ANNUAL REPORT
OF
PROGRAM ACTIVITIES

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NATIONAL INSTITUTE OF
NEUROLOGICAL DISEASES AND BLINDNESS

NATIONAL INSTITUTES OF HEALTH
PUBLIC HEALTH SERVICE
U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

U.S. National Institute of Neurological Diseases and Blindness. Report of program activities

ANNUAL REPORT - 1959

NINDB Office of the
Director Project Reports

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Collaborative Research	Masland
Epidemiology Branch	Kurland
Biometrics Branch	Goldstein
Extramural Programs	Seger
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II. Field Investigations and Pilot Projects	Seger
III. Graduate Training Grants	Seger
IV. Special Traineeship Program	Hartman
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11(c)	The Relation of Pyridoxine (Vitamin B_6) to Certain Seizure States----------	McKhann
12(c)	Isolation, Fractionation, characterization and Metabolism (Synthesis, Turnover, etc.) of Ribonucleic Acids in Brain----------	Sporn Dingman
13(c)	Cerebral Protein Metabolism and Turnover in Tissue Slices incubated in vitro--------	Wherrett
14(c)	The Histopathological and Chemical Investigations of Neuromuscular Disorders----------	Shy Wanko Haase
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Electroencephalography and Clinical Neurophysiology Branch

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Serial No.	Title	Principal Investigator
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NINDB-NA-NC-7	Species Differences in Cholinesterase Activity of the Central Nervous System----	Brightman
NINDB-NA-NC-8	Metabolism of the Nervous System: Quantitative and Cytochemical Studies-----	Albers
NINDB-NA-EP-1	Pathogenetical Factors in the Development of Myelopathies----------	Cammermeyer
NINDB-NA-EP-4	Introduction of a New Chemical Fixative for Tissues----------	Malm
NINDB-NA-FN-1	A Study of the Auditory Afferent and Efferent Systems----------	Rasmussen
NINDB-NA-FN-5	The Comparative Anatomy of the Efferent Cochlear Bundle in Selected Submammalian Vertebrates; an Experimental Study--------	Boerd
NINDB-NA-FN-6	Further Studies on the Efferent Component of the Vestibular Nerve----------	Gacek
NINDB-NA-FN-7	An Experimental Study of the Ascending and Descending Fiber Connections Between Corpus Geniculatum Mediale and Auditory Cortex----------	Rasmussen
NINDB-NA-PP-1	Ecological Study of Rhesus Monkeys on Cayo Santiago, Puerto Rico----------	Koford
NINDB-NA-PP-2	Normal Reproductive Function in the Rhesus Monkey----------	Jacobson Vollman
NINDB-NA-PP-3	Maturation in Infant Rhesus Monkeys and Care Required for Rearing Them----------	Jacobson
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NINDB-NA-PP-5	Neurological Evaluation and Electro-encephalographic Studies in the Monkey (Macaca Mulatta) Asphyxiated at Birth----------	de Arellano
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NINDB-NA-PP-8	Multiple Alpha-rhythm Cortical Responses Resulting from Single Shock Cerebellar Stimulation----------	Combs Dennery
NINDB-NA-PP-9	An Evoked Potential Study of Connections Between the Diencephalon and the Sigmoid Gyri----------	Dennery Combs
NINDB-NA-PP-10	Physiological Studies of Fetal and Newborn Monkeys----------	Dawes
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NINDB-B-1	Ionic Permeabilities of the Squid Giant Axon Membrane. Experiment and Analysis--	Moore Taylor
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ANNUAL REPORT

Calendar Year 1959

National Institute of Neurological Diseases and Blindness
National Institutes of Health

The Director's Report

The 1959 Annual Report of the National Institute of Neurological Diseases and Blindness contains the Director's Report; the Reports of the Chief of the Extramural Programs Branch, Dr. Gordon H. Seger; the Annual Report of the Clinical Director, Dr. G. Milton Shy; the Annual Report of Basic Research, Dr. Robert B. Livingston; the Annual Report of Collaborative Research, Dr. Richard L. Masland; and summary reports of branch and laboratory chiefs.

The Annual Report also brings notification of the request of the Director of Basic Research for reassignment. At the completion of three years as Director of Basic Research, Dr. Robert B. Livingston, in accordance with a previous commitment, feels that a change of leadership is desirable at this time.

Since these individual reports include comprehensive commentaries on the total 1959 program, the Director's report will confine itself in the main to new trends and developments and to events of special importance in the overall program during the year.

DEVELOPMENT OF THE OVERSEAS RESEARCH PROGRAM

In September 1959, the overseas program of the Institute received increased emphasis as Dr. Pearce Bailey relinquished his position as Director of the NINDB to become Director of International Neurological Research, a newly created Institute post. His decision highlights the recognition of the tremendous opportunity which exists for the establishment of a closely knit and carefully coordinated worldwide program of attack against disorders of the nervous system. Dr. Bailey has established headquarters in Antwerp, where he will maintain close liaison with the World Federation of Neurology which also has headquarters in that city. The objectives of his mission have been summarized as follows:

The primary mission of this overseas assignment is to strengthen the NINDB-NIH program for the diagnosis, prevention, treatment or rehabilitation of crippling neurological and sensory

disorders. A special focus shall be placed upon research promising to supply clues to the basis of serious neurological and sensory disorders that are prevalent in the United States.

In his overseas assignment, Dr. Bailey will give attention to two immediate aims. The first is to survey, evaluate, and report on international scientific talent and facilities for research and training in neurological disorders. The second is to study and develop methods for the application of this potential to the organization of promising collaborative projects in international geographical clinical pathology. Many scientists now believe that the discovery of clues to the treatment of obscure metabolic diseases and certain other disorders of the nervous and sensory systems depends upon international studies of their nature and frequency in relationship to genetic and environmental factors in diverse geographic regions and populations throughout the world. This mission is concerned with the development of such collaborative undertakings. The objective is not to undertake general solicitation of individual research grant applications.

Certain methods are directed toward the strengthening of the total international research effort as follows:

1. The implementation of a program for the dissemination and exchange of medical and scientific news related to neurological and sensory disorders.

2. The initiation of a program for establishing a full-time technical staff at the WFN secretariat in Antwerp.

3. The organization of international neurological congresses and symposia.

4. Establishment of a liaison and working relationship with the World Health Organization, UNESCO, and other international bodies.

5. Establishment of a liaison between NINDB and overseas investigators.

6. Giving technical advice and aid to foreign countries desiring to organize more effective research and training programs in neurological and sensory diseases.

7. Seek contributions and support of countries other than the United States to an international research effort in neurological and sensory disorders.

The program of the World Federation of Neurology has moved ahead rapidly during the closing months of 1959. Problem commissions, comprising men of international stature in various fields of neurology, have been formed, and meetings of these commissions have already taken place for the discussion of important areas of concern. New personnel have been recruited for a full-time central staff in the WFN, and this organization is planning a leading role in the development of a cooperative comparative study of the geographic neuropathology of cerebrovascular diseases. The WFN has been recognized as a nongovernmental agency in official relations with the World Health Organization.

DIGEST OF EXTRAMURAL PROGRAMING HIGHLIGHTS

The Institute has continued its cautious development and strengthening of broad cooperative and collaborative programs. In fact, 1959 marks the first year of actual study of patients within the collaborative project for the study of cerebral palsy and other sensory and motor disabilities of childhood. During the year, approximately 5,000 women and 2,500 children have been observed under this program. The study is showing increasing strength and cohesiveness as individuals from within the collaborating institutions meet regularly with the central staff for purposes of protocol development, methodology testing, and quality control.

The central staff for this project, which in January 1959 numbered 67, has been strengthened to a full complement of 104. Facilities for the storage of serum specimens have been developed to accommodate over 120,000 vials. Facilities for neuropathological examination of specimens also have been established.

Further extension of this program in the immediate future will depend upon efforts to strengthen the so-called extensive phase of the project--those methods which may obtain less detailed information from large groups of women and their offspring. Through such a mechanism some indication of the representativeness of the experience in the collaborating institutions and increased data for statistical analysis will be obtained.

Two important cooperative studies of cerebrovascular diseases have also reached the stage where findings are becoming available. Seven institutions are collaborating in a study of the effectiveness of anticoagulants in the treatment of cerebrovascular diseases. Initial reports of the study of 600 cases have been prepared, and it is evident that this group will provide significant facts for the rapid and accurate evaluation of the effectiveness of this means of therapy in various types of cerebrovascular diseases.

Twenty-two different institutions are cooperating in a study of intracranial aneurysms, and the records of well over 1,000 cases are now in hand. An overall mortality rate of 40 percent highlights the extreme seriousness of this disease, and indicates that our presently available therapeutic measures still fall far short of the desirable. It is encouraging, however, that important advances in neurosurgical techniques, including the improvement of anesthetic agents and the use of hypothermia, are greatly reducing the mortality of surgical procedures for aneurysms as well as for intracranial tumors.

Another important cooperative venture is concerned with the development of accurate screening techniques for the early diagnosis of glaucoma. Four institutions are working together toward the development of a reliable mass screening technique, and an evaluation of methods now available. The increasing effectiveness of these glaucoma detection methods, and the availability of drug therapy, are greatly reducing the impact of this important cause of blindness. The improvement of surgical techniques for the treatment of retinal detachment, and for cataract removal, are reducing blindness from these causes. Improved diagnostic and therapeutic measures for toxoplasmosis are expected to reduce the toll of blindness from certain types of uveitis.

The year 1959 has shown an important and growing emphasis in problems of speech and hearing. Expenditures in these two areas increased by over 60 percent. The development of accurate techniques of audiometry is greatly improving our ability to distinguish between those forms of communications defects which result from hearing loss from those which are attributable to abnormalities of the higher brain centers. Basic research is also increasing our knowledge of the mechanisms of hearing and of hearing loss. Similarly, the newer techniques of neurophysiology are providing important new information regarding the speech mechanisms and the disturbances of coordination and movement which underlie certain forms of speech disorder.

Significant increases in the NINDB training program are now beginning to provide trained personnel to undertake badly needed research in this important and long-neglected field--a field which is a very large factor in special disabilities of children of school age.

The above special programs highlight the overall steady growth of the grants program. At the end of December 1958, there were 913 active research grants; at the end of December 1959, 1,107. Grant support amounted to $10,844,100 in fiscal year 1958; $16,855,000 in fiscal year 1959.

In 1959 continued emphasis on the training of scientists has provided an increased reserve of manpower to carry forward the country's research programs of the future. Programs in clinical neurology and ophthalmology have not enlarged, although moderate budget increases have been allocated. Increases in the clinical specialties have occurred in pediatric neurology--a reflection of heightened recognition of the serious gap in trained personnel for this important area of clinical neurological research--and in otolaryngology, another area of serious neglect. It is a source of great satisfaction to record the continuing growth of the important training programs in sensory physiology and of other basic sciences supportive of neurological research. The objective of these specialized programs is to provide this form of training for clinicians interested in broadening their research resources, and for Ph.D.'s in basic science wishing to prepare themselves for careers in medical research. Expenditures in these important areas have almost tripled during 1959.

MAJOR OPPORTUNITIES FOR NEUROLOGICAL RESEARCH

At this particular point in time, it is apparent that the development of certain new techniques has opened a wide vista for research in neurological disorders. The opportunity now exists to exploit special techniques of physics and chemistry which have only recently become available. The prevention and cure of neurological disorders will be hastened to the extent that we find the means and resources to encourage the prompt application of these techniques to the solution of our problems.

The first important research area is the general range of neurochemistry. In a number of important neurological conditions, there is strong reason to believe that the fundamental basis of the disorder lies in some abnormality of general body metabolism, or of the enzyme systems of certain selected individual cells. In this category, one might mention amyotrophic lateral sclerosis, Parkinsonism, some forms of "idiopathic" epilepsy, myasthenia gravis, and certain forms of neuropathy. Increased knowledge of tissue metabolism, and the search for specific abnormalities of metabolites either by microchemistry, histochemistry, or in vitro analysis, provide avenues through which our ability to cure such important diseases as these listed above may be achieved.

Closely related is the study of molecular biology and cytogenetics. Important new techniques and discoveries in these fields lend hope that the solution of such conditions as the hereditary ataxias, muscular dystrophy, the leukodystrophies, the lipoidoses, as well as of the inborn errors of metabolism with generalized disturbances, may achieve solution.

A second major opportunity lies in the field of immunology. This expanding field now includes not only the study of the immune reaction itself, but the use of the immune response and of antibodies as a tool for biochemical analysis. Of outstanding concern in this area is the problem of multiple sclerosis and some of the acute encephalomyelitides to which it bears a close relationship. It is probable that certain of the infectious neuritides are also influenced by inflammatory reactions and sensitivity responses. The search for an infectious agent in multiple sclerosis, and the elucidation of the immune responses of the central nervous system and their experimental nullification are important avenues toward the solution of these major neurological diseases.

The search for new clues toward finding the cause and prevention of neurological diseases can be greatly strengthened by broad scale epidemiological investigations, which represent the third area where expanded activities are needed. Such epidemiological investigations are pertinent to the problem of cerebrovascular diseases, multiple sclerosis, and amyotrophic lateral sclerosis. Closely related is the increasing concern over environmental health hazards. Under NINDB direction, such hazards were high-pointed this year through the investigation of an outbreak of "Minimata disease"--now shown to be the result of mercury poisoning, presumably because of improper disposition of industrial wastes. To what extent less dramatic disorders are being brought about is not evident.

For the strongest possible development of programs in these important areas, it is urgent and essential to bring well-trained nonmedical specialists into medical research. Solution of this type of medical problem is most likely to develop when basic scientists with great skill in the techniques of physics and chemistry can be provided an opportunity for stable working conditions in close relationship with clinicians having broad knowledge of the problems of neurological disorder. It would appear that three measures would be required if this ideal is to be achieved.

The first requires continued development and expansion of training programs in the basic sciences underlying neurological research. The second requires the establishment of a mechanism whereby stable support for both medical and nonmedical investigators can be assured. The final measure envisages the establishment of centers within which a multidisciplinary attack against various disease entities can be mounted. The productivity of the Clinical Center, National Institutes of Health, is a clear example of the gains to be reached when one establishes a research environment of this sort.

INTRAMURAL RESEARCH

The direct intramural research program remained approximately stationary in 1959. Those expansions which have taken place have been exclusively in the collaborative research area housed off campus in rental facilities at Silver Spring. There is at this time no additional clinical or laboratory space for basic or clinical research expansion at NINDB. The only exception lies in additional construction of neurosurgical operating facilities, expected to be completed in 1960.

The productiveness of the direct intramural research program is strikingly presented in the reports of the Directors of Basic Research and Clinical Research.

The intramural program continues to demonstrate the tremendous achievements which result from bringing together within one institution a group of scientists representing many branches of knowledge and skill, but working closely together toward the solution of fundamental problems of medical science. Within the clinical research area, there have been three major centers of emphasis.

The first is an overall study of epilepsy and the convulsive process. This broad study includes careful analyses of chemical disorders and changes in normal and epileptic brain tissue, and changes of excitability brought about by alterations in amino acid levels in brain tissue. It includes clinical evaluation of seizure patterns, which correlate closely with electroencephalographic observations, and is valuable in demonstrating the focus of origin of the seizure in patients with focal epilepsy. The epileptic process is being studied by means of the microelectrode technique, through which scientists are demonstrating the character of the electrical discharges associated with the seizure. Results of surgical therapy, particularly centering on temporal lobectomy, complete the range of this broad study of convulsive disorders.

The second large area of activity relates to muscle disorders. Starting with basic classification of muscle disease, by both clinical and neuropathological examination, studies include detailed investigation of muscle chemistry, using the newer techniques of immunochemistry, and correlating chemistry with the production of specific lesions by specific antibodies. Special pathological studies include the observation of degenerating tissue, the study of the distribution of neural fibers and motor endplates, in normal and abnormal muscle, and the study of the process of regeneration by the use of tritium-labeled thymidine. Finally, the study of neuromuscular conduction has led to the development of new therapeutic agents in myasthenia gravis, which offers some promise of improving our armamentarium for that disease.

The study of eye diseases encompasses a particularly broad program. Basic physiological investigations are concerned with the nature of impulse initiation--the chemical and physical phenomena which accompany response to light stimulus. Such investigations are carried out in varying selected animal species from Limulus to tree squirrel and humans, both normal volunteers and diseased.

No less than five broad programs are concerned with the study of intraocular tension and the mechanisms altering fluid transfer. The chemistry and pathology of the cornea, and similar studies of the lens, are closely related to concern with cataract and opacities. Finally, the study of inflammatory processes in the eye, the diagnosis and treatment of toxoplasmosis and other forms of uveitis, is included in this broad range of investigations. These brief summaries can give merely the shallowest glimpse of the program, the depth, breadth, and intensity of which can be appreciated only through perusal of the detailed reports of the individual investigators.

Review of the basic science program also attests to its breadth of view. This year has seen important new advances in our knowledge of the nature of the nerve impulse, and the mechanism of synaptic transmission. In the area of neurochemistry, our knowledge of the routes of synthesis of important complex lipids of the nervous system is rapidly extending. From the Laboratory of Neuroanatomical Sciences come new observations on the complex developmental processes of the nervous system, the processes of nerve regeneration, and the intricate pathways concerned with coordination and with hearing.

The Perinatal Laboratory in Puerto Rico this year reached the stage where an adequate number of animals were available for a broadened program of study of the physiology of pregnancy, and of the developmental processes of the nervous system in primates. A broader utilization of this important facility will be possible from now on.

Although this program, as outlined above, is proving extremely productive, it will be less inspiring in the future unless ways are found for developing within the Institute broader programs to capitalize on the new developments in neurochemistry, immunology, and epidemiology, discussed in this report. The creation of additional research facilities and office space, within which the various activities of this Institute can be closely coordinated and interrelated, could be a most important measure in strengthening the overall program and increasing the efficient utilization of its critically short manpower.

RUSSIAN MISSION TO NINDB

During 1959, this Institute continued to contribute to the Russian exchange program through serving as host to the Russian mission visiting the United States.

Three Soviet scientists working in the area of research on the nervous system visited this country under the provision of a January 1958 agreement between the United States and the Union of Soviet Socialist Republics to exchange missions in different fields of medical sciences. They made a 30-day survey of U.S. research developments in the physiology and pharmacology of the nervous system. They spent three days visiting the National Institute of Neurological Diseases and Blindness before visiting research centers throughout the country.

The members of the group were: Dr. Sergey Viktorovich Anichkov, Professor, Head of the Department of Pharmacology, Sanitary-Hygiene Medical Institute at Leningrad, and spokesman for Pharmacology achievements in the U.S.S.R.; Dr. Vladimir Sergeyevich Rusinov, Head, Department of Physiology and Pathology of the Nervous System, Institute of Neurosurgery of the U.S.S.R. Academy of Medical Sciences; and Dr. Vasiliy Vasil'yevich Zakusov, Director of the Institute of Pharmacology and Chemotherapy of the U.S.S.R. Academy of Medical Sciences.

At the end of their tour, they said their impression of neurological research in this country was that the United States is doing more work in the subcortical areas and the U.S.S.R. is doing more with the cerebral cortex. They suggested that perhaps new findings might be made if the emphasis were reversed.

They indicated a great interest in the work this country is doing on the electronic investigation of single cells and said their work related more to the effect of drugs on the body as a whole.

All three scientists indicated that they were glad to know that we were conducting not only applied but basic research relating to the pharmacology of the central nervous system. However, they said that they had observed that most of the search for new drugs in this country is done by industry rather than universities.

During the course of their stay, they visited the research facilities of a number of medical institutions which included: Johns Hopkins University, Baltimore; the Neurological Institute, New York; Cornell University, New York; University of Utah College of Medicine; U.C.L.A. School of Medicine; Washington University School of Medicine, St. Louis; University of Wisconsin, Madison; Harvard Medical School, Boston; Massachusetts Institute of Technology, Boston.

The group received publicity and press coverage at most every point of their tour. A final, well-attended press conference arranged by NINDB, was held March 7 at the Shelburne Hotel, New York City.

They expressed the hope that their visit had strengthened the friendly feelings between the scientists and the peoples of the two countries and expressed deep thanks for the many individuals who had been kind to them during their stay.

INFORMATION

The Information Office of NINDB is engaged in producing materials to support and implement a broad program of public and professional information and education. This past year, the work has included the preparation and distribution of press releases, press conference materials, exhibits, radio and television broadcasts, films, nonperiodical publications, speeches, and articles. The office has also arranged press conferences, press interviews, and picture stories. It has edited many manuscripts and has answered inquiries of all types.

Among the publications completed in 1959 are: "Little Strokes--Hope Through Research," "Brain-Damaged Children and The Collaborative Project," and "Highlights of Progress in Research on Neurological and Sensory Disorders." Nearing completion are four other publications: "Parkinsonism," "Cerebral Palsy," "Cataracts and Glaucoma," and "Mongolism."

The Information Office also prepares reports, articles, and speeches to fill requests from individual Congressmen, Congressional Committees, the Department, Public Health Service, the National Advisory Council, and voluntary health agencies. In 1959, these included: the neurology section of the Senate Documents on International Chronic Neurological Disorders (Epidemiology) and International Research; background statements on disorders of vision, cerebrovascular diseases, hearing and speech, neurological disorders of childhood, and the Collaborative Project; in addition, statements were prepared on the problem, progress, and the potential gains for epilepsy, blindness, cerebral palsy and mental retardation, and cerebrovascular diseases.

Interviews were arranged and press releases distributed to scientific journals and the press. Included were: the Associated Press, United Press International, New York Times, Medical News, Life, Time, Newsweek, Parade, Saturday Review, Scope, and Science Service as well as professional journals. Detailed arrangements were also made for 17 Institute lectures.

During 1959, the Information Office replied to approximately 1,000 letters including Congressional inquiries; mailed out several thousand individual pamphlets (not including bulk orders); and replied to more than 2,000 telephone inquiries from the public. Finally, the Information Office edited and cleared many articles and scientific manuscripts, and gave assistance in the construction, revision, and refurbishing of three scientific exhibits and the revision of the amyotrophic lateral sclerosis film for the Epidemiology Branch. Five NINDB exhibits appeared at 21 showings to professional and scientific groups.

