

In all, 62 lots of influenza vaccine were tested for potency in 1959. A really satisfactory, rapid, potency test is still a great need in the control of influenza vaccine. Efforts to improve and simplify the hemagglutination test for potency continues. While the demand for adenovirus vaccine is less than that for influenza vaccine (only 14 lots of adenovirus vaccine having been submitted for release in 1959), work on different methods of determining potency has continued. One method involving the immunization of guinea pigs and the use of their sera in a serum-virus neutralization test in known numbers of HeLa cells has been studied and shows promise.

VIRUSES—GENERAL

Ribonucleic acid (RNA) from mouse-virulent and mouse-avirulent strains of poliovirus was studied by injecting it intraspinally into mice. The results suggest that the infective RNA for Type I poliovirus carries the genetic characteristics which determine the degree of neurovirulence.

Work with tumor materials, including human tumor materials, continues and considerable effort has been expended on the SE polyoma virus. This tumor agent was recovered in tissue culture from AKR mice, and present studies are in the direction of using it as a model for the recovery of a virus or viruses from human neoplasms.

Another tumor agent being studied is the "sarcoma 180" agent. It has been found that mouse sarcoma 180 harbored polyoma virus while tissue culture-passed 180 did not. An interesting observation with the sarcoma 180 agent has been the occurrence of hydrocephalus after 3–6 weeks in

about 30 percent of Swiss mice inoculated one day after birth.

Some effort continues to be spent in attempts to cultivate the agent of hepatitis. Tissue-culture methods are being used with known infected material.

During the past year an evaluation of the degree of neurotropism of the seed virus used in the manufacture of yellow-fever vaccine (17–D strain) was carried out in monkeys. Of 30 monkeys inoculated intracerebrally, 29 showed no signs of encephalitis. Thus, the seed virus comfortably meets the proposed World Health Organization requirements that not more than 20 percent of the test animals shall develop encephalitis. It should be noted, however, that all of the monkeys showed lesions in the brain and spinal cord on histological examination. No lesions were noted in the viscera.

The comparative inactivation characteristics of a number of animal viruses exposed to ultraviolet light indicated that inactivation rate and slope of inactivation curve could be correlated with the individual viruses, but not with their size as in the case with bacteriophage.

An examination of the relative susceptibility of animal viruses to inactivation by the photodynamic action of toluidine blue permits the classification of the viruses studied as follows:

Susceptible	*Resistant*
T2r+ coliphage	Poliomyelitis I, II, and III
T3 coliphage	ECHO–1
Eastern equine encephalomyelitis	Coxsackie A–9 and B–2
Vaccinia	
Adenoviruses 3, 4, and 7	
Simian virus 1	
Rabies	
B-virus	
ECHO–10	
Measles	
Fibroma	
Myxoma	
SE polyoma	

Photodynamic treatment of mixtures of B-virus and poliomyelitis virus destroys all B-virus infectivity (within the limits of sensitivity of the test system) but does not significantly reduce the infectivity of the poliovirus. This process appears to be of practical importance as a possible step in the preparation of live poliovirus vaccines.

A cognate study, but one not related to DBS programs, was the determination of the effect of sunlight as a variable affecting the efficacy of sodium pentachlorophenate (NaPCP) as a molluscicide in pools and streams. The findings serve to explain the unexpectedly poor results in certain areas of the world when NaPCP was used in shallow streams in an effort to control the vector of schistosomiasis.

These results on photodynamic inactivation appear to be of considerable practical importance and studies are in progress which are designed to elucidate the mechanism of this process.

MISCELLANEOUS

Studies were continued on latent infection of stable tissue-culture cell lines with L-forms of bacteria with the object of eliminating these microorganisms from the cell cultures. Although a variety of antibiotics and conditions of treatment have been used, no permanent solution has been possible.

A semiautomatic diluting machine has been developed for making serial dilutions in serological tests. Evaluation of the machine (fabricated by the NIH instrument section) using a dye indicates that it is capable of accurate dilution in 12 simultaneous operations.

An active factor (or factors) with strong antibiotic action against *Staphylococcus aureus* and *B. subtilis* has been demonstrated in abalone juice. This observation is being followed by a study of extracts from other marine creatures.

Laboratory of Bacterial Products

This laboratory continued to operate with a deficiency of staff members during 1959. However, the position of Chief, Section on Allergy and Sensitivity, will be filled at the beginning of 1960. Attention will be directed towards developing specific standards for allergenic products, with emphasis first on ragweed pollen. In this connection there will be liaison with the "Committee for the Standardization of Allergens," which is functioning within the extramural activities of NIAID.