MEETINGS AND SYMPOSIA

During the calendar year, 1959, a number of important meetings and symposia were held under NINDB auspices. Among these were the following: a three-day conference on Neuromechanisms of the Auditory and Vestibular Systems (June 11-13); a conference on Postgraduate Training in Otolaryngology (May 14); conferences on Glaucoma (April 13), Uveitis (September 18), Research in Otopathology (October 19), a Cooperative Study on Aneurysms (December 9); and planning conferences on Cerebrovascular Diseases (December 8), and Drug Therapy Research (September 16).

NINDB also cooperated in sponsoring a number of conferences. Among these were: The Second International Symposium on Myasthenia Gravis held in Los Angeles, April 18-19, and co-sponsored by NINDB and the Myasthenia Gravis Foundation, Inc.; an International Symposium on the Encephalitides held in Antwerp, May 10-14, and sponsored by WFN, Centre Interuniversitaire de Recherches Neuropathologiques, and the NINDB; a Geomedical Conference, under the auspices of the WFN Commission on Biometry and Genetics, held in Copenhagen in June and sponsored by WFN, NINDB, and the Danish Multiple Sclerosis Society.

The proceedings of most of these meetings are now in press. In addition, in 1959, three books were published which summarized the proceedings of previous NINDB symposia. These are: The Process of Aging in the Nervous System, Allergic Encephalomyelitis, and The Biology of Myelin. In addition, members of the NINDB staff have published books on Neurology of Infancy and Neurochemistry of Epilepsy: Seizure Mechanisms and Management.

ANNUAL REPORT
Calendar Year, 1959
Direct Training
NATIONAL INSTITUTE OF NEUROLOGICAL DISEASES AND BLINDNESS
NATIONAL INSTITUTES OF HEALTH

Funds under this activity ($50,000) provide for the support of the in-service training program of the Institute. This program makes it possible for the Institute to secure qualified staff for some of its operations by training younger scientists in particular skills necessary to carry on certain program operations.

In cooperation with the Communicable Disease Center in Atlanta, Georgia, the Institute is presently supporting training in the broad concepts of epidemiology. Basic training and experience in the field of the more acute and widespread communicable diseases offer an introduction to the more complex and protracted epidemiological problems of neurological and sensory disorders and chronic diseases.

Training is obtained in universities and other appropriate institutions in order to supplement already acquired experience and academic training where special needs exist in the total neurology research program.

Short-term courses, generally of a specific technical nature, are also undertaken in this activity.

The following is a list of personnel who received training under this activity during the calendar year 1959:

Name	Institute	Purpose
Dr. Milton Alter	Columbia Presbyterian Med. Center, New York	Training in neurological disorders
Dr. Bernard Agranoff	Institute for Cellular Chemistry, Munich, Germany	To study the mechanisms involved in the formation of acetylcholine and in the relationship between acetylcholine and certain important lipid factions of the nerve membrane.

Name	Institute	Purpose
Dr. John Van Buren	Freiburg Clinic, Germany	Training in stereotoxic techniques.
Dr. Asao Hirano Dr. Anthony Fons Dr. Harry Jameson Dr. Captaine Thompson	Communicable Disease Center, Atlanta, Ga.	Training in epidemiology and statistics.
Dr. C. Spyropoulos	Varian Associates, Instrument Div., Palo Alto, Calif.	Lectures and laboratory sessions in Nuclear Magnetic Resonance - Electron Paramagnetic Resonance Spectroscopy.
Mr. Eckart Wipf	Indiana University, Bloomington, Indiana	American Society for Public Administration Course
Mrs. Ronica Schwartz	Friden Inc. School, Rochester, New York	Training in the programming of the data processing models of the Friden Flexowriter.
Dr. Mary Marques	Walter Reed Army Medical Center, Washington, D. C.	Course in Application of Histochemistry to Pathology.
Mr. Jerome Kern Miss Dorothy Starr Mrs. Shirley Chun Wong Mr. Robert Rawles	U. S. Dept. of Agriculture Graduate School NIH, Bethesda, Md.	Course in Introductory Virology. General review of animal viruses infecting humans, with emphasis on the laboratory properties of these viruses.
Mrs. Blanche Vincent	U. S. Dept. of Agriculture Graduate School NIH, Bethesda, Md.	Introductory course in Human Genetics.
Mr. Robert Rawles	U. S. Dept. of Agriculture Graduate School NIH, Bethesda, Md.	Course in General Bacteriology.
Mr. Albert Cantu	U. S. Dept. of Agriculture Graduate School Georgetown Univ., Washington, D. C.	Course in Basic principles of laboratory animal care.

ANNUAL REPORT
Calendar Year, 1959
Collaborative Research
National Institute of Neurological
Diseases and Blindness
National Institutes of Health

The function of the Collaborative Research Program of the Institute is to conduct a collaborative program of research, wherein the Institute serves as the planning and integrating axis for programmed research on institutional, geographic, and disciplinary levels, and includes laboratory and other professional research and services such as epidemiology, biometry, neuropathology, and virology.

Personnel 1960

Unit	Positions
OD	17
Proj. Serv.	20
Biometrics	34
International	0
Virology	7
Neuropath.	6
Kuru	5
Epidemiology	15
TOTAL	104

Budget 1960

Personal Serv.	713,500
Other Objects	420,500
Contracts	260,000
TOTAL	1,394,000

At the present time, the major emphasis of the collaborative program is the study of perinatal factors in relation to cerebral palsy and other defects of the nervous system. However, there are several independent activities, and they are covered in more detail in the individual reports of the Branch Chiefs of Epidemiology and Biometry.

Further studies of Kuru continue to emphasize the devastating potential of this disorder. Although this condition is clearly limited to an ethnic group in New Guinea, several other conditions showing a very close pathological similarity have been observed in other parts of the world. There appears to be a strong familial factor suggesting a genetic basis, but this has not been entirely established on the basis of present knowledge. Detailed population studies are in progress. In addition, continuing efforts to determine toxic or infectious agents are in progress, and are being combined with intensive studies of the chemical and pathological changes of this ~~bazaar~~ bizarre and destructive disorder.

In general, the results of the collaborative research area continue to demonstrate the mutual advantages for research and scientific development which stem from a close collaboration between members of the Public Health Service and University and research centers throughout the world.

PERINATAL STUDIES

The Problem

An understanding of when and how brain damage takes place is essential in the effort to achieve the eventual prevention of cerebral palsy, mental retardation and related disorders of infancy and childhood. The source of the damage is now believed to lie in the period surrounding birth--from conception through labor and delivery and until about one month after birth.

It is estimated that there are approximately four and a half million mentally retarded persons in this country. About one third of these are children and the disorder afflicts about 126,000 children each year. In addition, some 550,000 persons are afflicted with cerebral palsy, two to five percent of our school children are reported to have speech disorders, four percent have hearing impairment, and some 35,000 school children are legally blind.

In an attempt to find the answers to these large national problems, a collaborative research program related to cerebral palsy, mental retardation and other defects in children was officially begun January 1959, after two and a half years of intensive preparation.

This is the first large-scale effort to collect information on all factors which might conceivably relate to these neurological disorders and the first research program ever undertaken to collect and analyze this information before rather than after such disorders develop. The NINDB is serving as coordinator and central laboratory for the project.

Accomplishments This Past Year

The central services for the collaborative perinatal project have been expanding this past year and will continue to expand during 1960. There have been two major objectives: first, to forge a strong and closely knit organization of the collaborators and the central office; and second, to refine the data collection procedure.

To build a well knit organization, small working subcommittees have been established in special areas of the study. These have included obstetrical, pediatric, and neurological examinations; psychological testing; and interviewing. The members of the subcommittees have worked closely at Bethesda with the central staff, and in some instances, have served as staff members for the development of certain facets of the project. This association has not only increased the actual participation of the collaborators in the central planning, but has aided in coordinating the total program.

In addition to the active working sessions, thirty-five meetings involving principal investigators in various areas of the study have been held this year. Plans have been reviewed, protocols established, and policy decisions made regarding the scientific development of the project.

Recognizing the importance to the study of accurate and complete reporting, the reliability and validity of protocols have been further developed and tested during the past year. A carefully refined protocol for listing accurate and detailed family health and socio-economic data has been worked out. A series of obstetrical examinations has been critically reviewed, and the obstetrical subcommittee has established procedures to achieve accurate recording of these essential data. A detailed protocol for the examination of the placenta has been developed, and a manual for placental examination is now available. The manual is accompanied by kodachrome slides depicting the characteristic changes to be reported.

A protocol for the neurological examination of the newborn infant has been developed and has been subjected to a test-retest analysis. On the basis of these findings, important changes in the procedure have been recommended. A training film demonstrating the technique of the examination of the newborn has now been completed. Also, a rigorous pretest of the developmental examination for the eight-month old infant has resulted in various procedural changes.

A procedure has been established for the collection of

blood specimens from the pregnant woman for virus studies. A coldroom is now equipped at NIH and blood specimens from patients studied this year are being carefully filed for future virus studies. Under contract, antigens of some 40 known viruses are being prepared. More than three-quarters of these are now available and it is anticipated that within the next six months, antigens for all these agents will be on hand. The team of scientists responsible for conducting the serological studies is now in training.

In addition to the 5500 mothers and 4200 babies studied in the pretest phase of the project, 3300 mothers and 1800 babies had been studies this year in the final study series as of October 1. The project also envisages the study of other groups of women and their offspring not delivered in the collaborating institutions. This will aid in determining the representativeness of the pregnancy experience in the collaborating institutions and will broaden the statistical base of the project by bringing into it additional numbers of damaged cases.

In December, 1958, arrangements were made with the Columbia University School of Public Health and Administrative Medicine to explore the needs and methods of this 'extensive phase' of the collaborating project. In January, a conference of some twenty outstanding investigators was held to outline the needs and methods of this phase of the study. Since then, a small working committee has been formed which is concerned with the further development of these methodologies. To date, two ongoing pretests have evolved.

The Columbia University group is now studying the methodology for case finding in the New York area. Their objective is to determine methods of detecting cases of cerebral palsy, mental retardation, blindness, deafness and other neurological disabilities, and relating the findings in these patients to the prior recorded events of pregnancy and labor. This study will also aid in collecting accrued figures of prevalence and incidence, and in evaluating the sample being collected at the New York Medical College and Columbia University School of Medicine.

In another study, with the Kaiser Foundation and the University of California at Berkeley, women receiving prepaid medical care are participating in a study similar to the collaborative project but with less detailed observation. This will make possible a larger sample, approximately 8,000 women a year.

As a result of these activities, and under the surveillance of the planning committee, a well-rounded total program is evolving. Its objective is to evaluate those factors in pregnant women which influence the health of their children and interfere with the achievement of their full potentiality.

The following table indicates the support for the collaborating perinatal project; grants awarded are on the attached sheet:

	1959	1960	1961
Extramural	$3,452,058	$4,203,122	$3,732,734
Intramural	700,000	1,161,000	1,200,000
Totals	$4,152,058	$5,364,122	$4,932,734

Institution								
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
University of Minnesota	7/1/59	21,746	---	207,946	276,467	76,467	276,467	
New York Medical College	3/2/59	---	168,840	190,440	298,299	292,669	[illegible],563	
Medical College of Virginia	9/1/58	125,232	---	155,967	[illegible]24,566	113,369	113,369	
Children's Hospital, S.F.	4/1/59	62,640	66,700	[illegible]24,250	35,000			
Children's Hospital, Phila.	10/1/59	94,361	---	111,889	[illegible]57,179	245,455	203,498	
Pennsylvania Hospital	6/1/59	159,955	137,358	319,344	228,451	228,451		
University of Oregon	11/1/59	---	147,946	300,131	376,981	300,131	300,131	
Johns Hopkins University	9/1/59	282,569	---	355,819	355,819	355,819	355,819	
Boston Lying-In Hospital	10/1/59	237,592	---	354,002	429,078	[illegible]34,145	433,718	
Charity Hospital	1/1/59	---	168,167	262,725	262,725	262,725	262,725	
Columbia University	1/1/59	---	150,000	241,103	270,658	[illegible]62,608	[illegible]63,298	
Children's Med. Ctr., Boston	9/1/58	---	---	103,646	87,320	99,418	102,818	112,386
Children's Hospital, N.Y.	12/1/59	---	---	108,664	148,000	148,000	148,000	148,000
University of Tennessee	9/1/59	---	---	142,481	239,600	239,600	239,600	239,600
		$1,389,527	$1,101,506	$3,452,058	$4,203,122	$3,732,734	$3,356,423	$[illegible],403
							(N.Y. 64-	$[illegible],417

ANNUAL REPORT
Calendar Year 1959
Epidemiology Branch
National Institute of Neurological
Diseases and Blindness
National Institutes of Health

In general, the basic program of this Branch is still concerned with the frequency, geographic distribution, and population selectivity of neurological and sense organ diseases. Numerous genetic and definitive epidemiologic studies have been developed in conjunction with this basic program. The nature of the disorders under study and the need to define the inheritance factors of the affected "hosts" has resulted in a broad and diversified genetics program and a recent recommendation for the formal organization of the Section of Genetics within the Branch. Several new activities of genetics personnel are dependent on cytological and biochemical laboratory facilities. Since these facilities are not available at our present quarters, laboratory space has been temporarily provided by other programs at NIH on a cooperative research basis. The resultant displacement and the constant movement of our personnel between an office in Silver Spring and laboratory, library and conference facilities in Bethesda has resulted in considerable disruption of our program. Any success registered during the year has been made in spite of this difficulty; but the probable gain in recruitment and research potential should be considered if the space policy met the basic needs which are so obvious to our staff.

Our part in the investigations of several localized epidemics of acute neurologic disease in which toxic agents have been incriminated, has focused our attention on the need for an organized program in experimental and clinical neurotoxicology. However, here too, the lack of a suitable laboratory facility has resulted in dispersion of so many facets of our investigations, that schedules and commitments, so often dependent on outside resources, have been difficult to meet; outbreaks which we believe could have been solved promptly by our personnel have suffered because of the lack of appropriate research facilities.

Our interest in geographic isolates and particularly the low cost but productive research of the genetic and neurological conditions of phenomenal incidence on Guam continues. Long-term epidemiologic and genetic follow-up studies as well as detailed clinical, pathological and anthropological investigations among the Chamorros are under way.

In cooperation with the Commission on Biometry and Genetics of the World Federation of Neurology, the Branch is developing standardized procedures and reporting methods for population comparisons of morbidity from neurological diseases. International mortality statistics are also being evaluated in cooperation with the Biometrics Branch, NINDB.

Since problems of nomenclature and classification are basic in morbidity surveys, the Branch is cooperating in efforts to simplify the Neurologic Section of the AMA Standard Nomenclature and the International List of Diseases and Causes of Death. Part of this effort is concerned with the development of a useful classification system for out-patient services.

The relatively small professional staff of this Branch represents an unusual combination of scientific disciplines whose activities are widely distributed geographically. Half of its professional staff are in the field; others provide laboratory and administrative support for the distant operations as well as direct field, clinical, and laboratory research locally and teaching responsibilities at N.I.H. In addition, the staff provides many supportive functions for the Office of the Director, as well as the usual evaluation of reports and applications for grants and fellowships.

The Branch maintains a cooperative field investigation and teaching program with the Epidemiology Branch, Communicable Disease Center. Before completing his tour of duty in June, 1959, Dr. Howard D. Siedler, an Epidemic Intelligence Service Officer assigned by CDC to this Branch, completed his report on the recent poliomyelitis and aseptic meningitis epidemic in Washington, D. C. He also completed reports on the investigation of multiple sclerosis prevalence in Duxbury, Massachusetts, and a report on an investigation of congenital anomalies in Alexandria, Virginia. His field evaluation of multiple sclerosis prevalence in Farmington, Maine, failed to disclose anything out of the ordinary. Other projects in which he was engaged included the collaborative study on the possible teratogenic effects of Asian Influenza, and also "Minamata" disease. The Asian Influenza study is nearing completion; preliminary results fail to show any effect of the virus on the incidence of prematurity, abortions or neonatal deaths. The teratogenic effect, if any, must await refined statistical analysis of data which are still being collected.

Dr. Siedler's replacement from the Communicable Disease Center, Dr. Stanley Faro, continues the Asian Influenza study; he has been engaged particularly in the neurotoxicological effects of alkyl mercury compounds as part of the Minamata Disease study. There is reason to believe that these compounds, contaminating shellfish in Minamata Bay, Japan, may have been responsible for the epidemic of serious neurologic disease in that community. Efforts are being made in cooperation with other Laboratories in the Public Health Service to determine whether the possibility of a similar threat exists in the vicinity of waterways close to United States vinyl chloride production plants using mercury compounds as catalysts. Dr. Faro is also engaged in developing survey procedures for a new study on the geographic distribution of uveitis.

Dr. Robert S. Krooth who was stationed on Guam has completed several reports on his work on diaphysial aclasia, Parkinsonism and amyotrophic lateral sclerosis in the Marianas. The population on Guam

and neighboring islands still provides a wealth of clinical information and experience. At the same time, pathological and other specimens are available on short notice for cooperative research activities at NIH, CDC, and for the collaborators in various universities in the United States and abroad. The island is at a crossroads of the Western Pacific and provides unusual opportunities in standard epidemiologic practice for our field workers as well. The excellent cooperation of the Government of Guam and the Navy Hospital facility continues. It is proposed that the facilities of this research center be expanded for further use of all Institutes of the National Institutes of Health.

In cooperation with other laboratories at NIH, Dr. Krooth is studying the excretion rate of beta-isoaminobutyric acid in gout, the relationship of phenylthiourea tasting to thyroid disease, and is applying the technique of tissue culture to metabolic problems in selected genetic diseases. In conjunction with Dr. Harold Fullmer, NIDR, members of the Branch are pursuing studies which have demonstrated abnormalities in collagen formation of amyotrophic lateral sclerosis patients.

On Guam, Dr. Krooth was followed by Dr. Asao Hirano, a highly skilled neurologist and neuropathologist, who has joined our research center as a Visiting Scientist. He is particularly concerned with determining the nature of recently described neurofibrillary changes in postmortem specimens of amyotrophic lateral sclerosis and Parkinsonism on Guam, and he will continue the genetic follow-up studies of patients and controls in the large registry on the Island.

Dr. Milton Alter who is completing a year of training in clinical neurology at the Neurological Institute in New York has reported the results of a comparative study of multiple sclerosis prevalence in Halifax, Nova Scotia, Canada; and Charleston, South Carolina, United States. The prevalence is 2.5 times greater in Halifax and, for the white populations alone, about 2 times as great in Halifax. There is no apparent reason for the geographic difference. Dr. Alter has also participated in descriptive studies of the epidemiology and genetics of myasthenia gravis and is reporting an unusual set of twins, one of whom had myasthenia gravis.

Dr. Anthony Fons is developing survey procedures for a descriptive study of cerebral vascular disease in the population of Rochester, Minnesota. Records of the Mayo Clinic, the cooperating unit in this investigation, provide excellent coverage of diagnosed cerebral vascular disorders. Dr. Fons also plans a study on the prognosis of subarachnoid hemorrhage prior to the present surgical era to provide baseline data for comparison with the surgical procedures now being recommended.

A neurologic section should be ready for organization at the end of this year. The proposed head for the Section will be available in 1960 and in the interim, the Branch Chief will continue in this activity.

Difficulties in recruiting an epidemiologist with training in ophthalmology have again delayed the proposed study on phlyctenulosis in the Alaskan native population and the development of an ophthalmologic epidemiology program.

Dr. Ntinos Myrianthopoulos, geneticist, continues in his investigations of Parkinsonism, schizophrenia, and amyotrophic lateral sclerosis. The Parkinsonism study is an attempt to determine whether there is a genetic mechanism in the sensitivity or resistance to the development of Parkinsonism by phenothiazine ataraxic compounds In cooperation with the Laboratory of Blood Products, NIH, the association of blood groups in patients with amyotrophic lateral sclerosis is being evaluated. Dr. Myrianthopoulos is also carrying on a project with the cooperation of the Neurology Service, Clinical Center, to study the existence of congenital anomalies in offspring of patients with myotonic dystrophy; there is reason to believe that such anomalies appear more frequently in the offspring of such patients than would be expected by chance. He is also pursuing several other studies as follows: peroneal muscular atrophy (the question of penetrance and detection of the genetic carrier state by nerve conduction velocity tests); incidence and prevalence of Tay-Sachs disease among the Jewish and non-Jewish population of the United States from mortality statistics, and with Dr. Rowley he has completed a report on a study of Huntington's chorea in monozygotic twins.

In addition to several of the preceding studies in which he participates, Dr. Kurland is responsible for continuing the study with the National Research Council and the Veterans Administration on the natural history of acute retrobulbar neuritis and multiple sclerosis. Data now available indicate that only about 8 per cent of patients with retrobulbar neuritis develop multiple sclerosis 10 - 15 years after the bout of acute optic neuritis.

The future program of the Branch is, in large measure, dependent on the organizational structure of the Institute, the sectional development which has been recommended and the assignment of space suitable for the combined field investigations and laboratory programs in the Branch. If our space and organizational needs are met, we can anticipate an appreciable extension of our research, particularly as this relates to geographic distribution and the clarification of the role of environmental and inherited factors in the etiology of neurological and ophthalmological disorders. Our staff of experienced epidemiologists and geneticists will be prepared to explore, expeditiously, situations of unusual incidence and particularly geographic isolates of disease as these are discovered.

The close relationship of genetics and epidemiology in this Branch provides unique opportunities for the simultaneous study of populations, the individual patient, and biochemical factors in disease. It is proposed to clarify the interplay of environment and heredity in chronic disease and to assess the genetic endowment of populations as these relate to disease predisposition. The genetics program can be expected to provide important contributions through the estimation of gene frequencies and mutation rates, the detection of heterozygous carrier states, the elucidation of the selective advantage of genotypes and the biochemical and cytologic nature of inherited disease. The epidemiologic investigations have demonstrated a need for an experimental and clinical neurotoxicologic program in the Branch. Furthermore, our field studies can be expected to help complete descriptions of clinical disease since broad population studies are likely to uncover subclinical, minimal and atypical cases and unusual familial aggregates which are not obvious in standard clinical procedures.

In closing, it is difficult to reconcile the many disruptive moves and unsuited facilities assigned to the Branch with the tremendous cost and effort represented in the training and experience of the scientists who form the nucleus of our extensive and somewhat unique program. If we are to attain the complete fulfillment of the scientific achievements of which we are capable, the logistic support of the administrative leaders of this Institute and of the N.I.H. is essential.

PUBLICATIONS

Published or in Press

1. Kurland, Leonard T.: The Incidence and Prevalence of Convulsive Disorders in A Small Urban Community. Epilepsia, June, 1959

2. Engel, King; Kurland, Leonard T.; and Klatzo, Igor: An Inherited Disease Similar to Amyotrophic Lateral Sclerosis With A Pattern of Posterior Column Involvement. An Intermediate Form? Brain, Vol. 82, Part II, 1959, pp 203-220.

3. Kurland, Leonard T., and Alter, Milton: The Current Status of the Epidemiology and Genetics of Myasthenia Gravis. Accepted for publication in Symposium of the Second International Congress for Myasthenia Gravis.

4. Deacon, Walter E.; Alexander, Leo; Siedler, Howard D.; and Kurland, Leonard T.: Multiple Sclerosis in A Small New England Community. Accepted for Publication in New England Journal of Medicine.

5. Fullmer, H. M.; Siedler, H. D.; Krooth, R. S.; and Kurland, L. T.: Dermal Changes in Patients Affected with Amyotrophic Lateral Sclerosis. Submitted to Neurology for publication.

6. Mackay, R. P. and Myrianthopoulos, N. C.: Multiple Sclerosis in Twins and Their Relatives. Preliminary Report On A Genetic and Clinical Study. Arch. Neurol. Psychiat. 80:667-674, December, 1958.

7. Myrianthopoulos, N. C. and Mackay, R. P.: Multiple Sclerosis in Twins and Their Relatives: Genetic Analysis of Family Histories. Accepted for publication by the American Journal of Human Genetics.

8. Myrianthopoulos, N. C. and Pieper, S.: The ABO and RH Blood Groups Among the Chamorros of Guam. Am. J. Phys. Anthrop., June, 1959.

9. Myrianthopoulos, N. C. and Rowley, P.: Monozygotic Twins Concordant for Huntington's Chorea. Accepted for publication by Neurology.

10. Myrianthopoulos, N. C. and Stevens, H.: Genetic Counseling in Medicine. Med. Ann. District of Columbia 28:256, 258, December, 1958.

11. Myrianthopoulos, N. C.: Genetics and Public Health. Accepted for publication by Public Health Reports.

12. Siedler, Howard D.; Kalter, Seymour S.; Thrupp, Laurie D.; Utz, John P.; Finucane, Daniel L.; and Parrott, Robert H.: Outbreak for Type III Paralytic Poliomyelitis in Washington, D. C. in 1957. Accepted for publication in the American Journal of Hygiene.

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13. Siedler, Howard D.; McGough, Thomas F.: Excess Congenital Anomalies in Alexandria, Virginia. Accepted for publication in Public Health Reports.

14. Pieper, Samuel J. and Fields, William S.: Failure of Amyotrophic Lateral Sclerosis to Respons to Intrathecal Steroid and Vitamin B_{12} Therapy. Neurology, Vol. 9, No. 8, August 1959.

REPORTS (Unpublished data)

15. Alter, Milton; Allison, R. S.; Talbert, O. R.; Kurland, L. T.; and Godden, J. O.: Geographic Distribution of Multiple Sclerosis: A Comparison of Prevalence in Charleston, South Carolina, U.S.A., and Halifax County, Nova Scotia, Canada. To be published.

16. Krooth, Robert S.: Amyotrophic Lateral Sclerosis and Certain Related Problems on the Island of Guam.

17. Krooth, Robert S.: Southeast Asia Trip Report.

18. Okinaka, Shigeo; McAlpine, Douglas; Kurland, Leonard T., et al: The Prevalence of Multiple Sclerosis in Northern and Southern Japan, A Description of Surveys in Sapporo on the Island of Hokkaido, and Kumamoto on the Island of Kyushu. To be published.

19. Alter, Milton, and Talbert, Rheet. Myasthenia Gravis in One Member of A Monozygotic Twinship. To be published.

ELECTED TO MEMBERSHIP

American Epidemiologic Society, April, 1959. Dr. Kurland.

Chairman, Commission on Biometry and Genetics, World Federation of Neurology, June, 1959. Dr. Kurland.

Consulting Editor, World Neurology, June, 1959. Dr. Kurland.

Assn. of Military Surgeons, June, 1959. Dr. Kurland

Clinical Assistant Professor, Neurology Department, Georgetown University, Washington, D. C., October, 1959. Dr. Kurland

PHS-NIH
Individual Project Report
Calendar Year 1959

PART A.

1. Project Title: Investigations of Chamorros on the Island of Guam

Principal Investigators: Leonard T. Kurland
Robert S. Krooth
Asao Hirano
Samuel J. L. Pieper
Nathan Malamud
Jose Torres

Other Investigators: James H. Pert, American Red Cross
H. Eldon Sutton, Department of Human Genetics
Univ. of Michigan Medical School
Oliver Smithies, University of Toronto
Medical School

Cooperating Units: Department of Human Genetics, University of
Michigan Medical School
Connaught Medical Research Laboratories,
University of Toronto

Man Years: Total - 3 1/4
Professional - 1 1/4
Other - 2

Project Description:

The phenomenal incidence of several disorders among the Chamorros of Guam offers, in this unique and isolated setting, an excellent opportunity for clinical, genetic and epidemiologic investigations. Major emphasis continues on amyotrophic lateral sclerosis; other studies are underway to delineate an organic brain syndrome resembling Parkinsonism, Jakob-Creutzfeldt disease and presenile dementia. Diaphysial aclasia, gout and diabetes also appear to be unduly prevalent and preliminary studies of these disorders are underway.

Amyotrophic Lateral Sclerosis

A registry was compiled of the immediate relatives of a group of proven cases of amyotrophic lateral sclerosis, and the immediate relatives of control persons known to be free of the disease but matched to each patient by age, sex, and village of residence. A genetic analysis was then performed on this registry to determine whether ALS was occurring with exceptional frequency among the relatives of affected. The analysis revealed that the patients had about ten times as many living affected relatives as one would expect if there were no family concentration of the disease. Moreover, the patients had significantly

fewer living relatives than the controls, and the living relatives of patients averaged significantly younger than the living relatives of controls. It was possible to show that the patients had fewer living relatives in age groups where the mortality from ALS is high, suggesting that members of the patients' families may have been selectively eliminated by the disease.

Neuropathologic studies to date, performed in collaboration with Dr. Nathan Malamud of the Langley Porter Institute for Neuropsychiatric Research, have revealed in both ALS and Parkinsonism patients, a neurofibrillary change which is unaccompanied by senile plaques and which is widely distributed in the brain. This finding is preliminary and is receiving further study.

Cooperative studies on the biochemistry of ALS and Parkinson's disease include:

a) urine chromatography for amino acids
b) starch gel electrophoresis of the serum proteins
c) starch gel electrophoresis of the cerebrospinal fluid protein.

With respect to amino acid chromatography and electrophoresis of the serum proteins, the result revealed no essential difference between patients and controls. The data on the cerebrospinal fluid protein is not yet available. These investigations have yielded, as a by-product, some interesting biochemical data bearing on the anthropology of the Chamorros of Guam. Detailed immunogenetic studies were performed on 100 Chamorros and the rate of isonicotinic hydrazide inactivation was determined on 20 of these people to help further characterize the Chamorros as a people.

The high prevalence of ALS offers a unique facility for therapeutic studies. Baseline life expectancy and progression of disease rates are available. Trends of new drugs are readily accepted by the affected population and their families. Among recent trials reported are the unsuccessful tests of intrathecal cortisone and vitamin B_{12} by SA Surgeon Samuel Pieper and Dr. William Fields, Baylor University.

The uses of the Guam Research Center in other investigations are described in Project No. 2, "Dermal Changes in Patients With Amyotrophic Lateral Sclerosis."

Parkinsonism

The incidence may be high but is still uncertain for an organic brain syndrome characterized by increased rigidity and tremor but differing from the usual Parkinson's disease in its earlier age at onset, its more rapid course, and frequently observed dementia. There are numerous instances of family history of ALS in these cases and of "Parkinsonism" in ancestors of ALS patients. The interrelationship of

these disorders and of presenile dementia and Jakob-Creutzfeldt disease is being intensively studied by Dr. Asao Hirano, Visiting Scientist assigned to the Research Center on Guam.

A genetic study of "Parkinsonism", with 18 patients and 18 controls on the registry, has also been developed. Here, however, the results were more equivocal than in the ALS cases, due partly to the small sample size.

The Parkinsonism and ALS registries are being continued for 5 years to accumulate further experience on the genetics of these diseases.

A study of the effect of artane on the dysdiadokinesis of Parkinson's disease was completed. A hand punch counter was used to record the maximum number of voluntary thumb movements over a 30-second interval. No effect of orally administered artane on the patients' scores was noted over several months, or on the hourly scores following a single parenteral dose. Similar results were obtained with dextroamphetamine, atropine, and a placebo (acetylsalicylic acid).

Diaphyseal Aclasia

Genetic studies were carried out on diaphysial aclasis, a rare disease, due to an autosomal dominant gene, and characterized by multiple usually benign osteochondromata. 21 living cases of this disease, involving 7 families were collected on Guam, reflecting a prevalence which is almost certain to be far greater than any previously noted. A number of interesting findings have been obtained from the data, including the sudden appearance within families of tumors at unique sites. The X-ray films on the patients are now being reviewed at the National Institutes of Health.

Other Disorders

Preliminary clinical and statistical studies on gout and diabetes were also started on Guam in cooperation with interested members of the NIH Staff.

PART B.

Pieper, Samuel J. and Fields, William S.: Failure of amyotrophic lateral sclerosis to respond to intrathecal steroid and vitamin B_{12} therapy. Neurology, Vol. 9, No. 8, August 1959.

Part III

Annual Project Report

Calendar Year 1959

PART A.

2. Project Title: Dermal changes in patients affected with amyothrophic lateral sclerosis

Principal Investigator: Dr. H. Fullmer (NIDR)

Other Investigators: Dr. H. Siedler (NINDB)
Dr. R. S. Krooth (NINDB)
Dr. L. T. Kurland (NINDB)

Man-Years: Total - 1 1/2
Professional - 1/2
Other - 1

Project Description:

A large number of skin biopsies and skin from patients with ALS and from controls were stained by the peracetic acid-aldehyde fuchsin-Halmi method recently developed by Fullmer. The majority of ALS patients showed changes in the dermis of their skin. The most specific of these changes involved the size and morphology of collagen fibers. It is believed that the patients have both an increased rate of elaboration and an increased rate of destruction of collagen.

The changes noted were not uniformly present over the whole integument, and seemed correlated with the clinical severity of the disease. It is not at present known whether they reflect another facet of the basic disease process, or whether they are purely secondary to the muscle atrophy, neuronal degeneration, etc. Investigations on this last point are continuing.

PART B:

Publications other than abstracts from this project:

Fullmer, H. M., Siedler, H. D., Krooth, R. S., and Kurland, L. T. (1959). A dermal connective tissue disorder in amyotrophic lateral sclerosis. Presented at meeting of American Academy of Neurology and submitted for publication in Neurology.

Individual Project Report
Calendar Year 1959

PART A.

3. Project Title: The ABO and Rh Blood Groups Among the Chamorros of Guam

Principal Investigator: N. Myrianthopoulos

Other Investigator: Samuel J. L. Pieper

Man-Years: Total - 2/32
Professional - 2/32

Project Description:

A study to determine the phenotypic and genotypic frequencies of the ABO and Rh groups among the Chamorros of Guam, with emphasis on anthropologic and genetic implications.

The low frequency of Rh negative blood among the Chamorros casts some doubt on the hypothesis that amyotrophic lateral sclerosis on Guam was introduced from Spain about 1700.

PART B.

Myrianthopoulos, N. C. and Pieper, S., Jr. The ABO and RH Blood Groups Among the Chamorros of Guam with Reference to some Anthropoligic and Genetic Problems in the Area.

Accepted for publication by the American Journal of Physical Anthropology, June 1959.

PHS-NIH
Individual Project Report
Calendar Year 1959

PART A.

4. Project Title: The Association of Blood Groups to Amyotrophic Lateral Sclerosis

Principal Investigator: N. Myrianthopoulos

Other Investigators: P. Schmidt, LBBP
Leonard T. Kurland

Cooperating Units: Laboratory of Blood and Blood Products, Division of Biologic Standards

Man-Years: Total - 4/32
Professional - 4/32

Project Description:

A pilot study to determine if there exists any selection for a specific blood type among patients with a motor neurone disease.

Present Status. The medical facilities of the Army, Navy, Air Force and Veterans Administration, the Guam Research Center, and many practicing neurologists are participating in this project by providing blood and saliva specimens for analysis. Specimens from about 400 cases and controls have been collected.

PART B.

None

PART A.

5. Project Title: The Effect of Climate and Other Environmental factors in the Prognosis of Multiple Sclerosis One Aspect of A Broader Investigation of the Natural History of Multiple Sclerosis (Collaborative Project)

Principal Investigator: Leonard T. Kurland

Other Investigators: Gilbert Beebe and Dean Nefzger, Follow-up Agency, National Research Council
J. F. Kurtzke, Chief, Neurology Service, V. A. Hospital, Coatesville, Pennsylvania
Thomas Auth, Neurology Department, Veterans' Administration, Washington, D. C.
Benedict Nagler, formerly Veterans' Administration (now Lynchburg Training School and Hospital, Colony, Virginia)

Cooperating Units: Follow-up Agency, National Research Council, Washington, D. C.
Veterans' Administration, Washington, D. C.

Man-Years: Total - 1/4
Professional - 1/8
Other - 1/8

Project Description:

Retrobulbar neuritis has been reported to progress to multiple sclerosis in 45-50% of affected individuals within 10 to 15 years. It had been hoped that correlation of residence with RBN might show whether some climatic or residential status influenced prognosis with respect to multiple sclerosis.

In the large population of military personnel with RBN, 1940-1945, only about 8 per cent have developed M. S. to date, according to V. A. records. Physical examinations of patients to verify this is required. If the incidence of M. S. is as low as the V. A. reports indicate, the main objective of the investigation cannot be achieved but the low incidence of M. S. will be worth reporting.

The project to analyze other factors in the Natural History of Multiple Sclerosis is continuing.

PART B.

None

PHS-NIH
Individual Project Report
Calendar Year 1959

PART A.

6. Project Title: Epidemiologic Investigations of Multiple Sclerosis and Other Neurological Diseases in Charleston County, South Carolina, and Halifax, Nova Scotia

Principal Investigator: Milton Alter

Other Investigators: R. S. Allison, Neurological Department, Royal Victoria Hospital, Belfast, Ireland
Rhett Talbert, Professor of Neurology, Medical College of South Carolina
John Goddard, Asst. Professor of Preventive Medicine, Dalhousie University, Halifax, N. S.
Leonard T. Kurland

Cooperating Units: Medical College of South Carolina, Charleston, S. C.
Dalhousie University, Halifax, N. S.

Man Years: Total - 1
Professional - 1/2
Other - 1/2

Project Description:

The incidence and prevalence of multiple sclerosis and other diseases of the nervous system was determined for the populations of these two communities. Data are being analyzed and reports are being prepared on the frequency of multiple sclerosis, myasthenia gravis and other diseases of the nervous system in the communities and for subgroups of their respective populations. Clinical reports based on unusual cases observed in these communities are also being prepared. These include a study of myasthenia gravis in twins and an hereditary cerebellar ataxia with cataract formation. Multiple sclerosis prevalence is about 2.5 times greater in Halifax than in Charleston. The rates for the white population alone are about two times as great. Within each community there is no clear cut racial selection for the disease.

PART B.

Alter, Milton, Allison, R. S., Talbert, O. R., Kurland, L. T., and Godden, J. O.: Geographic Distribution of Multiple Sclerosis: A Comparison of Prevalence in Charleston County, South Carolina, U.S.A. and Halifax County, Nova Scotia, Canada. To be published.

Alter, Milton and Talbert, Rhett: Myasthenia Gravis in One Member of A Monozygotic Twinship. To be published.

Kurland, Leonard T. and Alter, Milton: The Current Status of the Epidemiology and Genetics of Myasthenia Gravis. Symposium of the Second International Congress for Myasthenia Gravis. In press.

PHS-NIH
Individual Project Report
Calendar Year 1959

PART A

7. Project Title: The Prevalence and Incidence of Multiple Sclerosis in Duxbury, Massachusetts

Principal Investigator: Howard D. Siedler

Other Investigators: Walter Deacon, Duxbury, Massachusetts
Leo Alexander, Boston, Massachusetts
Leonard T. Kurland

Man Years: Total - 3/8
Professional - 1/4
Other - 1/8

Project Description:

The preliminary investigation of the frequency of multiple sclerosis in Duxbury, Massachusetts, was determined because of the suspected high frequency of M.S. among its residents. Although the incidence and prevalence are high, the factor of chance in selection rather than local environmental situation cannot be ruled out. Further studies in the vicinity of Duxbury and some additional genetic investigation in this region are indicated.

PART B

Publications other than abstracts from this project:

Deacon, Walter E.; Alexander, Leo; Siedler, Howard D. and Kurland, Leonard T: Multiple Sclerosis In A Small New England Community. To be published in The New England Journal of Medicine

PHS-NIH
Individual Project Report
Calendar Year 1959

PART A.

8. Project Title: Multiple Sclerosis in Twins and Their Relatives

Principal Investigator: R. P. Mackay, University of Illinois

Other Investigator: N. Myrianthopoulos

Cooperating Agencies: University of Illinois

Man-Years: Total - 8/32
Professional - 2/32
Other - 6/32

Project Description:

To determine whether any hereditary factors are involved in the causation of multiple sclerosis by studying the occurrence of the disease among monozygotic and dizygotic twins and their relatives.

The first phase of the study was completed in 1958. The second phase, which will involve the reexamination of all twins, will begin in 1961.

PART B.

Mackay, R. P. and Myrianthopoulos, N. C.: Multiple Sclerosis in Twins and their Relatives. Preliminary Report on a Genetic and Clinical Study. Arch. Neurol. Psychiat. 80: 667-674, 1958.

Myrianthopoulos, N. C. and Mackay, R. P.: Multiple Sclerosis in Twins and their Relatives: Genetic Analysis of Family Histories. Accepted for publication by the American Journal of Human Genetics.

PHS-NIH
Individual Project Report
Calendar Year 1959

PART A

9. Project Title: Cerebral Vascular Diseases: A Study of Their Natural History in the Population of Rochester, Minnesota.

Principal Investigator: Anthony Fons

Other Investigator: Clark Millikan

Cooperating Unit: Mayo Clinic, Rochester, Minnesota

Man Years: Total - 1 1/32
Professional - 1
Other - 1/32

Project Description:

The unique community medical services facility offered by the Mayo Clinic and its affiliated hospitals will provide almost complete coverage of diagnosed cerebral vascular disorders in patients and at autopsy. Past medical histories are unusually detailed and accurate; follow-up facilities are excellent. A descriptive study of various clinical and pathological forms of cerebral vascular disease and details on selectivity by population characteristics will be carried out first. Studies on prognosis of subarachnoid hemorrhage prior to the present surgical era will be made to provide baseline data for comparison with various surgical procedures now recommended. The relationship of cerebral vascular disease with past medical history and particularly with other vascular diseases will be determined.

PHS-NIH
Individual Project Report
Calendar Year 1959

PART A

10. Project Title: Paralytic Poliomyelitis and Aseptic Meningitis Syndrome in Washington, D. C., and Surrounding Counties During 1957

Principal Investigator: Howard D. Siedler

Cooperating Agencies: D. C. Health Department

Man Years: Total - 1/2
Professional - 1/4
Other - 1/4

Project Description:

The report of studies concerning paralytic poliomyelitis and aseptic meningitis syndrome in Washington, D. C. and surrounding counties in 1957, has been completed. The outbreak originated in a small Southwest quadrant of the city where the population is predominantly negro and of lower socio-economic standing. Paralytic disease manifested a marked selectivity for preschool age children from this group, the same pattern observed in the Chicago 1956 epidemic. Aseptic meningitis syndrome in the Washington group was believed to be associated in the majority of patients with poliovirus. This syndrome in the county group was associated with poliovirus in only 1 of 11 instances where viral cultures were positive.

PART B

Siedler, Howard D; Kalter, Seymour S; Thrupp, Laurie D; Utz, John P; Finucane, Daniel L; and Parrott, Robert H. Outbreak of Type III Paralytic Poliomyelitis in Washington, D. C. in 1957. For publication in the American Journal of Hygiene.

PHS-NIH
Individual Project Report
Calendar Year 1959

PART A.

11. Project Title: Evaluation of Possible Teratogenic Effect of Asian Influenza Virus (Collaborative Project)

Principal Investigator: Leonard T Kurland

Other Investigators: E. Goldstein, Biometrics Branch, NINDB
Howard D. Siedler
Stanley N. Faro

Cooperating Units: 7 Cooperating Universities and Hospitals
Johns Hopkins
University of Pennsylvania
University of Ohio
Baylor University
Charity Hospital
Hartford Hospital and State Univ. of New York School of Medicine
University of Maryland

Man-Years: Total - 2
Professional - 4/32
Other - 1 7/8

Project Description:

Certain virus infections during early pregnancy are known to have an adverse effect on the fetus. The Asian influenza epidemic offered an unusual opportunity to assess the teratogenic effect, if any, of this strain of influenza virus. Histories have been collected and serological specimens have been obtained from about 8,000 women in 15 centers. For the purposes of this study the histories and sera from mothers of affected offspring and controls will be evaluated; those from 3,500 mothers in 7 medical centers will be utilized for the study.

All bloods have been analyzed and all histories received; coding should be finished by January of 1960 and the report should be completed by next Spring.

PART B.