Studies relating to pertussis products, cholera vaccine, Schick-test toxin, immunological re-

sponses to multiple antigens, and fibroma-myxoma viruses were continued.

Introduction of poliomyelitis vaccine into the well-established triple antigen product, diptheria and tetanus toxoids combined with pertussis vaccine, presented a number of control problems which required solution. Because changes in manufacturing procedures of the pertussis component in particular were made in order to preserve potency of the poliomyelitis components of the combination, problems in potency, toxicity, stability, and sensitization (mouse) of pertussis vaccine were introduced. In particular, the load of potency and toxicity testing of pertussia vaccine was increased materially. A study relating to the latter factor has been initiated.

During the year considerable progress was made in developing a reproducible quantitative mouse-protective test for potency evaluation of cholera vaccine. With this test, one basis of reference for the study of field-trial vaccines will be available. It remains to be determined, however, whether there is a direct relation between potency as judged by such an animal test and human protection. This study along with others on various serological responses to the vaccine and rapid biochemical tests for rapid identification of cholera vibrios are being coordinated insofar as practical with the emerging SEATO Cholera Research Program.

Work on establishing a reference diphtheria toxin for the Schick test is coming to an end. There has been a great need for such a reference for a number of years, but its establishment has been complicated and time-consuming. As this particular effort draws to a close, attention is being directed to staphylococcal products—a group of biological products which have been neglected for some years.

A study of the various media used in the sterility test in different countries for detection of the presence of fungi and yeast in biological products and of the optimum temperature of incubation was initiated. Need for additional data was brought out during the revision of the sterility test procedures which were set forth in recent amendments to the PHS Regulations concerning biological products and during participation with a Study Group appointed by the World Health Organization for formulation of international requirements for sterility of biological substances.

The observations on the relation of thermoresistance to virulence among fibroma and myxoma viruses has attracted attention in relation to the general phenomenon of virulence and temperature of propagation, a phenomenon exhibited by polioviruses in the so-called "t marker." The benign fibroma virus is incapable of reproducing above 38° C., but after transformation caused by myxoma DNA, the virus has the characteristics of malignant myxoma virus and is capable of reproducing at higher temperatures.

Laboratory of Blood and Blood Products

The rapid expansion, in the last few years, of the number of licensed establishments* coupled with the increasing complexity of methods and tests employed in production has created a need for some central organization to assist in disseminating the latest information, helping with technician training programs, and distributing adequate reference standards. As time and staff are available, this laboratory is working in each of these areas with some success. This almost amounts to an "extramural program" having as its goal the raising of the standards of achievement of the establishments.

The laboratory has begun a program, in cooperation with several national groups, of storing blood of rare and unusual types. This blood is to be available in limited amounts to meet actual transfusion needs. In this way, donors of known rare type can donate at their convenience against remote future needs. In addition, this program offers an excellent opportunity to evaluate under actual operating conditions the best procedures for storing, shipping, and administering blood that has been stored for long periods at extremely low temperatures by a technique developed in this laboratory.

Also in collaboration with outside groups, this laboratory has accumulated a supply of different bloods of rare types which will be used as reagent cells.

At the request of the Office of Civil and Defense Mobilization, dried grouping and typing serum

*There are now more than 400 operating units involved in the production of blood and blood products.

samples from the Civil Defense stockpile were tested to determine their suitability for use after the expiration of the dating period. Although in this case it was not possible as a result of the tests to recommend that the dating period be extended, this type of collaboration with other Government agencies has led to savings in the blood and blood-products stockpile.

It is of interest to note that this laboratory is cooperating with the American Standard Association and the International Standards Organization in an effort to achieve standardization of disposable transfusion equipment.

The laboratory research program is designed to obtain information on the properties of blood and blood products which is needed for improvement of existing control procedures or for new tests required for adequate control of new products. The increasing specialization and uniqueness of the new products, particularly in the field of coagulation components, creates a further need for a research program oriented toward the investigation of these products.

During the past year some expansion of the investigation of components of the coagulation system and diagnostic reagents was possible. It is hoped that it will be possible to expand the cooperative research program on the long-term stability of albumin now being carried out with the Department of Defense and the Office of Civil and Defense Mobilization.