None

PHS-NIH
Individual Project Report
Calendar Year 1959

PART A

12. Project Title: Excess Congenital Anomalies in Alexandria, Virginia

Principal Investigator: Howard D. Siedler

Other investigators: Thomas F. McGough

Man Years: Total - 8/32
Professional - 2/32
Other - 6/32

Project Description:

In February, 1959, there were 12 infants reported to have been born with congenital anomalies in the Alexandria, Virginia, Hospital among a total of 303 deliveries. The source of these reports was routine birth certificates. This abrupt increase over the usual monthly experience prompted an investigation which centered about correlation procedures with other events of the prenatal period and probability of this incidence of anomalies occurring by chance.

Present Status: The project has been completed and a report has been prepared for publication.

PART B

Siedler, Howard D; McGough, Thomas F: Excess Congenital Anomalies in Alexandria, Virginia. (Accepted for publication in Public Health Reports.)

PHS-NIH
Individual Project Report
Calendar Year 1959

PART A

13. Project Title: Experimental Studies of the "Minamata Disease"

Principal Investigator: Leonard T. Kurland

Other Investigators: Stanley N. Faro
Gert Lacquer, NIAMD
Olaf Mickelsen, NIAMD
Howard D. Siedler

Cooperating Units: National Institute of Arthritis and Metabolic Diseases
Occupational Health Program, Cincinnati, Ohio
Chesapeake Biological Laboratory, State of Maryland, Solomons, Maryland
Texas Game and Fish Commission, Austin, Texas

Man Years: Total - 12/32
Professional - 6/32
Other - 6/32

Project Description:

An unusual neurological illness attacked at least 75 persons in fishing villages near Minamata Bay in Kyushu Island, Japan, between 1953 and 1956. It was manifested by numbness in extremities and around the mouth, slurred speech, unsteady gait, constricted visual fields, progressive amentia and psychosis. Case fatality was 35 per cent; all affected have permanent neurological or intellectual deficits. As a result of epidemiological and other studies made by Japanese scientists, it was concluded that the cause of Minamata Disease was probably a toxic chemical compound contained in the effluent which flowed into Minamata Bay from a nearby factory. Recent Japanese investigations suggest that an organic mercury compound used as a catalyst in vinyl chloride production may be the cause of Minamata Disease. It is believed that this compound is either formed or concentrated in shellfish and other marine life when the mercury compound is ingested by the marine organisms. This disease is of interest because it may aid in the understanding of the mechanisms of toxic encephalopathy. We are conducting feeding experiments with shellfish from Minamata Bay provided by scientists at the University of Kumamoto, Japan, to help establish the exact cause of the disease. Studies are also underway to compare the relationship of the seafood poisoning and organic Hg poisoning in animals.

Project 13.

There is reason to believe that in the production of vinyl chloride in the United States, American industry has taken extreme precautions to prevent contamination of nearby waters used for commercial fishing. If this is clearly established, knowledge of these methods of handling toxic agents will be transmitted to authorities in Japan concerned with preventing any recurrence of the epidemic there.

PHS-NIH
Individual Project Report
Calendar Year 1959

PART A

14. Project Title: Phlyctenulosis in Alaska Natives

Principal Investigator: Robert Phillips, AHRC

Other Investigators: Joseph Shelton, AHRC
Leonard T. Kurland

Cooperating Unit: Arctic Health Research Center

Man Years: Total - 1/32
Professional - 1/32

Project Description:

Corneal scars from phlyctenulosis are present in about 40 per cent of the Alaska Native population. The distribution with respect to geography, diet, incidence of otitis media, the rate of tuberculosis infection and tribal customs, is to be determined. The incidental effect of prophylactic isoniazide on the incidence rate of acute phlyctenulosis will be studied in conjunction with the current study on prophylaxis in tuberculosis. This project was scheduled to go into its full-scale program this year but has been delayed because of difficulty in recruiting an epidemiologist with experience in ophthalmology.

PHS-NIH
Individual Project Report
Calendar Year 1959

PART A

15. Project Title: Study of the Incidence and Geographic Distribution of Uveitis

Principal Investigator: Stanley N. Faro

Other Investigators: Henry P. Wagener, Mayo Clinic, and Leonard T. Kurland

Cooperating Unit: The Veterans Administration, Wash., D. C.

Man Years: Total - 3/32
Professional - 2/32
Other - 1/32

Project Description:

There have been few studies reported on the incidence, prevalence and distribution of uveitis. The incidence for several recent years in the population of Rochester, Minnesota, will be determined through the records of the Mayo Clinic. Plans are being developed to study the geographic distribution from nationwide VA Hospital discharge data.

PART B

None

PHS-NIH
Individual Project Report
Calendar Year 1959

PART A

16. Project Title: Applications of The Technique of The Tissue Culture to Metabolic Problems in Selected Genetic Diseases

Principal Investigator: Robert S. Krooth

Collaborating Investigators: Dr. H. Eagle (NIAID)
Dr. J. Darnell (NIAID)

Man Years: Total - 1/2
Professional - 1/4
Other - 1/4

Project Description:

In 1954 Book and Kostman first suggested the value of the technique of tissue culture in human genetics - based on their experience with infantile genetic agranulocytosis. The technique for manipulating cultures and the chemical composition of the media have been developed so that it is feasible to inquire into the metabolic properties of a number of genetic diseases.

At the present time, actively growing marrow and skin cultures are being maintained from patients with hereditary neurological diseases as well as from patients with certain metabolic disorders. However, the technical perfection of skin cell culture will require considerable work. We hope to detect the accumulation in cell cultures of visible intracellular metabolic accretions. We are working with Gaucher's diseases and certain other histocytic and lipid storage states. As of this writing, however, our immediate interests continue to be concerned with perfection of technique.

PART B

None

PHS-NIH
Individual Project Report
Calendar Year 1959

PART A

17. Project Title: Possible Association of High Beta-isoaminobutyric Acid Excretion With Gout

Principal Investigator: Robert S. Krooth

Other Investigators: Dr. J. E. Seegmiller (NIAMD)
Dr. A. Grayzel (NIAMD)
Dr. H. Eldon Sutton, Univ. of Mich. Med. School

Man Years: Total - 1/4
Professional - 1/8
Other - 1/8

Project Description:

Beta-isoaminobutyric acid occurs as an intermediate product in the physiologic degradation of thymine. It may also occur as a metabolite of valine though at present this pathway appears less certain and less important than the former one. The amount excreted appears to depend in each case upon the blood level, which in turn is a function of the total miscible pool. Whether one is a high or a low excretor is largely independent of age and diet, and appears to be mainly genetically determined. The formal genetics, however, havenot yet been satisfactorily worked out.

Persons with polycythemia vera, leukemia, and certain other neoplastic diseases and mongoloid idiots (who have a high incidence of leukemia) put out very large quantities of the acid, apparently irrespective of their genotype. Probably one reason for this is that individuals who turn over cells rapidly have a high rate of DNA turnover, and thus a high rate of thymine degradation. A high rate of thymine degradation may result in elevated steady state pools of intermediate thymine metabolites including perhaps beta-isoaminobutyric acid.

Talbot has recently urged that about 10 percent of all gouty patients he sees eventually develop a neoplastic disease, particularly neoplasms of blood. Presumably the mechanism for the hyperuricemia here is the same as that postulated above.

An effort is now being made, in collaboration with the investigators listed, to:

1) compare the frequency of high beta-isoaminobutyric acid excretors among gouty and non-gouty patients.
2) compare the clinical and genetic features of the disease in high and low excreting patients with gout.
3) follow high and low excretors with gout prospectively to see if neoplastic processes occur with different frequency in the two groups.

If this amino acid is elevated in blood and body fluids when DNA turnover is increased, then it may eventually prove useful in the early detection of certain tumors. Recent technical developments, notably improvements in the Stein-More column, may soon make accurate determination of the acid in the cerebrospinal fluid feasible. In this circumstance, levels of the acid may one day complement existing diagnostic methods in the detection of some of the intracranial tumors. However, such possibilities can be approached with greater confidence when more is known about the metabolic significance of the acid in diseases where the tumor freely communicates with the peripheral blood.

As of this writing, 18 gouty patients and three persons without gout have had urine chromatography in Dr. Sutton's laboratory. Only two patients (both gouty) had an elevated excretion of BAIB. Of these two, one patient had polycythemia vera with secondary hyperuricemia and gout, thus illustrating the association of high BAIB excretion with blood neoplasms. Following the exclusion of this patient, the frequency of high BAIB excretors among patients with presumed primary gout is one out of seventeen, which is of the same magnitude as the population frequency in this country.

No other abnormalities distinguishing the urine chromatograms of the gout patients from the controls has thus far been noted. The chromatograms are still being reviewed.

PART B

None

PHS-NIH
Individual Project Report
Calendar Year 1959

PART A

18. Project Title: Relation of Ability to Taste Phenylthiourea and Related Compounds to Thyroid Disease

Principal Investigator: Robert S. Krooth

Other Investigators: Dr. T. E. Rall (NIAMD)
Dr. Bernard Witkop (NIAMD)

Man Years: Total - 1/4
Professional - 1/8
Other - 1/8

Project Description:

Twenty-five years ago Fox discovered that some persons are able to taste phenylthiourea (and certain related compounds) while others cannot. Shortly thereafter Blakeslee and Snyder showed that inability to taste is largely determined by a recessive Mendelian gene. In the past 15 years numerous investigators have found that many compounds in this family have appreciable antithyroid activity and some occur naturally in food. Recently Harris and Kalmus (in 1949) and Kitchin, Howel-Evans, Clarke, and McConnel (in 1959) have noted that non-tasters occur with undue frequency among patients with non-toxic goiter. The latter authors have noted also that tasters are exceptionally common among patients with diffuse toxic goiter. One possibility is that the trait causes differences in dietary habits with respect to naturally occurring goitrogens. It seems more likely, however, that some individuals (tasters) taste these compounds because the cells of their body, including the taste buds, metabolize thiourea-like substances differently from non-tasters. Differences in the metabolism of this class of dietary goitrogens may explain why non-tasters appear susceptible to non-toxic goiter and tasters to toxic thyroid disease. Thus (in this case at least) variation in tasting ability reflects metabolic differences having significance at remote organs -- outside the nervous system.

In collaboration with Dr. T. E. Rall, an effort is being made to associate the clinical features of goitrous patients with their taste status, both in the case of toxic and non-toxic goiter. It is suspected, for example, that non-tasters with non-toxic goiter may form a more uniform group than tasters with non-toxic goiter.

In collaboration with Dr. Bernard Witkop, we are inquiring further into the chemical nucleus conferring this taste-activity on organic compounds. A resonating system relating carbon, sulfur, and nitrogen appears necessary. This problem once solved, may have general implications in the field of taste physiology.

PART B None

PHS-NIH
Individual Project Report
Calendar Year 1959

PART A.

19. Project Title: The Detection of the Genetic Carrier in Peroneal Muscular Atrophy by Conduction Velocity Studies

Principal Investigator: N. Myrianthopoulos

Other Investigators: M. Lane, NINDB

Man-Years: Total - 4/32
Professional - 2/32
Other - 2/32

Project Description:

A study to determine whether the genetic carrier (whether unaffected children of affected parents or individuals showing lack of penetrance) can be detected by slow peripheral nerve conduction velocity. A slowing of conduction velocity in the peroneal, ulnar and other nerves has been repeatedly demonstrated in patients with peroneal muscular atrophy.

PART B.

None

PHS-NIH
Individual Project Report
Calendar Year 1959

PART A

20. Project Title: The Question of Penetrance in Peroneal Muscular Atrophy

Principal Investigator: Ntinos C. Myrianthopoulos

Man Years: Total - 1/32
Professional - 1/32

Project Description:

A study of families with peroneal muscular atrophy to determine whether the reduction in penetrance in this disease, as described in the literature, can be substantiated after vigorous neurological examination.

Present Status

With the collection of two very extensive pedigrees the study has now moved on to a new procedure for the detection of the genetic carrier state: the measurement of conduction velocity of peripheral nerves in members of the families. (See Project No.19)

PART B

None

PHS- NIH
Individual Project Report
Calendar Year 1959

PART A.

21. Project Title: Genetic and Clinical Investigations in Myotonic Dystrophy

Principal Investigator: N. Myrianthopoulos

Other Investigators: J. Caughey, NINDB

Man-Years: Total- 6/32
Professional- 4/32
Other- 2/32

Project Description:

A study based on history and examination to determine if children of patients with myotonic dystrophy show congenital anomalies more frequently than expected; if there exists a significant difference in the occurrence of congenital anomalies between children of parents with the complete syndrome and children of patients who show only cataract; if the sex of the affected parent has any effect in the production of congenital anomalies among the children; and what is the reproductive fitness of persons affected with myotonic dystrophy.

PART B.

None

Individual Project Report
Calendar Year 1959

PART A.

22. Project Title: The Detection of the Heterozygote in Cerebroretinal Degeneration (Amaurotic Family Idiocy)

Principal Investigator: N. Myrianthopoulos

Other Investigator: G. Brecher

Cooperating Unit: Clinical Center, NIH

Man-Years: Total - 2/32
Professional - 2/32

Project Description:

Examination of a large number of peripheral blood smears from patients with the infantile form of cerebroretinal degeneration, and smears from their parents and siblings, failed to disclose any diagnostic changes in the peripheral blood. Examination of blood smears from patients with the juverile form of the disease disclosed very striking changes in the lympocytes in the form of large vacuoles in the cytoplasm. The investigation is now concentrated on the juverile form.

PART B.

None

PHS-NIH
Individual Project Report
Calendar Year 1959

PART A

23. Project Title: Some Epidemiologic Features of Tay-Sachs Disease

Principal Investigator: N. Myrianthopoulos, NINDB

Man-Years: Total - 2/32
Professional - 2/32

Project Description:

A study to determine the incidence and prevalence of Tay-Sachs Disease among the Jewish and non-Jewish populations of the United States by mortality statistics.

Present Status: Mortality statistics for the years 1954, 1955 and 1956 have already been collected and mortality data for 1957 have now become available. Information concerning the ethnic origin of patients is being implemented and the data are being made ready for analysis.

PART B.

None

PHS-NIH
Individual Project Report
Calendar Year 1959

PART A.

24. Project Title: Multiple Congenital Anomalies in three children of a family

Principal Investigator: N. Myrianthopoulos

Other Investigators: R. Hamburger
C. Cheney

Cooperating Units: Yale University School of Medicine .
Grace-New Haven Hospital

Man-Years - Total - 2/32
Professional - 2/32

Project Description:

To study genetically, clinically, anatomically and pathologically what appears to be a new syndrome of multiple congenital abnormalities in a New England family. These were identical in three successive children, all of whom died within one month after birth.

PART B.

None

PHS-NIH
Individual Project Report
Calendar Year 1959

PART A.

25. Project Title: Huntington's Chorea in Monozygotic Twins

Principal Investigator: N. Myrianthopoulos

Other Investigator: P. Rowley, NINDB

Man-Years: Total - 2/32
Professional - 2/32

Project Description:

A comparative study of the onset, course and clinical findings of Huntington's chorea in a pair of female monozygotic twins with emphasis on some pathological findings and eugenic problems in the family of the twins.

PART B.

Myrianthopoulos, N. C. and Rowley, P. T. Monozygotic Twins Concordant for Huntington's Chorea in a Family with Chorea and Mental Illness. Accepted for publication by Neurology.

PHS-NIH
Individual Project Report
Calendar Year 1959

PART A.

26. Project Title: Parkinsonism - Ataraxic Drugs Study

Principal Investigator: N. Myrianthopoulos

Other Investigators: Leonard T. Kurland
A. Kurland, Spring Grove State Hospital

Cooperating Unit: Spring Grove State Hospital

Man Years: Total - 24/32
Professional - 8/32
Other - 16/32

Project Description:

A study to determine the occurrence of Parkinsonism among the relatives of two groups of patients: those who show Parkinsonian symptoms on high therapeutic dosages of certain phenothiazine derivatives, and those who prove to be resistant to the side effects of these drugs. The two groups of patients, actually patients and controls, have been selected from the patient population of Spring Grove State Hospital. The collection of data is almost completed; analysis will follow.

PART B.

None

PHS-NIH
Individual Project Report
Calendar Year 1959

PART A.

27. Project Title: A Survey of Schizophrenics Among the Relatives of Schizophrenia Patients

Principal Investigator: N. Myrianthopoulos

Cooperating Unit: Spring Grove State Hospital

Man-Years: Total - 18/32
Professional - 2/32
Other - 16/32

Project Description:

To determine the occurrence of schizophrenia among the relatives of patients who have already been selected for another project (26) and to determine the role of inheritance in schizophrenia. This project is a by-product of project No. 26 and has the advantage of overcoming some of the biases involved in selecting an adequate sample. The collection of data is almost completed; genetic analysis will follow.

PART B.

None

ANNUAL REPORT
Calendar Year 1959
Biometrics Branch - Collaborative Research
National Institute of Neurological
Diseases and Blindness
National Institutes of Health

A. SCOPE OF PROGRAM

The interests and involvement of the Biometrics Branch during the calendar year 1959 may roughly be divided into three major areas:

1. Collaborative Project of Cerebral Palsy and Other Neurological and Sensory Disorders of Infancy and Childhood.

2. Statistical consultation and/or service given to investigators outside of NINDB on other projects.

3. Statistical consultation and/or service furnished to clinical and basic research investigators at NINDB in the areas of neurology and blindness.

Accomplishments achieved and problems encountered during the course of 1959, as well as proposed future objectives in each of the above three areas, are reviewed below:

1. Collaborative Project of Cerebral Palsy and Other Neurological and Sensory Disorders of Infancy and Childhood.

In the intensive phase of the Collaborative Project, it is anticipated that some 14 participating programs will contribute 50,000 pregnancies over five years to account for a minimum of 40,000 live births available for at least a six-year followup. The purpose of this prospective approach is to relate factors in the gravida (genetic, family health, medical, socio-economic, prenatal history, etc.) to outcome of pregnancy. In 1958 the various institutions had been completing pretest forms of which the purpose was to train local personnel and determine whether the types of data requested could feasibly and reliably be secured. Cases from a given institution had their unique serial numbers. This was considered the pretest case number which identified the patient and the institution. After pretest forms were completed, they had been sent by the institutions to the Biometrics Branch for editing and critical review. For each institution there were prepared periodically evaluations of the

quality of these completed forms and suggestions for improvement which were taken up with each institution separately. There was no attempt to pretest case collection or methodological procedures. It became evident that the pretest forms would need considerable modification before they were suitable for full-scale investigation. As a result, statisticians of the Branch worked in close cooperation with Project Services Branch members and with the staff of the Bureau of Social Science Research, Washington, D. C., and various consultants, and devised forms to secure more meaningful and reliable data in the obstetrical, pediatric, and socio-economic-genetic areas that would also be more amenable to coding and tabulating procedures.

In January 1959, "final" study forms, covering the various aspects (prenatal, labor, delivery, neonatal, etc.) were distributed to each of the participating institutions. Where an institution's case selection procedures, the quality of its data collection procedures, and its completed forms were considered by the institution to be of sufficiently high calibre, it was permitted to start enrolling so-called "final" study cases with a new identifying serial number series. Not all institutions believed that they qualified immediately for such "final" series numbering. They continued using the pretest series numbering system. As the year progressed and they were confident that their procedures and forms had improved sufficiently, such procedures and forms were reviewed by the central offices and, if acceptable, the hospital was permitted to change numbering systems. Unfortunately, however, some institutions switched to "final" study case numbering even when evaluation of case selection procedures, data collection procedures or quality of completed forms indicated that much improvement was still in order. Data received on pretest cases and on "final" study cases have been tabulated separately. As of October 31, 1959, some 5500 gravidas and 4200 babies have been processed in the "pretest" series. In the "final" study series as of the same date, approximately 3300 gravidas and 1800 babies have been processed.

Perhaps the largest single effort of the Biometrics Branch relating to the Collaborative Project is the processing of data collected in the course of the study. The only feasible approach to summarization of the wealth of data being collected is to codify the pertinent information initially in relatively broad and simplified categories, but so as to permit identification of abnormal findings and factors for control purposes. More detailed codes can then be applied to "abnormal" cases and adequate controls, as required. The design of the initial "coarse" codes are now in the process of development. For 10 of the study forms the coarse codes and their related editing procedures have been completed. Full processing of some of the study records for which editing and coding procedures have been developed is under way. Processing will proceed as rapidly as additional clerical staff can be hired and trained.

During the year formal questionnaires were sent to each institution requesting specified information as to its case selection procedure, covering the following aspects: (1) the segment of the hospital population, such as clinic or private, from which study cases are drawn; (2) of such segment admitted or seen for prenatal care or delivery, the eligibility of all such patients for selection as study cases; if all not eligible, the types of patients routinely or occasionally excluded from possible selection in the study sample (basis for exclusion); (3) the point in time of case selection; (4) the description of the case selection procedure separately for cases receiving prenatal care and for those reporting for delivery only, indicating where done, by whom, methods used for handling special situations (such as refusal to partitipate), and the sampling ratio; and (5) the approximate number of patients per year selected into the study. In general, these descriptions of case sampling procedures were in accordance with recommendations given by members of the Biometrics Branch on visits to the institutions. Where an institution is not registering 100% of its cases into the study, a systematic sampling procedure has been instituted that will furnish the number of pregnant women annually that the institution can adequately process with the facilities, space, and personnel at its disposal.

A procedure was instituted during the year by the Biometrics Branch whereby for all obstetrical patients, not just those being registered into the study, a form (OB-1) is completed and sent to the central office by the institution. This form will furnish information for each institution on obstetrical population characteristics, particularly with respect to such items as age, marital status, race, gestational age at time of registration for prenatal care, etc. As a result of this information, the Biometrics Branch will be better able to recommend special sampling procedures, where desired, so that it may be possible to reach certain objectives for the study as a whole without losing others.

It was desired to arrive at some estimation of the percentage of repeat pregnancies (i.e., women registered more than once for the study) to be expected in the study from each institution over the five-year period during which gravidas are taken into the study. This estimation was necessary so that it might be possible to determine what proportion of repeat pregnancies could be permitted over-all into the study without reducing the number of different gravidas to a level that would be insufficient to uncover or demonstrate statistical significance for true etiological associations. This becomes particularly important in connection with a stated objective of the study to determine the relationship of unknown and unsuspected factors in the gravida to the etiology of neurological and sensory disorders in the neonate. Questionnaires were devised by the Biometrics Branch and sent to each institution, requesting information as to the employment of a

unit history number, availability of punch cards for any of the last ten years of obstetrical admissions, bearing information as to unit history number, year of delivery, age, and race. On the basis of the returns, decisions could be made as to methods by which the desired data could be secured from each institution.

It is planned to prepare summary statistical reports periodically during the course of the study. These reports will include summary tabulations for each institution and for all the institutions combined. Due to the complex nature of this study, and the multitude of variables to be analyzed, it would not be feasible nor practicable for these reports to be prepared in great detail. These summary tabulations will be in the form of relatively simple distributions, such as two-way classifications for the items of major importance and interest. It is planned to set up dummy tables to be referred to the collaborating institutions for review, suggestions for additions or deletions, and for purposes of indicating priority in connection with the periodic feed-back. It is not contemplated to prepare detailed, periodic analytical reports, describing associations or correlations of perinatal events with the development of neurological and other sensory disorders until data on a sufficient number of pregnancies and outcome have been collected to allow for meaningful interpretation. However, on occasion, tabulations and interpretations in conjunction with any special analyses of central core data could be prepared. The reports or tabulations, which are to be prepared periodically, will also be utilized to determine the general distribution of the populations sampled in the different institutions with respect to the variables under consideration. They will be used, in addition, on a continuing review basis to point up possible major differences between institutions which may be due either to random variations, differences with respect to population sampled, or differences among the institutions in the procedures, techniques, and methods of data collection. In the interpretation of these data, consideration must be given to these possible sources of differences.

The continuing examination of data will provide valuable clues leading to a review of the procedures to determine through what mechanisms the observed deviations from the general experience are occurring within a given institution's population. It may well be that deviations in the procedures employed in the collection of data may account for inconsistencies.

During the course of the year the Branch Chief attended meetings of the Advisory Board of the Collaborative Project and of the Ad Hoc Review Board. He and other Branch members attended meetings of the Project Directors, as well as conferences and workshops devoted to consideration of the study forms and data received dealing with the following aspects of the Project: (1) Socio-economic-genetic, (2) prenatal, (3) labor and delivery, (4) pediatrics, (5) neurological, (6) psychological, and (7) pathological. In addition, numerous meetings

of small working groups and special meetings with outside consultants were attended by staff members in the capacity of statistical consultants. Every institution in the Collaborative Project was visited at least once during the year by Branch staff, either as members of Project Site Committees, or as statistical consultants on procedure in any of the above aspects of the Collaborative Project or related ancillary studies. The purpose of these visits was to obtain information regarding the characteristics of the hospital populations, case selection procedures, selection of children for standardization of the Bayley Psychological Test, processing of patients, completion and editing of study records, and other problems of a statistical nature with regard to the various aspects of the study. These visits tended not only to "educate" personnel in the collaborating institutions in the statistical aspects of the study, but also to enhance the appreciation of the Branch staff of the difficulties encountered in the application of some study forms and of the need of possibly different approaches.

Branch members work closely with medical and other professional members of the Project Services Branch, NINDB, in setting up study data forms which are suitable for processing and data reduction, and in devising codes and manuals of procedure and instruction. Due to lack of sufficient medical staff in the Project Services Branch, the periodic review of forms for completeness, consistency, legibility, and quality has been almost completely a responsibility of the Biometrics Branch. The staff also prepared statistical analyses and reports for working groups and conferences. For instance, in connection with a test-retest of the neonatal neurological examination to determine the reliability and adequacy of the items, techniques, and the form, a Branch staff member directed the collection, editing, coding, and tabulating of the data, prepared the analysis, and wrote a report given at a meeting of neurologists. Interestingly enough, primarily stimulated by the result of this analysis, two additional test-retest studies (revised neonatal neurological examinations and the one-year neurological examination) are being planned. Additional examples are an analysis of the one-minute Apgar scores, analysis of data from the pediatric study forms dealing with cyanosis and jaundice in the newborn, etc. Further evaluation of incoming data, such as data from the obstetrical and gynecological examinations of the gravidas have been accomplished through simple tabulations of various items so that percents of given characteristics found in one institution may be compared with the others.

Branch members have participated in training sessions for interviewers by explaining the need for carefully and uniformly collected data and its relationship to the role of the Biometrics Branch in the Project.

Branch members have also worked closely with procedure analysts of the Project Services Branch, NINDB, and the Statistical Processing Branch, NIH, in the preparation of procedures to be used in the collection of data centrally, storage of forms, and establishment of necessary controls so that up-to-date information in number and types of forms received and patients processed by institutions may be available on a current basis.

The Branch Chief presented a paper on "Some Service Benefits to a Community from a Long-Term Research Program" at the Health and Welfare Council's Citizen's Conference, Philadelphia, Pennsylvania, in September 1959. He was also co-author of the following publications:

Pollack, E. S.; Person, P. H., Jr.; Kramer, M.; and Goldstein, H. Patterns of Retention, Release, and Death of First Admissions to State Mental Hospitals. Public Health Service Publication No. 672. Public Health Monograph No. 58. Washington, D. C., U. S. Government Printing Office, 1959, 53 PP.

Many statistical problems remain to be solved. Among these are:

1. The establishment of a suitable sampling procedure adapted to the situations of each institution and to the needs of the Project. Such procedures may be geared to the need for oversampling on certain high risk variables and for patients seeking prenatal care early in pregnancy; and to the need for including a certain proportion of repeat pregnancies;

2. The completion of appropriate codes and manuals of procedure for each aspect of this study;

3. The further development of adequate methodological tests of reliability and validity of the data secured;

4. Inauguration of methods for periodic feedback of tabulated data to the institutions;

5. Further planning for data analysis.

The Branch is involved in two studies, in which it has given consultation and/or service, which are indirectly related to the intensive phase of the Collaborative Project. In one of these, coordinated by the Epidemiology Branch, NINDB, a collaborative prospective study of the relationship between Asian Flu during pregnancy and the occurrence and course of neurological sequelae in

the offspring at birth or within one year of age, the Biometrics Branch has been involved as the central statistical agency. During the year, some 3500 cases from seven institutions were edited, coded and submitted for machine processing. The tabulation and analysis of these data are the responsibility of the Branch. Consultation relating to the sampling of serological specimens in the study has also been given.

In the other study, Branch members have worked with the Director of Research and Statistics, Baltimore City Health Department, and his associates, in planning a study, "Smoking and Prematurity," part of which was financed by contract. The purpose of this study is to determine the incidence of prematurity among offspring of gravidas who smoke and among those who do not smoke, and to include within these groups other co-variables, such as work history, education, blood grouping, and personality characteristics. To date, some 2200 gravidas have been interviewed. Of these about 1160 have delivered. It is expected that an analysis of these delivered women and their offspring will be available sometime in January 1960. The final analysis for the total study should be ready late in 1960. Preliminary data completed on 450 cases, available to date, fail to show for the smokers any significant increase in the incidence of prematurity over non-smokers. There is no difference in the average birth weights of babies born to the two groups nor is there any significant difference between the average Neuro-Psychiatric scores of the groups. The smokers and non-smokers differ with respect to the percent of primipara (lower in smokers), the percent with pre-eclampsia (lower in smokers), and work history during pregnancy (greater in smokers). These factors will be further investigated with increased numbers of cases for analysis.

Staff of the Branch have consulted with the Director, Research and Statistics, Health Insurance Plan of New York City (HIP) and his associate, in planning a study to test the reliability of certain data secured on the past medical and family health history of the gravidas. This would be done by comparing the data secured from the HIP gravidas, by interviewers using Collaborative Project forms, with data available from the HIP prenatal records.

Exploratory discussions with officers of Group Health, Incorporated, Washington, D. C., have taken place to determine the feasibility of setting up studies, under contract, to test the reliability of certain data furnished by Group Health, Incorporated, obstetrical clients as compared to data on records available from that Association.

The accomplishments given above relate to the intensive phase of the Collaborative Project. In view of the possibility that the incidence of certain neonatal deficits is so low that even 40,000 live births will not yield enough cases to demonstrate statistical significance, an extensive phase has been postulated. In this phase a number of approaches are envisioned whereby the cases available for study in given categories may be increased. For instance, it is expected that, in those communities

in which collaborating institutions are located, attempts will be made to relate retrospectively, during the course of the Collaborative Project, the occurrence of neurological disorder to prenatal, labor, and delivery data available from hospital records, vital statistics records, etc. Although the number of cases will be increased, the data available will not be as comprehensive nor as detailed as that in the intensive phase. To the Columbia University School of Public Health and Administrative Medicine has been assigned the task of planning methodology and approaches of the extensive phase.

Branch staff members participated in the Planning Conference at West Point, New York, in January 1959, to explore the various aspects of the extensive phase.

Additional studies, valuable to the conduct of the extensive phase, may be executed by utilizing copies of punch cards of all live births, fetal deaths, and neonatal deaths, purchased by contract from cooperating State and local health departments for the cities concerned with the intensive phase. Such a study of fetal wastage in New York City is currently under way on a joint basis with the New York City Health Department. It is concerned with the tabulation and analysis of approximately 380,000 punch cards for the period 1955-56. It is expected that a number of valuable leads will come to light as a result of this investigation. A contract has been negotiated with the New York State Health Department for similar punch cards for the Erie County area, covering the population served by the Children's Hospital, Buffalo, New York. Approximately 108,000 punch cards for the period 1954-57 are concerned. Negotiations are under way to conclude similar contracts with the Minnesota State Health Department for the Hennepin-Ramsey County area, covering the population served by the University of Minnesota Hospital, Minneapolis, Minnesota; with the Oregon State Board of Health for the Portland City area, covering the population served by the Multnomah County Hospital and University of Oregon Medical School Hospital, Portland, Oregon; and with the Philadelphia City Department of Public Health for the Philadelphia City area, covering the population served by the Pennsylvania Hospital, Philadelphia, Pennsylvania. Attempts will be made to undertake similar or related studies during the coming year in other cities in which the collaborating institutions are located.

2. Statistical consultation and/or service given to investigators outside of NINDB on other projects.

The following represent the type of consultation and/or service rendered during 1959 to outside investigators on other projects:

(a) Evaluation of study design of a retrospective study of etiology of cerebral palsy in Chicago, supported by an NIH grant, and consultation given on the limitations and methods of analysis of data accumulated as well as data to be collected on various aspects of the study.

(b) Consultation on study design, development of adequate tabulations, procedures, and design and construction of codes given to the medical director of a research foundation, in connection with a retrospective study of the etiology of mental retardation among children in Chicago.

(c) Consultation on study design of a retrospective study of the etiology of cerebral palsy in several urban areas in Minnesota.

(d) Preparation of a mathematical model and appropriate tables for determining sample size and interpreting results in an investigation of the proportion of identical twins among twins with a certain placental structure.

(e) Consultation and statistical analysis of data dealing with hormones and cholesterol in chickens.

(f) Consultation on the use of "Health Unit Daily Records" and on the statistical procedures necessary in a study of the effects of influenza inoculation on respiratory illness among NIH employees.

(g) Consultation and service in setting up a study design and study forms for a proposed collaborative study of the etiology of uveitis.

Evaluation of the study design and merits of several projects submitted for research grants to the Easter Seal Research Foundation, United Cerebral Palsy, and the Office of Vocational Rehabilitation has also been rendered at the request of the Director of the Institute. These applications for research grants have been concerned with "The Role of Neonatal Jaundice as a Cause of Preventable Physical and Mental Handicap," "Study of Western Region's Needs and Resources in the Field of Training Teachers for Handicapped Children," "A Comparative Study of Selected Mental Abilities of Cerebral Palsied Children with Varying Degrees of Perceptual Disabilities," "The Results of Stereotaxic Cerebral Surgery and Rehabilitation Procedures Upon Individuals Vocationally Handicapped by Parkinsonism, Cerebral Palsy, and Other Dyskenesias."

In addition, a number of proposed projects to be submitted for research grants in connection with the so-called "extensive" phase of the Collaborative Project were reviewed in order to evaluate their study designs. These projects were titled: "Community Obstetrical Study Follow-Up," "Study of Etiological Factors of Cerebral Palsy," "Relationship of Cigarette Smoking to Perinatal Loss," "Perinatal Factors of Health and Neurological Sequelae."

Review of a manuscript entitled: "A Survey of Blind and Partially-Sighted Children in California, Spring 1956" was requested by Public Health Reports prior to acceptance for publication.

To determine possible studies which the Branch might undertake jointly with the Veterans Administration on matters of interest to NINDB, Branch members met with the Chief, Biometrics and Evaluation Division, Reports and Statistics Service, Veterans Administration. Ideas were explored relative to the types and methods of study of research valuable to NINDB that could be undertaken with the types of data available to the Veterans Administration.

A "working group," composed of members of the Biometrics Branch and the National Office of Vital Statistics, has met periodically to explore ways in which joint projects of benefit to both NINDB and NOVS might be undertaken. Initial discussions have lead to a proposal, which is being worked up, whereby follow-back and record-anchored studies and surveys conducted by NOVS could be related to the Collaborative Project.

Branch staff attended a meeting of the Sub-committee on Records of the Committee on Maternal and Child Care of the American Medical Association, Philadelphia, Pennsylvania, in August 1959, to determine ways in which the Collaborative Project might be of help to the Sub-committee in drawing up proposed prenatal, labor, and delivery forms that might be recommended as standard for hospital use.

Statistical advice has been given, upon request, by the staff to various individual investigators in such matters as the role of virus infections in pregnancy, methods of determining the zygosity of twins, and the investigation of the prognostic value of certain pathological findings in the placenta.

Members of the Branch staff presently serve on the following committees, thus being, in effect, channels of communication whereby problem areas in the neurological field become known to the Branch and whereby the Branch's field of interest and activity become known to other investigators:

(a) Statistical Advisory Committee to "A Study of Use of Statistics on Maternity and Newborn Infant Care in Hospitals."

(b) Advisory Committee on Epidemiology and Biometry (NINDB-Liaison).

(c) Committee on Nomenclature and Biometrics of the American Academy of Neurology.

(d) Ad Hoc Committee on Mental Retardation of NIMH.

(e) NIH Advisory Committee to National Health Survey (NINDB-Liaison).

(f) Panel for the Study Group on Guide Material for Comparable Studies on Maternal and Perinatal Events Reported on Vital Records.

The Branch Chief was designated by the Director, NINDB, to represent the Institute at the annual meeting of the National Rehabilitation Association in Boston, Massachusetts, in October 1959.

3. Statistical consultation and/or service furnished to clinic and basic research investigators at NINDB in the areas of neurology and blindness.

Below are indicated the units in NINDB that received statistical aid from the Biometrics Branch in 1959 and the problems involved:

(a) Laboratory of Neuroanatomical Sciences.

Review and criticism of manuscript of study dealing with "A Probabilistic Model of Social Organization" in a monkey colony in Puerto Rico.

(b) Medical Neurology Branch.

Consultation and analysis of data on the comparison of force velocity and force shortening curves of muscles under different drugs.

(c) Surgical Neurology Branch.

Assistance in testing the significance of differences between body temperatures of a group of brain operated chimpanzees and a group of controls.

(d) Ophthalmology Branch.

Consultation and assistance in computation of regressions and tests of significance on the relationship between measures of venous pressure and intraocular pressure before and after the administration of diamox.

Consultation of the analysis and interpretation of data on comparisons of the effectiveness of daraprim on various categories of uveitis patients.

Statistical assistance in curve sitting, tests for "goodness of fit," analysis of variance, and variance ratios on squirrel eye response data.

(e) Office of Director, Collaborative Research.

Statistical analysis of gene frequencies obtained from blood test data.

(f) Epidemiology Branch.

Joint study of world-wide mortality from neurological and related disorders. Efforts to obtain official mortality statistics from some 40 countries on 26 specific disease entities in the International Classification of Diseases have resulted in the collection of usable data from more than 25 of these countries, although they are not all in the desired detail. For most of the countries, age-adjusted mortality rates for the various conditions have been calculated which may shed light on geographic patterns of mortality from diseases of the central nervous system.

Technical assistance and analysis of prevalence data, by race, based on a survey of multiple sclerosis in a community.

Consultation and assistance with problem of computing an overall chi-square combining the information on a genetic hypothesis in multiple sclerosis for different age groups.

(g) Publications and Reports Section.

Consultation and technical services provided on statistical material relating to perinatal problems in this country and abroad.

B. PROPOSED FUTURE OBJECTIVES

To date, the energies of the Branch have been directed to the aspects of the program mentioned above. With an increase in staff, it is expected that other aspects of proposed Branch activities relating to the development of a program for statistical data on prevalence, incidence, and mortality due to neurological and sensory disorders, will be approached. It is also hoped that a program to design and implement studies in order to investigate the relationship of prevalence, incidence, and mortality of such disorders to various biological, genetic, and environmental factors, will be undertaken cooperatively with the Institute's Epidemiology Branch and other interested agencies.

C. RECRUITMENT

Budget for fiscal 1960 includes 34 positions (12 professional-statistical positions and 22 nonprofessional clerical-stenographic positions). To date, 10 of the 12 professional positions and 19 of the 22 clerical-stenographic positions have been filled. The personnel ceiling of 34 positions is 22 positions (2 professional and 20 non-professional) less than the minimum needed to be able (a) to furnish statistical consultation and guidance required in connection with the

Collaborative Project; (b) to furnish consultation and service upon request from clinical and basic research scientists in neurology and blindness at the Clinical Center; (c) to keep up with editing and coarse coding of incoming forms of the Collaborative Project at the present level, and (d) to have statistical-clerical help available for the many analyses requested by the obstetrical and pediatrics working groups, and by medical and other professional staff members of the Collaborative Project.

One of the serious problems facing the Biometrics Branch is the heavy load being carried by the statisticians on staff. Although it has not been too difficult to secure nonprofessional help, much frustration has resulted from the many attempts to recruit professional persons. The great shortage of adequately trained bio-statisticians, plus the increased need for such personnel resulting from increased funds available for medical research, has made it virtually impossible to expect success in recruitment efforts. A conscientious and continuous recruitment effort has been under way for more than a year to hire additional statisticians, particularly those with some training in public health. Not only have the efforts of the Personnel Branch, NIH, been enlisted, but also those of the employment offices of the American Public Health Association and of the American Statistical Association. All of the schools of public health in the country were contacted by mail and a number were visited personally. It appears that, although the training programs for such personnel has been somewhat stimulated by the grants available from NIH, the noncompetitive salaries allowed under Civil Service prove quite unattractive.

Although the number of professional statisticians has only increased from 8 to 10 over the past year, the number of nonprofessional employees has increased from 5 to 19. This would mean that the staff of the Branch has more than doubled during the past year. The space allotted to the Biometrics Branch is inadequate for the staff at present on hand, not to mention the fact that fast and continuous expansion is urgent. The inconvenience of being off the NIH reservation with respect to tabulating facilities will, no doubt, continue to grow as the need for such services increases.

A staff member was sent for training at the Basic and Special Features 650 Programming School in order to secure background as to possible ways to establish programs for computer operations in the types of statistical operations amenable to this system. Another staff member attended the Flexowriter Programming and Procedure School This training would permit the most efficient use of the Flexowriter machine in preparing programmatic tapes and exploiting the machine's capabilities in data origination and copy production.

It is hoped that it may be possible to send one or two statisticians for advanced training in biostatistics that will be offered during the summer of 1960 at the University of Minnesota School of Public Health. It was not possible to take advantage this summer of the many fine biostatistics courses offered by the University of Michigan School of Public Health because of the extreme understaffing of statisticians in the Branch.

[illegible] REPORT
Calendar Year, 1959
Extramural Programs Branch
National Institute of Neurological
Diseases and Blindness
National Institutes of Health

I RESEARCH GRANTS

1. Program developments

During the year a continued expansion has occurred in the number of research projects and programs seeking to discover the cause and cure of neurologic and sensory disorders. An increased appropriation of 39% for the various programs, effective July 1, 1959, has permitted the awarding of all worthwhile research projects recommended for approval. Some new and important programs have been started, and certain on-going programs have been strengthened.

Numbers of grants: - As the year ended (December 1959) there were 1,107 active research grants. A year ago at this time there were 913 active research and field investigation grants. This represents a net gain of 21.2% in the year-to-year increase in grant numbers.

During the year 259 new projects were started. There were 62 projects which terminated during the year, either because continuation was not requested, or due to lack of success in competitive review. The 259 new projects that were started this year required a total of 3.96 million dollars to fund; hence, the average new grant was in the amount of $15,200. The 259 new grants were selected from a group of 496 applications that requested a total of 9.46 million dollars. Expressed in percentages, this means that 53.0% of the applications for new projects were successful, but at only 42% of the total funds sought.

During the year requests to renew 125 on-going research projects were reviewed. Of these, 105 (or 84%) were approved in the amount of 2.00 million dollars; hence, the average competing continuation grant was in the amount of $19,200. In addition, 95 out of 123 requests for funds to supplement on-going and active research projects were paid.

In summary, the Council this year recommended approval for 459 competing applications in the amount of 7.60 million dollars. The year-to-year increase from the previous year is 55 applications of all types (13.6% increase) for an additional $580,000 in funds (8.3% increase in dollars approved).

After a rapid increase over a 3 year period, the individual studies related to the cerebrovascular disorders appear to have arrived at a plateau. Current support is at a level of 3% of the grant funds. However, studies related to epilepsy (7%), multiple sclerosis (6%), and muscular dystrophy (6%) are increasing in numbers at about the average rates. Very little interest is apparent in neurotropic viruses, or other aspects of infectious and post-infectious neurological disorders. This is an area that requires activation. Possibly the symposium on the encephalitides held in Antwerp in the spring of this year will result in renewed interest.

Problems related to hemiplegia and accidents in general have expanded recently. This may be due to the spectacular, but as yet nondefinitive, studies on nerve and spinal cord regeneration. Dr. Campbell and co-workers at Columbia University have perfected the techniques that may lead to meaningful advances of a practical nature in this field.

Studies on the neuroendocrine relations, and on the autonomic nervous system use about 6% of the grant funds, and are expanding at about the average rate. This program is somewhat hampered by being very general in nature and not readily associated with a single disease category.

Studies on disorders of vision use about 17% of grant funds. Very active programs are underway into the cause and care of glaucoma, cataracts, retinopathy and the mechanisms of visual perception. No studies on trachoma are supported although uveitis is being actively investigated. Hearing and equilibrium studies are expanding very rapidly, in numbers. There are currently 19 grants for projects on the neurological aspects of speech disorders, supported by $300,000 in grant funds. This program is increasing rapidly.