Among the continuing activities of the laboratory are such projects as the following:

To search for the rare antibodies to the human blood factors which result from the antigenic stimulus associated with pregnancy, blood transfusion, and natural exposure, and to develop better methods for use in this area.

To establish standards for the antigenicity and viability of the formed elements of the blood. It is interesting in this regard to note that an increasing stock of rare red-blood cells is being built up to meet transfusion needs. Such stored cells, after suitable preparation, have been successfully administered in quantity.

To determine the changes which occur in the physical and chemical properties of various human plasma proteins following physical and chemical treatment incidental to processing and to investigate the properties, mechanism of action, and assay of blood coagulation components and related systems. The stability measurement of albumin stored for five years shows that definite changes of unknown clinical significance take place at storage temperatures above 5° C. These findings have permitted the extension of the dating period of albumin stored at low temperatures.

To develop improved control procedures and standards by evaluating the present control procedures and manufacturing methods insofar as these affect the safety, purity, and potency of biological products derived from human blood.

One rather utilitarian result of some of the interests of the laboratory has been the development of an efficient economical and lightweight shipping container which is now in routine use for the shipping of labile biological materials.

Not the least of the activities and responsibilities of this laboratory is the operation of the Clinical Center Blood Bank. This operation which provides suitable blood, sometimes in considerable quantity, for use in the Clinical Center programs serves as a useful extension of the interests of the laboratory in the standards for blood for transfusion and in connected research.

Laboratory of Control Activities

This laboratory is responsible for dealing directly with licensed establishments in relation to the licensing and control of biological products. It is supported by sections on reference standards, control tests, and pyrogens. Its activities include:

Determination of eligibility of establishments and of individual biological products for license. This determination is made on the basis of a review of the integrity of management, the physical facilities for manufacture and testing of products, the scientific and professional qualifications of personnel, and the evidence of continued safety, purity, and potency of products for which an application for license is being evaluated.

Supervision of annual and special inspections of licensed manufacturers and of establishments for which an application for license has been made.

Releasing of individual lots of biological products for distribution by manufacturers on the basis of review of manufacturer's and NIH tests, and on other data relating to the safety, purity, or potency of the individual lot of the product.

The establishment and distribution of physical biological standards, reference preparations, and control materials.

Review of requirements and regulations now in effect for such constructive revision as needed, and development of requirements and regulations needed for new products.

Maintenance of close working relations between this laboratory and the other laboratories of the Division to insure continuous knowledge of (1) all activities and data needed for licensing of establishments and new products and (2) the testing and release of individual lots of products currently licensed.

The scope of activities carried out by this laboratory may be appreciated by the fact that during the period December 1958–December 1959 a total of 8,220 control tests were carried out to insure the sterility, safety, potency, and purity of biological products.

A summary breakdown of these tests as they pertain to various activities is as follows:

	Tests
Routine Products for Release	5,557
Inspection Samples	1,298
Cooperative Service Tests	1,063
Complaint Investigations	46
Research	256

In addition 885 samples were tested for pyrogenicity.

The data obtained served as the basis of recommendations for acceptance or rejection of the individual lots examined.

During the same period, 3,409 lots of biological products were submitted for release by licensed manufacturers. Of these, 3,329 lots were released, 31 lots rejected, and 47 lots withdrawn from consideration of release by the manufacturers.

To maintain an adequate supply of physical reference standards for use by the licensed manufacturers in their official control testing, it is necessary to prepare and standardize new liquid lots from the primary dried stocks. The number of lots prepared and standardized during the year were: antitoxins, 12; serums, 4; vaccines, 5; toxoids, 4. A total of 134 tests were required to complete a satisfactory standardization of these lots. These tests include flocculation reactions, animal protection tests, animal potency tests, neutralization tests, and a number of specialized tests for specific products.

Standard and reference preparations and cultures are freeze-dried for greater stability during storage. The following were dried between December 9, 1958, and November 30, 1959:

	Ampules
Cultures	924
Serums	1,608
Vaccines	832
Viruses	998

Official standards, reference, and control preparations currently maintained include 64 items.

The following standard and reference preparations were distributed to commercial pharmaceutical manufacturing houses, health departments, universities, and foreign countries:

Antitoxins	424
Serums	1,993
Vaccines	2,963
Toxins	189
Cultures	264
Viruses	254

A culture collection of organisms used in the production and testing of biological products is maintained by this laboratory.

U.S. GOVERNMENT PRINTING OFFICE: 1960