New Programs: - Several new programs have been started during the year, in response to clearly defined needs, and aimed at solution of important neurologic and sensory problems.

Among protocol-type group studies, special attention should be given to the decision of the Council to favor a large collaborative cerebrovascular project in Europe, involving several countries and related primarily to neuropathology and epidemiology of strokes. A grant of $100,000 has been recommended to permit the development of a satisfactory protocol and procedure. Another protocol study is nearly organized and ready to be launched on the evaluation of methods for detecting uveitis. Preliminary plans are being worked up to organize similar cooperative studies on diabetic retinopathy,

and on the [illegible] of hearing disorders.

Expansion of work on drug therapy for neurologic disorders will undoubtedly occur as a result of a major conference on the subject, funds for which will be made available this year. Similarly, plans to develop a program of active grant support in ionizing radiation effects on neurologic and sensory response was launched by the holding of a relatively large conference.

2. Research Development.

A. Conferences: - During the year two conferences were sponsored by the NINDB through research grant funds. (1) "Neurophysiological Mechanisms of the Auditory and Vestibular System" was held in Bethesda on June 11 - 13, 1959. A series of 20 papers were presented, including 4 from European scientists. (2) "Symposium on the Encephalitides" was held in Antwerp on May 10 - 14, 1959. In this discussion the problems of classification, definition and diagnosis of post infectious neuritides were worked out. Publication is expected in the near future.

In addition a grant was made to the Society for Crippled Children for partial support of the International Congress on Welfare to Crippled Children, to be held in 1961.

B. Publications: - The transactions of a conference held in 1957 and supported by the NINDB, were published this year by the Hoeber-Harper Press. The title is "The Biology of Myelin" edited by S. R. Korey. Fifty-one people are listed as participants.

In 1959 reprints of 744 papers that appeared in scientific journals were placed on file by NINDB grantees. These were supported by 412 research grants.

C. Contributions from Specific Awards: - The year revealed no single spectacular development. Rather, many excellent publications reveal the slow but definite progress toward program objectives. Certain contributions have been summarized in the following pages, although these do not necessarily represent the ones which future research may show to be the most important.

In a review article Drs. Wolf and Cowen of Columbia University discussed the relative importance of spirochetal, protozoan, viral and bacterial infections of the nervous system occurring in the perinatal period. They conclude that despite the great number of infective agents which may enter and damage the central nervous system in the perinatal period, infection must still be considered to be one of the less common causes of pathological

changes in the brain at this time of life.

Drs. Silverman, Klebein and Fowler have reported on a thorough investigation of a group of 192 prematurely born infants, and followed this with neurologic and psychometric examinations when the children attained the age of two years. It was demonstrated that approximately one quarter of these survivors of premature birth exhibited signs of neurologic deficit and mental retardation at the age of two years, and that language and speech disability was evident in about 80 percent of these children. These findings strongly suggest that the problem of brain damage among premature infants is one which requires at least equal consideration with the problem of reducing the mortality rate among these newborn infants.

Basic studies are continuing to identify and determine the role of the organic constituents of nerve fibers and axoplasm in the physiological function of nerve. A long range study at M.I.T., under the direction of Dr. Francis O. Schmitt, is being made to ascertain the amino acid content of the axoplasm of the squid, and recently this group has been able to detect a number of amino acids which had not previously been described in this material. Other fundamental investigations are proceeding on the effects of X-irradiation upon the nervous system. Dr. Kenneth Brizzee, of the University of Utah, is engaged in studies of X-irradiation of the developing cerebral cortex in cats and rats throughout prenatal and postnatal development, maturation and aging. Dr. Samuel Hicks, of the Harvard Medical School, uses radiation as a tool in studying the development of the mammalian nervous system. He has found that doses of about 200r quickly kill primitive non-mitotic embryonal neural cells. The radiosensitive cells are the building blocks of the brain. Drs. Vogel and Haymaker, of Cornell University and the Armed Forces Institute of Pathology, have made some very interesting observations concerning demyelinization and the effects of irradiation on neurones.

Studies of epilepsy and related convulsive disorders are increasing in number, and several significant contributions have appeared in the literature during the past year as a result of work supported by NINDB. Drs. Peters, Vanderahe and Hebman, of Xavier University and the University of Cincinnati, have used the technique of localization of biologic function to provide a clearer understanding of various convulsive phenomena. By ablations, the influence of 5 major embryologic divisions of the central nervous system of the salamander-telencephalon, diencephalon, mesencephalon, rhombencephalon, and spinal cord - on induced seizures was tested. Neurologic tests performed five or ten

using both wick electrodes and conventional microelectrodes. Turning to clinical investigations, an interesting technique for light coagulation utilizing indirect ophthalmoscopy has been developed by Drs. Brockhurst, Wolf and Schepens of the Retina Foundation. They have devised an instrument for causing chorioretinal burns in the living eye, and have found that it offers a simple and efficient method for treatment of retinal lesions. Similarly, Drs. Campbell and Rittler, of Columbia University, have published results of their investigation indicating that flicker perimetry is a valuable diagnostic aid in cases of chronic simple glaucoma. Dr. Milton Flocks, of Stanford University, has found striking degenerative changes in the trabecular meshwork of eyes with primary and secondary glaucoma. He suggests that persons destined to have this disease have relatively vulnerable trabeculae which are injured by levels of intraocular pressure which ordinarily do not damage the trabeculae or optic nerve. When the curve of intraocular pressure reaches the point where enough trabecular degeneration has occurred to cause insufficiency of the pressure-regulating mechanism, progressive glaucoma is assured unless treatment is instituted. The aim of therapy in early glaucoma should be to lower the intraocular pressure sufficiently to prevent further trabecular damage, rather than to lower it enough merely to prevent damage to the optic nerve. Dr. Winston Roberts, of Bowman Gray School of Medicine, is also actively studying glaucoma, and has concluded from an eight year survey of over 200 patients that the technique of tonography offers criteria which make it possible to diagnose primary glaucoma before definite visualfield change can be measured, even with the most sensitive methods available.

At Northwestern University, Dr. George Shambaugh continues his pioneering studies in ear surgery. In a recent report he details the permanence of hearing results ten to seventeen years after fenestration for otosclerosis. When the results of 690 fenestrations were tabulated solely with regard to the final level of hearing at the most recent test, 43% were considered successful and 57% considered failures. Dr. Shambaugh makes the interesting point, however, that in over 4,300 fenestration operations performed by either him or his associates in the last 20 years, only 2 cases of proved closure of the fenestra occurred later than 2 years after operation.

Dr. Harold Schuknecht, at the Henry Ford Hospital, has demonstrated the presence of acetylcholinesterase along nerve fibers and at the bases of the hair cells in the organ of corti of the cat. He has also shown that the presence of acetylcholinesterase in the cochlea is dependent upon the integrity of the olivocochlear bundle. Fibers of the olivocochlear bundle

terminate upon or very close to the cochlear hair cells, and these fibers are cholinergic. Acetycholine liberated at the endings of the olivocochlear tract fibers may alter the excitability state of the hair cells or the acoustic nerve endings. Dr. Schulmecht theorizes that the amacrine cells of the retina, which are known to contain acetylcholinesterase, may subserve a function similar to that of Rasmussen's bundle in the cochlea. He feels that other sensory end-organs should be investigated for cholinergic efferent nerve fibers.

During the past year, support has been provided for several new projects in the field of speech disorders, vocal cord and laryngeal function, aphasia, etc. Of considerable interest is the work of Drs. Campbell and Murtagh, of Dartmouth Medical School, on the vocalizing function of the larynx. In a recent report they give details of the design and results obtained with a simulated larynx made of Gooch tubing. By using a high speed picture camera they were able to capture on film the various movements of this simulated larynx. They feel that the larynx should be photographed from the input side, as well as from above, to determine the effects of changes in the dimensions and contours of the inferior surface of the glottis. These may afford a rational basis for the known complex structure of the thyro-arytenoid muscle. The investigators hypothesize that the actual subglottic shape of this muscle may be the primary determinant of frequency of vibration of the vocal cords.

3. Major Problems

No major problem in the developing research grants program has been encountered. A minor disturbance in the programming and review process was created by transfer of the Field Investigation Committee to the Division of Research Grants, as described under Section 4. This has led to changes in personnel adverse to the best interests of this Institute, and which are still not resolved. In general, staffing is not yet adequate to fully exploit all the avenues of expension and programming opening up to us. If a program of foreign grants on a protocol basis becomes effective additional pressures on present staff will be felt.

4. Changes in the Program

Two important changes in technical administration matters occurred during the year, which will have an effect on the development of grant supported programs by this Institute. These changes relate, first, to the merging of the Institute's ad Hoc Cerebral Palsy Review Committee with the Human Embryological and Development Study Section of the DRG; and second, to the transfer of the Neurology Field Investigation Committee from the Institute

[illegible] grants. In the past, research grants [illegible] were [illegible] by a special [illegible] to differentiate them from the individual grants. This procedure has now been discontinued.

Delay in the use of research grant funds for foreign travel has been [illegible]. Now a grantee may use his funds for any travel, foreign or domestic, so long as it is in the interest of his research program and in accordance with the usual policy of his home institution. Approval by Council is not required. This, as indicated in last year's Annual Report, is the position favored by the Institute and Council.

The small grants program was discontinued at the end of last year, after a two year trial. This Institute and the Advisory Council had felt from the beginning that the small grants program could not be effective in accomplishing its objectives, and that this type of grant would be handled much better through the voluntary health agencies' grants programs.

5. Program Objectives for 1960

In the next year it is planned to expand the grants program into certain areas that are now thinly utilized for the support of neurologic and sensory disorders. Many of the chronic disease hospitals, schools for retarded children, homes for aged, and the like are filled with patient material that should be utilized for effective and original research problems. Every effort will be made to encourage research growth in this untapped and fertile area. It is hoped that centers for brain pathology registries will be organized in this connection.

Several conferences and symposia in specific research areas will be organized. One of these is the relation of low-level chronic radiation to changes in the CNS.

It is expected that a comprehensive report on the status of Neurologic Research in the U. S. will be completed this year by a Committee appointed by the Council and aided through a research grant. This report will be invaluable in ascertaining the proper balance in the grant support that goes into the various programs of the NINDB. Specifically, progress will be emphasized in expanded training [illegible] neurophysiology, basic research problems, mobility, clinical rehabilitation, effects of isolating pathways of the autonomic nervous system, and psychopharmacology.

6. Staff Assignments

[illegible]
[illegible] (from [illegible])
[illegible] (from [illegible])

Numbers of Research Grants

Council	New		Competing Continuations		Supplements		Totals	
	Requests	Approved	Requests	Approved	Requests	Approved	Requests	Approved
March 1959	$3,000,000 (139)	$1,165,000 (83)	$ 891,000 (51)	$ 634,000 (41)	$ 739,000 (36)	$ 363,000 (28)	$5,425,000 (231)	$2,162,000 (152)
June 1959	$3,115,000 (197)	$1,341,000 (87)	$ 670,000 (36)	$ 418,000 (30)	$1,243,000 (36)	$ 769,000 (28)	$5,771,000 (274)	$2,528,000 (145)
Nov. 1959	$3,340,000 (160)	$1,457,000 (89)	$1,483,000 (38)	$ 953,000 (34)	$ 913,000 (51)	$ 498,000 (39)	$5,768,000 (260)	$2,908,000 (162)
Totals	$9,455,000 (496)	$3,963,000 (259)	$3,044,000 (125)	$2,005,000 (105)	$2,895,000 (123)	$1,630,000 (95)	$16,964,000 (765)	$7,598,000 (459)
Approval Rate (dollars)		41.6%		65.8%		56.4%		44.6%
Approval Rate (numbers)		53.2%		84.0%		77.2%		60.0%

II. Postgraduate Training Grants (con't)

C. Ophthalmology Section

1. Program Developments

Two new programs in clinical ophthalmology were initiated in 1959, bringing the total number of clinical programs to 37. In contrast to the small increase in numbers of programs during the year, the dollar level of support has increased markedly from $874,945 at the beginning of the year to the current level of $1,034,178. The increase in support level has resulted from the approval of larger awards for continuation applications and the granting of supplemental awards for grants currently in effect. For the most part, these increases are related to augmentation of numbers of trainees receiving support, as well as to a rise in the individual stipend per trainee. Of the total awards made, $325,541 is earmarked for training stipends, being distributed among 325 trainees and 18 student clerks.

There were two grants for training in basic sciences related to ophthalmology in effect at the beginning of the year. One of these, for support of didactic, basic science teaching, is due to terminate before the end of the year. Of interest has been the establishment of a new grant for postgraduate training in sensory physiology at the University of Michigan. Training is to be offered in disciplines related to hearing, as well as to vision. As of the end of the year, total grant support in basic sciences related to ophthalmology will amount to $165,597.

2. Contributions Resulting from Particular Awards

It is to be recognized that contributions resulting from training for research, certainly in terms of outstanding research performed by trainees, may not be discernible for a number of years after initiation of a training program. For the present, all the training programs can be expected to do is increase the number of postgraduate trainees in ophthalmology going into academic medicine. In reviewing applications for continuation of current training programs, it is apparent that there has been at least a small trend toward academic and investigative pursuits on the part of some of the trainees.

3. Major Problems Encountered

One major problem continues to be that of keeping trainees,

relatively few full time paid positions available in medical schools to absorb the trainees. Moreover, the potentiality for economic gain in ophthalmology is probably so much greater in private practice than in investigation that it takes considerable dedication to research to turn to such an activity.

It therefore becomes necessary, and this is also a major problem related to the first, to establish training programs with definite research outlooks, which will inculcate the trainees with the spirit of research, perhaps thus lessening for them the attraction of private practice. For the time being, however, the investigative content of a number of the programs appears to be minimal.

In the basic science area, a major problem involves location of institutions and of departments capable of mounting a satisfactory training program at the postgraduate level.

4. Changes and Improvements in Program

a. Dr. Jerome resigned as Executive Secretary of the Ophthalmology Postgraduate Training Committee, in August. He was replaced by Dr. Barnard Shacter, in time for the September meeting of the Committee.

b. The following members of the Ophthalmology Committee completed their terms with the September meeting.

(1) Dr. F. Bruce Fralick, Chairman, Department of Ophthalmology, University of Michigan Medical School.

(2) Dr. S. Rodman Irvine, Clinical Professor of Surgery, School of Medicine, University of California, Los Angeles.

(3) Dr. Fred M. Wilson, Professor of Ophthalmology, School of Medicine, Indiana University.

c. New members who have accepted appointment to the Ophthalmology Postgraduate Training Committee for four year terms effective October 1, 1959 are:

(1) Dr. Edward W.D. Norton, Chairman, Department of Ophthalmology, University of Miami School of Medicine.

(2) Dr. James O'Rourke, Department of Ophthalmology, Georgetown School of Medicine.

(3) Dr. Richard C. Troutman, Professor, Division of Ophthalmology State University of New York, Downstate Medical Center.

(4) Dr. Bradley R. Straatsma, Associate Professor of Surgery (Ophthalmology), University of California at Los Angeles.

(5) Dr. Frederick C. Blodi, Associate Clinical Professor of Ophthalmology, State University of Iowa.

5. Program Objectives for 1960

In 1960 the objectives will threefold:

a. To bring about a better balance between basic science and clinical training programs. The development of knowledge regarding the nature of sensory diseases in humans, and the application of this knowledge to their alleviation clinically, is largely dependent on knowledge of the normal functioning of sensory organs. At present, the emphasis in the program has been primarily directed toward development of clinical investigators. If these are not to work largely in vacuo, however, it becomes essential that basic knowledge of sensory mechanisms be uncovered. To this end, therefore, greater emphasis will be placed on training of investigators for basic research.

b. To place further emphasis on investigative pursuits in the clinical training programs. Although there has been increasing recognition on the part of Program Directors that the primary function of the clinical programs is not clinical instruction per se, but training for clinical research, it is apparent that the research content of some programs is still minimal. It is proposed, during the coming year, to carry out a proselytizing campaign among the heathen to convert them to the true religion - research, at least as far as our training programs are concerned. As a corollary to this objective, it would be desirable to look into training approaches and techniques designed to motivate clinical trainees to become investigators.

c. To continue evaluation of the program needs. It is recognized that research in ophthalmology, and, indeed in vision in general, constitutes only a small segment of the overall research needs in biology and medicine. It is proposed to continue evaluation of the needs in this area, and to adjust the development of programs accordingly.

6. Staff Assignment

Bernard Shacter, Ph.D.
Executive Secretary, Ophthalmology
Postgraduate Training Committee

Ophthalmology Applications

Calendar Year 1959

March

	Amt. Req.	Amt. App.	No. Req.	No. App.
New or Revised	$ 78,432	$ 56,525	3	1
Continuations	205,150	132,519	4	3
Supplements	61,799	34,714	4	2
Total	$345,381	$223,759	11	6
	% Amt. App. 64		% No. App. 55	

June

New or Revised	$127,692	$ 55,000	2	2
Continuations	404,648	260,391	9	7
Supplements	38,575	31,845	2	2
Total	$570,915	$347,236	13	11
	% Amt. App. 61		% No. App. 85	

November

New or Revised	$ 9,747	$ 0	1	0
Continuations	178,931	94,395	5	3
Supplements	97,060	66,128	4	2
Total	$285,739	$160,523	10	5
	% Amt. App. 56		% No. App. 50	

GRAND TOTAL	$1,202,034	$731,517	34	22
	% Amt. App. 61		% No. App. 65	

1959 ANNUAL REPORT
of the
National Institute of Neurological
Diseases and Blindness

II TRAINING GRANTS

A. Clinical Neurology and Pediatric Neurology

1. Program Developments and Accomplishments

a. Clinical Neurology

The field which receives the largest amount of support in the NINDB training programs is Clinical Neurology. Sixty-one programs were in effect during 1959 with a dollar value of $2,321,037. Of these seven were activated in calendar 1959. Three grants were terminated during this period. This represents an increase of $783,127 over the previous year. This increase is accounted for almost wholly by the activation of new programs, increased numbers of trainees and also a general increase in the level of trainee stipends. The seven new programs were at a level of $178,444.

In addition to the above, two new programs in neuro-radiology were approved to begin in calendar 1960.

b. Pediatric Neurology

Seven programs with a dollar value of $118,071 are currently active. As pointed out in the 1950 annual report, sitpends are not a major element in the grants since those who specialize in this area have in most cases completed three years of advanced training and are therefore eligible and apply for Special Traineeships or similar types of senior awards.

2. Contributions Resulting from Particular Awards

The training programs supported by the NINDB have been in effect now for seven years. It is now possible to make some evaluation of the total program. Thus it is evident that in the field of Clinical Neurology we are approaching an optimum number of programs. Furthermore, it is now possible to discern which ones are training significant numbers of academically oriented neurologists. Those programs which fail to give evidence of productivity are being weeded out by denial of continuation grants. Review of continuation requests now takes place far enough in advance so that prolonged

terminal grants are no longer required.

Indeed it may now be said that in the field of clinical neurology, there is to be found among existing programs a hard core of productive ones which may be used for setting standards for new applications. This may be compared to a Big League and those wishing to join it will have to demonstrate that they have the men and capabilities for first rate performance.

3. Major Problems Encountered

The major problem still appears to be the provision of an adequate number of teacher-investigators in clinical neurology, pediatric neurology and related specialties. The increasing demands on the medical profession as a whole brought on by our explosively expanding population has been recently highlighted in the Bane Report (Physicians for a Growing America, Washington, 1959.)

The present pool of medically trained manpower is minimally adequate for present needs. Just to maintain the balance for our growing population will require not merely expansion of present medical teaching and research facilities but the creation of at least 20 new medical schools. This raises the question - Where will we find the personnel not only to provide necessary medical care but also to train new generations of doctors, surgeons and medical researchers? The question is legitimate and an answer is imperative.

There is no doubt that we must place increased emphasis on the development of new and improved training programs in Neurology (Clinical and Basic Science areas alike). Every effort must be made to meet the competition for the training of practitioners by encouraging those institutions and men who have shown a proven ability to attract and train teacher-investigators. Furthermore, efforts must be made to make academic careers more attractive through the creation of more full-time opportunities for well-trained and dedicated teachers and researchers.

4. Changes and Improvements Made

A significant step which will facilitate grants administration is the adjustment of starting dates of programs to coincide with the academic year. During this past year the Congress provided funds for this purpose and it is hoped that by 1961 all NINDB training programs will have been converted to this basis.

Dr. Lawrence Farber resigned as Executive Secretary of the Neurology Postgraduate Training Committee effective June 30, 1959. He was replaced by Mr. Morris C. Leikind who assumed his duties on July 1, 1959.

Neurology Postgraduate Training Committee and was attended by the following persons: Dr. Vahe E. Amassian, Dr. Orville T. Bailey, Dr. Maynard Cohen, Dr. Thomas W. Forman, Dr. Martin G. Netsky and Dr. Fred Plum.

5. Program Objectives for 1960

During the coming year, emphasis will be placed on improving the quality of existing programs in Clinical and Pediatric Neurology and related specialties. Furthermore the standards for new programs will be under continued scrutiny to make sure that training for clinical research and teaching is the primary objective.

Since the research program of the NINDB in the neurologic and sensory disease fields is dependent upon a continuing supply of well-trained career investigators, it is evident that the grant-in-aid training program is a vital and fundamental element in this research effort.

6. Staff Assignment

Mr. Morris C. Leikind, Executive Secretary of the Neurology Postgraduate Training Committee.

	June '59 Council		Nov '59 Council	
	Requested	Approved	Requested	Approved
Gen. Neur.				
new	$111,940 (3)	$ 59,065 (3)	$221,703 (5)	$ 41,958 (1)
cont.	144,113 (3)	66,888 (2)	238,666 (6)	121,267 (3)
suppl.	29,513 (3)	20,898 (2)	40,009 (3)	40,009 (3)
TOTAL	$285,566	$128,851	$500,378	$203,234
Ped. Neur.				
new	$ 18,956 (1)	$ 18,956 (1)	$ 11,854 (1)	$ 11,854 (1)
cont.	$ 13,203 (1)	------	------	------
suppl.	------	------	------	------
TOTAL	$ 32,159	$ 18,956	$ 11,854	$ 11,854
Neurosurgery				
new	------	------	------	------
cont.	------	------	------	------
suppl.	------	------	------	------
TOTAL	------	------	------	------
Neurophysiology				
new	$ 16,721	------	$ 50,115 (2)	$ 50,115 (2)
cont.	------	------	------	------
suppl.	------	------	------	------
TOTAL	$ 16,721	------	$ 50,115	$ 50,115
GRAND TOTAL	$334,446	$147,807	$562,347	$242,986

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Diseases and Blindness

II TRAINING GRANTS (cont)

B. Basic Sciences

1. Program Developments

In the field of the basic sciences of neurology, 34 programs were active in 1959 at a level of $954,317. One grant was terminated during the year. There were 16 new grants representing an increase of $445,240 in dollar value over the previous year.

The basic sciences represented include Neuroanatomy 6 grants, Neuropathology 14 grants, Neurophysiology 5 grants, Neurochemistry 4 grants and Neuropharmacology 5 grants.

2. Contributions Resulting from Particular Awards

Since, as indicated above, the basic science programs are quite new it is too early to have any indications of outstanding contributions resulting from research performed by trainees. It might be said that in their present state of development, the pipelines are being filled and so we must wait for a sizable number of trainees to come out of the pipeline and begin independent work before we can evaluate the results. Nevertheless, the increased number of programs is encouraging.

3. Major Problems Encountered

The critical shortage of trained manpower in the basic science fields continues to be serious. The major difficulty is primarily to find enough well qualified program directors who can offer, in properly equipped departments, the kind of post doctoral training required. Secondly comes the problem of finding trainees with an adequate background for advanced work and the desire for an academic career. In certain areas, such as neurochemistry for example, considerable basic training in chemistry, over and above that required of medical students appears to be a necessary prerequisite for post doctoral training. It is hoped that the predoctoral training programs of the DGMS will begin to supply an increased number of post doctoral candidates.

4. Changes and Improvements in Program

Already covered in (A) above.

5. Program Objectives for 1960

During the coming year the objectives will be to increase the number of basic science programs. Nevertheless, mere numerical increase is in itself not sufficient. The quality of the programs is of the utmost importance. The emphasis must be placed on types of programs designed to produce broad-gaged investigators competent to carry out independent investigations and with the qualities of good teachers who can attract, inspire and train new generations of scientists. The acquisition of technical skills in a narrow field cannot be regarded as a proper end for a post doctoral program.

Programming in this area thus becomes an educational task in acquainting the scientific community not only with the opportunities available but with the objectives to be attained.

6. Staff Assignment

Mr. Morris C. Leikind, Executive Secretary, Neurology Postgraduate Training Committee

Type	March '59 Council Requested	March '59 Council Approved	June '59 Council Requested	June '59 Council Approved	Nov. '59 Council Requested	Nov. '59 Council Approved
Pathology						
New	[illegible]	[illegible]	$114,045 (3)	$ 24,940 (1)	$ 25,171 (1)	$ 20,520 (1)
Cont.	$23,580	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Suppl.	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
TOTAL	$23,580	[illegible]	$114,045	$ 24,940	$ 25,171	$ 20,520
Anatomy						
New	$42,999 (2)	$44,295 (2)	$ 35,594 (1)	$ 35,594	[illegible]	[illegible]
Cont.	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Suppl.	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
TOTAL	$42,999	$44,295	$ 35,594	$ 35,594	[illegible]	[illegible]
Physiology						
New	$41,604 (1)	[illegible]	$ 22,109	$ 22,109	$ 24,840 (1)	$ 24,840 (1)
Cont.	69,682 (1)	$69,682 (1)	[illegible]	[illegible]	[illegible]	[illegible]
Suppl.	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
TOTAL	$111,286	$69,682	$ 22,109	$ 22,109	$ 24,840	$ 24,840
Biochemistry						
New	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Cont.	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Suppl.	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
TOTAL	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Pharmacology						
New	$85,229 (2)	$59,970	[illegible]	[illegible]	[illegible]	[illegible]
Cont.	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Suppl.	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
TOTAL	$85,229	$59,970	[illegible]	[illegible]	[illegible]	[illegible]
GRAND TOTAL	$263,094	$173,947	$171,748	$ 82,643	$ 50,011	$ 45,360

1959 Annual Report
of the
National Institute of Neurological
Diseases and Blindness

III. SPECIAL TRAINEESHIPS

1. Program accomplishments

The substantial growth made in 1959 by this program of individual awards for advanced research training is believed to be, to a great extent, a reflection of the success of Institute-supported Postgraduate Training Grant programs in providing a training climate favorable to the development of appreciation and respect for the importance of the researcher's role in medical progress. In this atmosphere the trainee with investigative talent is challenged by the unsolved problems presented by neurological disorders, and is motivated to undertake the additional years of specialized study necessary to make him a competent investigator.

A total of $1,409,660 was awarded to 170 trainees, representing an increase of a little more than 50% over 1958. NINDB Special Trainees are studying in 44 institutions in the United States, and 15 foreign institutions in Argentina, Canada, Denmark, France, Germany, Great Britain, Japan and Sweden; a total of 95 outstanding laboratory and clinical scientists are providing direction and guidance of the training so supported.

Postdoctoral training and experience of the 100 Special Trainees receiving initial support this year averaged 7 years and ranged from 3 to 26 years. Although about half of the trainees were from 3 to 6 years postdoctoral, seasoned investigators also availed themselves of the opportunity for developing new research techniques or concentrating on highly specialized problem areas. No fewer than 17% having 10 or more years postdoctoral experience made up this latter group.

Participation of basic scientists in the program has expanded during 1959; 13% of the trainees hold the Ph.D. as contrasted with the M.D. degree, an increase of 5% over 1958. Newly established Postgraduate Training Grant programs in basic science areas where manpower needs are especially acute have contributed to this increase. For example, a total of 17 awards for training in neurochemistry and 24 in neuropathology represents a doubling of the number of Special Trainees in these fields over 1958. Striking increases also occurred in neuropharmacology and neuroanatomy, which had in the past been represented by only one or two trainees.

Neurological deficits of the young continue to present a large and varied number of problems acutely in need of investigation, for which adequately trained researchers are not available. Therefore, the number of awards for training in pediatric neurology continued to grow, reaching 27 this year as against 18 in 1958

2. Contributions resulting from particular awards

An indication of the success of Special Traineeships in meeting program objectives is provided by a recent compilation of information about activities of trainees subsequent to training support.

Since inception of the program in 1956, 67 trainees had completed from 1 to 3 years of training under Special Traineeship auspices by June 30, 1959. Information is available to us from nearly 90% of this number, final reports from the trainees themselves, which are submitted a few months after the final training period has ended, constituting its main source.

Of the trainees about whom we have information, 77% hold appointments to essentially full-time academic posts with both research and teaching responsibilities--50% obtained positions subsequent to Special Traineeship training, and 27% returned to previously held positions, after leave spent in adding to their knowledge and skills in specialized research areas; 10% have academic appointments allowing some time for private practice; 3% are serving with the armed forces; 10% indicate private practice as their principal activity, with research as a minor activity at present.

As additional evidence of the research participation of Special Trainees, 32 NINDB Research Grants active at the present time have 27 Special Trainees named as principal investigators or co-investigators. Although the exact number is not readily available to us, it is known that Research Grants have been awarded to our Special Trainees by the other Institutes as well.

It is of interest that reprints of 27 published investigations resulting from Special Traineeship awards have been received, although a specific research project is not required as a part of the training program of the Special Trainee.

3. Major problems encountered

The only problem encountered this year is not a new one, and relates to the review of applications, which since 1957 has been an important function of our Postgraduate Training Committees. However, it has been apparent that undue delay in acting on applications seriously impairs the usefulness of the program, and when consideration of applications is restricted to the regular meetings of the training committees, which occur 3 times yearly, action on many applications is delayed, to the serious detriment of the program's usefulness. Accordingly, an ad hoc Interim Training Committee is convened 3 times yearly in the intervals between regular committee meetings. The ad hoc committee is composed of members of each of the Postgraduate Training Committees, appointed by their respective chairmen. Although consultants from the Ophthalmology and Otolaryngology Committees have evidenced some resistance to this procedure, largely because the number of applications in these areas is small compared with those from the neurological sciences, it is believed that as numbers become more nearly equal, this resistance may vanish. In comparison with the gains that have accrued to the program from application review by top-flight teacher-investigators, the difficulties associated with making procedural adjustments from time to time are minor indeed.

4. Changes and Improvements in program

An NIH-wide change in Research Fellowship stipends, Predoctoral, Postdoctoral and Special, resulted in the adoption of a new scale of Special Traineeship stipends, and the formulation of a new set of guide lines for deciding the amount of individual awards, for which the candidate's qualifications, and an estimate of what the training will cost provide a basis. Stipends now may range from $6,500 to $17,500, and this change has increased the average stipend from $7,247 to $8,500 per annum.

An increase in funds earmarked for this program from $1,500,000 to $1,750,000 was believed necessary in order to provide for needs arising from general expansion of the Institute's program with special emphasis on hearing and speech fields, and to provide for the payment of stipends at the higher level adopted this year.

5 Progress Objectives for 1970

Expansion of the program will be dependent on availability of funds either from private sources or grants for specific research investigations similar to those now supported by the Agricultural and Forestry Fund.

6 Volume of applications

Applications received-	291	$1,377,043
Applications approved-	140	1,097,000

Average grant: $8,000 per year

7) Short descriptions

Additional descriptions - Including changes in charges of research investigations and Research Relationship Officers

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1959 ANNUAL REPORT
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National Institute of Neurological
Diseases and Blindness

IV. RESEARCH FELLOWSHIPS

1. Program accomplishments

This program of awards, designed to support the research training of candidates qualified for investigative careers, continued at approximately the same financial level in 1959 as in 1958. However, the total amount available for Predoctoral and Postdoctoral Research Fellowships actually has been increased by nearly 50% as the result of transfer of responsibility for Post-sophomore and Foreign Fellowships to the newly created Division of General Medical Sciences.

A total of $535,264 was expended for 193 awards. Awards totalling $60,912 were made on an institutional basis to support 94 part-time students. The Institute takes no part in the administration of the Part-time Fellowship program beyond providing the funds for its support.

The amount spent for Predoctoral and Postdoctoral Research Fellowships in 1959 totalled $474,352, and represented an increase of a little more than $200,000 over 1958. The larger portion of this increase was devoted to Postdoctoral Research Fellowships. The expenditure of $337,614 provided for 56 awards to support research training during the years immediately following receipt of the Ph.D. or M.D. degree.

Predoctoral Research Fellowships, for training prior to and directed toward the Ph.D. degree, were awarded to 43 individuals in a total amount of $136,738.

2. Contributions resulting from particular awards

Detailed information about the activities and contributions of research fellows subsequent to completion of support is unavailable to us this year, as was true last year.

3. Major problems encountered

All the problems encountered in administering the Research Fellowship program this year have been major ones, and all were connected with changes in review and award procedures made last year.

These changes projected for 1959, which were delineated in the 1958 Annual Report, proved not to be an improvement, but rather a source of confusion leading to virtual frustration of the total Research Fellowship program's progress in meeting its primary objective. The principal difficulties were encountered when Discipline Panels (composed of intramural scientists) giving preliminary review to applications, and the NINDB Training Committees (Neurology, Ophthalmology and Otolaryngology), acting as Institute consultants, disagreed on the worthiness of certain applications for support. It is only recently that this issue has been clarified, with responsibility for final recommendation of Postdoctoral Research Fellowship awards being vested in the Institute's Training Committees, and final decision on Predoctoral Research Fellowships remaining the function of the NIH Discipline Panels.

4. Changes and Improvements in program

A. As of October, 1959, the Institute has been able to assume in fact, as well as name, more complete administrative control over Postdoctoral Research Fellowship awards, and at the same time to relinquish any but nominal control over awards at the predoctoral level. This appears to be a logical step, inasmuch as identification of categorical interests in predoctoral training is extremely difficult, if not impossible, while it is only at the postdoctoral level that the emergence of specialized research interests is perceptible.

Research Fellowship Review Branch continues to receive all fellowship applications and assign them to Institutes and Discipline Panels. Postdoctoral Research Fellowship applications are turned over to the Institute for review and recommendation for action by the Postgraduate Training Committees, and award and payment of those approved, with all the attendant procedures.

B. As of January 1, 1959, stipend levels were increased for Pre- and Postdoctoral Fellowships, to allow for advances in the cost of living which must be met if individuals are to continue in training for research careers in the medical sciences.

These changes are summarized in the following table:

Fellowship Level	Previous stipends and dependence allowance	Present stipends and dependence allowance
Predoctoral		
First year	$1600 + $350 per dependent	$1800 + $500 per dependent
Intermediate year	1800 + $350 " "	2000 + $500 " "
Terminal year	2000 + $350 " "	2200 + $500 " "
Postdoctoral		
First year	$3800 + $350 " "	$4500 + $500 " "
Second year	4200 + $350 " "	5000 + $500 " "
Third year	4600 + $350 " "	5500 + $500 " "

5. Program objectives for 1960

It is believed that in the coming year, it will be possible to stabilize the procedures relating to administration of Postdoctoral Fellowships, so that these awards may be used effectively in rounding out the total training program of the Institute.

Program expansion is not anticipated at present, since Postgraduate Training Grant funds are providing stipends for an increased number of postdoctoral fellows until they are qualified to apply for Special Traineeship support. It is believed that the Research Fellowship funds available now should be sufficient to meet the research training needs of those whom it may be highly desirable to support in departments where no training programs have been established.

6. Volume of applications

Applications reviewed:

Predoctoral Fellowships	41	
Postdoctoral "	64	
Total	105	

Applications awarded:

Predoctoral Fellowships	43	$136,738
(31 new; 12 renewals previously committed)		
Postdoctoral Fellowships	56	337,614
(42 new; 14 renewals previously committed)		
Part-time Fellowships	94	60,912
TOTAL	193	$535,264

Average cost of awards:

Predoctoral	$3,200 per annum
Postdoctoral	6,000 " "

7. Staff Assignments

Elizabeth C. Hartman - Training Analyst in charge of Special Traineeship and Research Fellowship programs.

Seventh Annual Report of Clinical Investigations

National Institute of Neurological Diseases and Blindness

1959

The Clinical Director's Report

At the time of completion of the seventh Annual Report of the Clinical Investigative Unit, an increase in all activities of the Unit ~~are~~ 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, 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, Texas. 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 the creation of 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 were 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 a period of six 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 six months, and hence 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 remains, in the Branch of Electroencephalography, Dr. Morillo, from Columbia, and Dr. Widen, who will be returning within the next week 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 included herein.

Specifically, the Branch of Medical Neurology reports as follows:

The Branch of Medical Neurology admitted, during the period covered by this Report, 269 patients. This is an increase of 54 patients over the previous year.

The total patient days was 6,981, an increase of 1,007 patient days. The average patient days was 26.0 days, a decrease of 1.8 days per patient. Two hundred, 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 of 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 actin 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 RNA in the C.N.S. This will be started in July, 1960, when Drs. Spora 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 latter presumably by metabolic conversions to other compounds such as γ-aminobutyric acid and glutamine. When cortical slices were incubated with 40 mM 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 mM. 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 six 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 non-chloride 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 swelling 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 non-damaged tissue, however, the Elliott-Heller formula may be used to estimate the cation distribution in the non-chloride space (? intracellular). It appears that neurons account for 75 percent of cortical non-chloride 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 exact similarly. This then allows an

estimation of the O_2 consumption of cortical neurons in terms of non-chloride 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 mM maolonate is added (blocks glutamic acid metabolism, see above) there is a decrease of protein-bound glutamic acid. Similar experiments with 10mM 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.

Finally, Dr. Tower and his group have utilized γ-aminobutyric acid to treat ten 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. 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. Semi-quantitative 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 ~~electrophoretically~~ 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 2000 µg/ml. after a second set of injections two months following the first. Circulating antibodies were identified by electrophoresis as γ-globulins. These antibodies were found to fall within one month after immunization to about 10 percent for the maximal level, and booster doses raised the titers in approximately ten 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 anti-clam 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. It is of interest that skin tests on patients demonstrate persistence of changed reactivity several months after immunization whereas the ephemeral nature of the circulating antibodies has disappeared. 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 a clearcut 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 crossover. 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. This has also been attempted in tissue cultures of muscle in which some effect has been demonstrated, but more precise studies are needed to define the conditions.

This break-through in immunochemistry has 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. 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 cargonic anhydrase. Other concentrations of "Diamox" are now under study. It is planned to do similar studies with diphenylhydantoin (Dilantin).

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 polypetides, 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. Complimentary 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 as 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 ultracentrifuge 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 of 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 suggesting 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 inter-related factors in cationic paralysis have also been concluded.

To follow the regeneration of muscle, tritium (H^3) labelled 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 manufacture 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 was 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 fallen in disrepute in many medical centers in the United States today. This Institute is now equipped 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 postulents. 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 of 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, imadazoleacrylylicholine, 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 trevelling 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 venous 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. Deoxydemethyl-lycoramine 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 is 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, in which standard biochemical procedures are used 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 appear 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 of these 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 terminated.

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 a 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 combination 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 eighteen 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 terminated 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 thirty 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 trans-sphenoidal 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 and of 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 in correlating these two techniques in this study also.

The last two studies of the Section of 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 temporal lobe epilepsy is now studied encephalographically, in such a way that a reliable comparison of the two temporal horns is thus obtained. Each temporal horn is filled separately, and afterwards examined in two ortogonal planes. Approximately twenty-some 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 two 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 are 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 state 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 an ultimate summary of a larger number of patients 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, resulting 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 extra-thyroidal 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 non-familial and familial non-thyrotoxic 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 anti-convulsant medication for centrencephalic

seizures. Patients were admitted and anti-convulsant medications were reduced to a minimum, whenever possible to do so without danger to the patient. After a short baseline period of observation, the patients were given either a placebo or JB 516 daily for four to six 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 four 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 has not been 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 micro-photography 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 Branch 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; and 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 eleven months covered by this Report, and twenty-four

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, and this will, in effect, terminate 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, however, this type of recording will be continued.

Dr. Ajmone Marsan and Dr. John Van Buren are continuing their studies in depth electrography in epileptic patients. This is a part of an over-all, 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, has 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 lateral geniculate was presumably chosen since it is a pure sensory relay nucleus. 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.). Pick-up 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 pre-stimulation 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 Ital. Biol., 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 non-specific 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 non-specific 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 fifty cats, and observations, concerning differences in latency, variability in responses, chances of unit activation by different stimuli, and the inter-effects 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 ten miscellaneous major procedures, the majority of which were performed for the relief of pain. Thirty publications were prepared during the period of this Report.

Twelve patients were referred from the National Cancer Institute, and were studied and eventually treated by hypophysectomy or stalk section. The endocrine and anatomical 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 radio-frequency 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 have a bilateral symmetrical aspecific metrazol burst associated with the 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 patient 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 bilateral 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 MINDB-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 has 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. These 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 supplied 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 four to forty-eight 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 % decrease

in the number of cells in the supra-optic 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 hypothermic 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 rostrolateral 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 follow-up 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 after-discharge 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 after-discharge, 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 surface 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 neurones 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 interneurones 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 were 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 interneurones. 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 such study, 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 fifteen 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, as may be seen in Project NINDB-68(c).

Dr. Dekaban's measurements of external and internal orbital distance in males and females from birth to adulthood are included in the book listed above. 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, an atlas has been completed 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, labelled 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 labelled proteins. The observations are made at time intervals, the cultures being subjected to various experimental conditions. The cells are observed in the bright-field, 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. twenty-four 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 six hours. This picture corresponds to the normothermic picture at thirty 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 semi-quantitative 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 twenty-four 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, which may be seen in project NINDB-64(c).

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. Christiansson, 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 four to five 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 complex may be one of the sites of automatism. The precise nucleus in such a complex remains yet to be discretely localized.

This, then, concludes the Reports from the Branch of Surgical Neurology.

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 suggest that the assumption of this function of the choroid is correct. The interrelation of these five factors ultimately combine 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 seems 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 done 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 neurones with the voltage clamp do support this assumption. To explain this apparent discrepancy, it may be 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 100-200 μ from the soma. He concluded, in the eye of the Limulus and in motoneurones 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, before leaving, 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 photosensitive 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 vitreous 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, that 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 fifteen 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 tri-racial 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 semi-translucent 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 horse-shoe-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, co-existing with the eye lesions. This was demonstrated by the Dental Department to also be a dyskeratosis.

Six projects of the Branch, Nos. NINDB-80(c), NINDB-82(c), NINDB-85(c), NINDB-86(c), NINDB-87(c), NINDB-91(c), and NINDB-94(c), demonstrated either a basic or clinical investigation relating to 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, and 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 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 #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 descriminated 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 one hour, and then placed in silver nitrate, and subsequently to 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 dilitation 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 anti-toxoplasmic 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 tenth 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 thirty patients with uveitis and ten 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 follow-up 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, and may be found in studies NINDB-88(c), NINDB-101(c), and NINDB-104(c). 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 microscopist 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 electronmicroscopic 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, NIAMD, 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 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. Paton 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 nine years previously.

Dr. Paton and Dr. von Sallmann 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.

This concludes the report of the Branch of Ophthalmology.

In closing it can be seen that the past year has been a productive one. After seven 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, the Central Intelligence Agency, 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 has been indeed extremely helpful during the past year, and the Clinical Investigative Unit acknowledges its debt to Drs. Masur, Chapman, and Farrier.

G. Milton Shy

G. Milton Shy, M. D.

National Institute of Neurological
Diseases and Blindness

November, 1959

SUMMARY

The Branch of Medical Neurology admitted, during the period covered by this Report, 269 patients. This is an increase of 54 patients over the previous year. The total patient days was 6,981, an increase of 1,007 patient days. The average patient days was 26.0 days, a decrease of 1.8 days per patient. Two hundred-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.

Two senior investigators will have left the Unit by the end of the calendar year. The first of these is the Associate Neurologist, Dr. Gunter Haase, who has accepted the Chair in Neurology at the University of Oklahoma; here he will have a chance to build a new neurological unit. Dr. Curtis resigned to go back into teaching, and his position as yet is not filled.

Two Visiting Scientists, Dr. Fritz Buchthal from Copenhagen, who is the Director of the Neurophysiological Institute of that City, and Dr. John Caughey who heads Neurology at the National University in New Zealand, were with the Institute for periods of time during from one to seven months. Their contributions can be seen in the projects reported below. Two Guest Workers were present also in the Neurological Branch. One was Dr. John Wherrett from the University of Toronto, who joined Dr. Tower in Neurochemistry; his work may also be seen outlined below. Recently from the same City, Dr. John Humphrey has joined the group as a Guest Worker, and he has been assigned to the laboratories of electromyography and muscle pathology.

The Branch of Medical Neurology reports specifically on the following projects:

The activities of the Section of 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 actin 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 RNA 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 latter presumably by metabolic conversions to other compounds such as γ-aminobutyric acid and glutamine. When cortical slices were incubated with 40 mM 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 mM. 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 six 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 non-chloride 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 swelling 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 non-damaged tissue, however, the Elliott-Heller formula may be used to estimate the cation distribution in the non-chloride space (? intracellular). It appears that neurons account for 75% of cortical non-chloride space and cortical potassium, but only 45% 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 non-chloride 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% the specific activity of the free pool glutamine. When 40 mM malonate is added (blocks glutamic acid metabolism, see above) there is a decrease of protein-bound glutamic acid. Similar experiments with 10 mM 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% 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% 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 effect of higher concentrations of α-ketoglutarate was not seen. Thus, as levels of the common precursor, α-ketoglutarate, rises the latter pathway (succinyl-Co A) is preferred. 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.

Finally, Dr. Tower and his group have utilized γ-aminobutyric acid to treat ten 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. 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. Semi-quantitative 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 electrophoretically.

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 2000 µg./ml. after a second set of injections two months following the first. Circulating antibodies were identified by electrophoresis as γ-globulins. These antibodies were found to fall within one month after immunization to about 10% for the maximal level, and booster doses raised the titers in approximately ten 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 anti-clam 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. It is of interest that skin tests on patients demonstrate persistence of changed reactivity several months after immunization whereas the ephemeral nature of the circulating antibodies has disappeared. 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 3%. 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 a clearcut 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% crossover. 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% impure. Under the present methods, which are 99% 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. This has also been attempted in tissue cultures of muscle in which some effect has been demonstrated, but more precise studies are needed to define the conditions.

This break-through in immunochemistry has 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. 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 3uM/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. 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 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. Complimentary 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 ultracentrifuge analysis.

The previous studies reported in 1961 indicated the enzyme activity was associated with the fastest component in the ultracentrifuge analysis. However,

purification by those 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 of 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 techniciams 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 suggesting 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 inter-related factors in cationic paralysis have also been concluded.

To follow the regeneration of muscle, tritium (H^3) labelled 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 manufacture 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% 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-l 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 was given. Approximately 18 such patients were studied in the current year, with over 200 penetrations.

It has become apparent that, 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 fallen 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 postulents. 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 of 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

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, imadazoleacrylylcholine, 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 travelling 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 venous 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,

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 lacks. 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. Deoxydemethyl-lycoramine 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 is 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, in which standard biochemical procedures are used 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 appear 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 of these 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 terminated.

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 a 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 combination 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 eighteen cases 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 terminated 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 thirty 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 trans-sphenoidal 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 and of 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 in correlating these two techniques in this study also.

The last two studies of the Section of 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 temporal lobe epilepsy is now studied encephalographically, in such a way that a reliable comparison of the two temporal horns is thus obtained. Each temporal horn is filled separately, and afterwards examined in two ortogonal planes. Approximately twenty-some 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 two 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 Boers; 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 are 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 state 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 an ultimate summary of a larger number of patients 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, resulting 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 non-thyrotoxic 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 anti-convulsant medication for centrencephalic

seizures. Patients were admitted and anti-convulsant medications were reduced to a minimum, whenever possible to do so without danger to the patient. After a short baseline period of observation, the patients were given either a placebo or JB 516 daily for four to six 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 four 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 has not been 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. Ercoth 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 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 micro-photography 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.

This concludes the major findings of the Branch of Medical Neurology.

Serial No. NINDB-1(c)
1. Medical Neurology
2. Clinical Neurochemistry
3. Bethesda, Md.
4. Same as 58-NINDB 27

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A:

Project Title: Amino Acid Metabolism in Normal and Epileptogenic Cerebral Cortex in Vitro.

Principal Investigators: Dr. Donald B. Tower and Mr. Edmund L. Peters

Other Investigators: Dr. Guy McKhann, Dr. John Wherrett

Cooperating Units: Dr. R. W. Albers, NINDB Lab. of Neuroanatomical Sciences.

Man Years: Patient Days: 0

Total:	0.8
Professional:	0.5
Other:	0.3

Project Description:

Objectives: To study the in vitro metabolism of amino acids and related compounds in brain tissue samples from experimental animals and from human patients operated on for focal epilepsy.

Methods: See previous reports on this project.

Patient Material: From NINDB patients admitted for [illegible] purposes.

Major Findings:

(1) The changes of free amino acids in slices of [illegible] cerebral cortex during incubation in vitro were investigated in detail with respect to time of incubation. The following table summarizes the findings:

	Initial Level	Changes from Initial Level at 15 ø	30 ø	45 ø	60 ø
Slice Total (Glutamic Acid + Glutamine)	11.4	+1.65	+4.85	+5.0	+4.85
Slice Glutamic Acid	7.75	+1.35	+4.1	+3.2	+2.4
Slice Glutamine	3.6	+0.3	+0.75	+1.75	+2.45
Medium Glutamate (≡ Glutamine)	5.1	-0.3	-1.2	---	-2.25
Slice Ammonia	2.1	-0.8	-0.65	-0.15	-0.35
Slice γ-Aminobutyric Acid	4.2	+0.2	+0.4	+0.5	+0.8

These results indicate a very rapid initial formation of glutamic acid, and a subsequent decline presumably by metabolic conversion to other compounds (e.g. γ-aminobutyric acid; glutamine). Most, if not all, of the glutamine increase during incubation appears to be accounted for by uptake from the medium (initial content there is leakage from slice during preparation). Only isotope studies can settle this question. The source of amino groups to support the pronounced synthesis of glutamic acid from its known precursor, α-ketoglutarate, is the chief problem posed by these data.

(2) Companion studies where slices were incubated with 40 mM malonate in the medium demonstrated striking differences: glutamic acid increased by30 to 6.5 µM over initial values and remained at this high level subsequently; glutamine rose little if at all; medium glutamate also changed little; slice ammonia did not decrease but rose slightly; and slice γ-aminobutyric acid rose by nearly 3 times as much as in control studies tabulated above. The mechanisms of malonate inhibition here are not yet clear beyond an evident block of glutamic acid metabolism, but this experimental situation is proving valuable as a means of "stabilizing" the free glutamate pool in incubated slices from its normal, rapid and active changing facets.

(3) A number of additional experiments were carried out with L-2,4-diaminobutyric acid, recently reported by Kessel to block further metabolism of γ-aminobutyric acid. No effect of this compound when incubated in vitro with normal cat cortex slices was observed (examining glutamate metabolism, glycolysis and oxygen consumption) in concentrations up to 40 mM. However administered in vivo to mice, seizures occurred 12 or more hours later. Brains of animals

with seizures exhibited *in vitro* a reduction in slice oxygen consumption (78% of controls and of injected mice without seizures) and a pronounced elevation of γ-amino-butyric acid levels (9.0 μM/g. compared to 5.6 in controls and 5.3 in injected mice without seizures). 2-dimensional paper chromatograms confirmed the latter and suggested increase of glutamine and decrease of glutamic acid in brains of mice with seizures. These studies were repeated on cats injecting 3 mM/kg of diaminobutyric acid. Seizures occurred in about 6 hours and brain slices from these animals incubated *in vitro* revealed the following: oxygen consumption 87% of expected values, corrected to the latter by added pyridoxal phosphate but not by added γ-aminobutyric acid; levels of γ-aminobutyric acid over twice normal with marked increase during incubation; very low initial glutamic acid (about 60% of controls) and very high initial glutamine (about 3 times controls) with some decline in glutamine and some rise in glutamic acid during incubation. The findings confirmed a distinct block of γ-aminobutyric acid metabolism, but also suggested ammonia intoxication of the brain (glutamine elevation), consistent with the known hepatotoxic acid of diaminobutyric acid. Further studies of this aspect have not yet been carried out, but are contemplated.

(4) Attempts to adapt an unpublished fluorimetric procedure for aspartic acid developed by Dr. O. H. Lowry proved completely unsuccessful, despite very extensive efforts. Thus no data on aspartic acid metabolism under normal or other conditions have yet been obtained. Utilization of various other enzymatic assays is now under investigation.

Significance of Project: The prosecution of this project is of fundamental importance to an understanding of the roles of the glutamic acid-aspartic acid group in neuronal metabolism and in the seizure process.

Proposed Course: To continue the above and related studies with special emphasis on the effects of various other metabolic inhibitors and on obtaining data on aspartic acid metabolism.

Part B Included: Yes

Serial No. NINDB-1(c)

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Individual Project Report
Calendar Year 1959

Part B:

Publications:

1. Tower, D. B.
The Effects of 2-Deoxy-D-Glucose on metabolism of slices of cerebral cortex incubated in vitro. J. Neurochem. 3: 185 (1958).

2. Tower, D. B.
Glutamic acid metabolism in mammalian central nervous system. p. 213 in Brucke, F. (Ed.) Biochemistry of the Central Nervous System (IV Internat. Biochem. Congr., v. 3), London, Pergamon, 1959.

3. Peters, E. L. and Tower, D. B.
Glutamic Acid and glutamine metabolism in cerebral cortex after seizures induced by methionine sulfoximine. J. Neurochem. 5: in press (1959).

4. Tower, D. B.
Neurochemistry of Epilepsy: Seizure Mechanisms and Management. Springfield, Thomas, 1959.

5. McKhann, G. M., Albers, R. W., Sokoloff, L., Mickelsen, O. and Tower, D. B.
The quantitative significance of the gamma-aminobutyric acid pathway in cerebral oxidative metabolism. In Roberts, E. (Ed.) Inhibition in the Nervous System and γ-aminobutyric Acid. London, Pergamon, in press.

Honors and Awards: None.

Serial No. NINDB-2(c)
1. Medical Neurology
2. Clinical Neurochemistry
3. Bethesda, Md.
4. Same as 53-NINDB-25(c)

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A:

Project Title: Electrolyte and Energy Metabolism in Normal and Epileptogenic Cerebral Cortex *in Vitro*.
Principal Investigators: Dr. Donald B. Tower; Mr. E. L. Peters
Other Investigators: Dr. Bushnell Smith
Cooperating Units: None
Man Years: Patient Days: 0
Total: 1.1
Professional: 1.0
Other: 0.1

Project Description:

Objectives: To study *in vitro* metabolism of electrolytes (potassium, sodium, chlorides, etc.) and of energy-producing cycles and components thereof in incubated slices of cerebral cortex from experimental animals and from human patients operated on for focal epilepsy.

Methods: See previous reports on this project.

Patient Material: Obtained from NINDB patients admitted for other purposes.

Major Findings: During calendar 1959 attention was devoted to problems of fluid spaces in the tissue slices and of effects of various *in vitro* conditions thereon. It was considered essential to gain more insight into these problems before proceeding with studies on human epileptogenic material or with the use of radioactive ion tracers.

(1) Fluid spaces calculated from chloride as the indicator were compared with spaces calculated from sucrose as the indicator on the same samples. Normal cortex and cortex from animals with experimentally induced seizures (methionine sulfoximine, Megimide) exhibited close correspondence or identity of fluid spaces determined by each method and slice swelling was limited to the chloride and sucrose spaces, in conformity with various previous reports by others.

(2) Similar studies on slices incubated with added glutamic acid, glutamine or asparagine in incubation medium exhibited a marked increase of the non-chloride (or non-sucrose) space during incubation, but addition of γ-aminobutyric acid produced no change in this space from normal controls.

Slices incubated under hypoxic conditions (10% oxygen) exhibited a marked discrepancy between chloride and sucrose spaces, indicating swelling of the non-sucrose (? intracellu space and influx of chloride into that space. This latter finding is consistent with several recent reports on intracellular shift of chloride during spreading depression and anoxia of cortex in vivo. The importance of carefully defining these changes before attempting to estimate "intracellular" cation concentrations is obvious.

(3) By comparing fluid spaces and electrolytes in incubated slices of subcortical white matter with those in incubated slices of cerebral cortex (same animal) it has been possible to estimate by the Elliott-Heller formula the relative distribution of non-chloride space and of potassium and sodium therein between neurons and non-neuronal cells in normal cat cerebral cortex. These data may be summarized as follows:

		Cortex		White
		Neurons	"Glia"	"Glia"
Non-Chloride Space (% of total vol.)	(I)	22	10	17
	(F)	30	12	19
Sodium (μEq./ml. non-Cl. Sp.)	(I)	90	42	42
	(F)	14	43	43
Potassium (μEq./ml. non-Cl. Sp.)	(I)	112	161	164
	(F)	180	168	174

[I = initial; F = Sample incubated 60 min.]

Two general conclusions may be derived from these data: first cortical neurons account for about 75% of cortical non-chloride space (or 32% of total cortical volume) and of cortical potassium in non-chloride spaces, but only 45% of cortical sodium in these spaces (final incubation values); and second, the extrusion of sodium and uptake of potassium normally observed during incubation of these cortical slices (see previous reports on this project) are limited to the neurons. The latter conclusion has important implications for cation turnover in cortex and for the observation made previously in this project that epileptogenic slices are unable to effect this cation shift during in vitro incubation. [It should be pointed out that the foregoing calculations have assumed astrocytes to comprise part of the chloride space on the basis of

recent electron microscope studies of incubated slices reported by de Robertis and by Luse].

(4) From the foregoing plus other data previously obtained in this project, it has been possible for the first time to estimate oxygen consumption (as a measure of energy metabolism) by cortical neurons and non-neuronal cells in terms of non-chloride space volume - a more satisfactory parameter than previous values based per unit weight or per cell. The values for cat cortex in terms of substrate metabolized (oxygen consumption/3) are as follows:

	Cortex		White
	Neurons	"Glia"	"Glia"
µM/hr/ml. non-Cl. Sp.	75	41	40

The higher metabolic rate of neurons is consistent with a number of previous studies, but in the present terms is highly suggestive of a direct relationship with the cation shifts illustrated above - a relationship already suggested in other contexts by Keynes. Further, the "extra" metabolism by neurons almost exactly equals the fraction of neuronal oxidative metabolism (33 µM/hr/ml non-Cl Space) attributable to the γ-aminobutyric acid pathway [See project 59-NINDB- 3 (c)], suggesting that the latter pathway, unique to neurons, may account for the higher metabolic rate of neurons compared to glia (which lack this pathway) and may be closely coupled with electrolyte metabolism.

Significance of Project: Energy metabolism is the basic factor in neuronal function and activity, and electrolyte metabolism, which is clearly dependent upon it, provides an important bridge between cellular chemistry and the functional activity of impulse conduction. The understanding of the factors involved is essential both for normal neuronal tissue as well as in hyperactivity status like seizures.

Proposed Course: To continue studies along lines indicated by 1958 and 1959 findings on both the electrolyte and energy aspects of the problem.

Part B Included: Yes.

Serial No. 59-NINDB-2(c)

PHS - NIH
Individual Project Report
Calendar Year 1959

Part B:
Publications:

1. Tower, D. B.
The effects of 2-Deoxy-D-glucose on metabolism of slices of cerebral cortex incubated in vitro.
J. Neurochem. 3: 185 (1958).

2. Tower, D. B.
Glutamic acid metabolism in mammalian central Nervous system.
p. 213 in Brucke, F. (Ed.) Biochemistry of the Central Nervous System (IV Internat. Congr. Biochem., v. 3), London, Pergamon, 1959.

3. Tower, D. B.
Neurochemistry of Epilepsy: Seizure Mechanisms and Management. Springfield, Thomas, 1959.

4. Tower, D. B.
Some neurochemical aspects of cortical neurobiology In Schade, J. P. and Tower, D. B. (Eds.) Structure and Function of the Cerebral Cortex (2nd Internat. Meet. Neurobiol.), Amsterdam, Elsevier, in press.

Honors and Awards: None.

Serial No. NINDB-3(c)
1. Medical Neurology
2. Clinical Neurochemistry
3. Bethesda, Md.
4. Same as 58-NINDB-30(c)

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A:

Project Title: The Metabolic Significance of γ-Aminobutyric Acid in Neural Tissue.
Principal Investigator: Dr. Guy M. McKhann
Other Investigators: Dr. Donald B. Tower, Mr. Edmund L. Peters
Cooperating Units: Dr. R. W. Albers, NINDB Lab of Neuroanatomical Sciences.
Man Years: Patient Days: 0
Total: 0.9
Professional: 0.7
Other: 0.2

Project Description:

Objectives: To study the metabolism of γ-aminobutyric acid in neural tissues, to study factors affecting such metabolism, and to deduce therefrom the significance of this compound in neural metabolism and function.

Methods: See previous project report and under Major Findings.

Patient Material: None.

Major Findings:

(1) The experimental studies reported in 1958, demonstrating that γ-aminobutyric acid can act as an effective substrate for cortical oxidative metabolism, have been extended by determining the quantitative contribution of the γ-aminobutyric acid pathway to total oxidative metabolism at the Krebs cycle stage. With pyruvate-3-C^{14} as tracer, the labelling of glutamic acid and γ-aminobutyric acid in incubated cat cortex slices was followed with duration of incubation and specific activities of the two compounds determined. Calculations from these data showed that 44% of the total substrate metabolized from α-ketoglutarate to succinate was metabolized via the γ-aminobutyrate pathway during 1 hr. incubation. Since the latter pathway is exclusively a neuronal pathway, it can be estimated that nearly 60% of neuronal oxidative metabolism proceeds via γ-aminobutyric acid.

(2) Data from the above study indicated that the proportion of oxidative metabolism directed via γ-aminobutyric acid varied in a sigmoid fashion during 1 hr. incubation, suggesting a reciprocal relationship with metabolism via the parallel succinyl-Co A pathway. Extensive studies have been carried out on the factors influencing the amount of substrate metabolized via γ-aminobutyric acid, utilizing cortical mitochondria and γ-aminobutyric acid-1-C^{14} and comparing $C^{14}O_2$ evolved to total oxygen consumption. Some α-ketoglutarate must be present for transamination with γ-aminobutyric acid if the latter is to be further metabolized. As the concentration of α-ketoglutarate is raised to 1 mM, metabolism via γ-aminobutyrate increases. However, as the concentration is further increased, a marked inhibitory effect is observed. In the presence of arsenite (which blocks α-ketoglutarate to succinyl-Co A metabolism) the inhibitory effect of higher concentrations of α-ketoglutarate is not present. Thus, it appears that there is a reciprocal relationship between metabolism via γ-aminobutyrate and via the parallel succinyl-Co A pathway, and that as levels of the common precursor, α-ketoglutarate rise, the latter pathway is preferentially favored.

(3) The metabolic significance of the γ-aminobutyric acid pathway *in vivo* in mouse brain has been investigated with Dr. Albers. Following parenteral injection of pyruvate-3-C^{14} the specific activities of brain glutamic acid and γ-aminobutyric acid have been serially determined. Preliminary results indicate labelling of glutamic acid and γ-aminobutyric acid from pyruvate-3-C^{14} is extremely rapid.

90 seconds after parenteral injection, the S.A. of γ-aminobutyric acid in brain is 50% that of glutamic acid. By 10 minutes after injection, the maximal S.A. of brain γ-aminobutyric acid is attained and is then some 20% higher than the maximal S.A. of brain glutamic acid. These findings strongly suggest that a compartmentation factor is involved, such that the labelled glutamic acid formed from labelled pyruvate has not during the time intervals studied equilibrated with all the cellular glutamic acid, and hence the labelled fraction has a much higher S.A. than that found for total glutamate and it is from this fraction that labelled γ-aminobutyric acid is formed. These interpretations are consistent with analogous data and interpretations reported by Waelsch for brain glutamine labelling from injected glutamic acid-C^{14}.

In view of the compartmentation factor, the metabolic rate of the γ-aminobutyric acid pathway in brain *in vivo* cannot be accurately calculated from present data, but the findings clearly indicate that the pathway is, if anything, more active *in vivo* than *in vitro*.

(4) Studies on various seizure preparations, notably with thiosemicarbazide and L-2,4-diaminobutyric acid as convulsants, strongly suggest that metabolism through the γ-aminobutyric acid pathway rather than the level of γ-aminobutyric acid itself is the significant factor. Thus in both thiosemicarbazide and diaminobutyric acid seizures, cortical oxygen consumption was significantly reduced, consistent with blocks respectively of the glutamic decarboxylase and γ-aminobutyric transaminase steps of the pathway by these agents. However, γ-aminobutyric acid levels were half normal with thiosemicarbazide and more than double normal with diaminobutyric acid. When seizures failed to occur after administration of either agent (presumably due to insufficient dose), none of these abnormalities occurred, indicating the close relationship of seizures to dysfunction of metabolism via the γ-aminobutyric acid pathway.

(5) The analytical problem of γ-aminobutyric acid levels in brain [Cf. 53-NINDB-30(c)] has been resolved satisfactorily. Studies by both Roberts and us have clearly shown that γ-aminobutyric acid levels continue to increase linearly with time after excision of brain samples, due to continued operation of glutamic decarboxylase anaerobically but failure of subsequent aerobically-dependent pathways for disposal of accumulating γ-aminobutyric acid. Comparable levels of γ-aminobutyric acid by fluorimetric and microbiological (Jakoby enzymes) assays have been obtained.

Significance of Project: The findings emphasize that the γ-aminobutyric acid pathway plays a major role in neuronal oxidative metabolism, and that its derangement is intimately associated with development of seizures. Study of the factors which regulate metabolism through this pathway may elucidate some of the regulatory factors in neuronal functioning. In addition the concept of compartmentation of metabolic activity among different types of cells and among different intracellular loci has far-reaching implications, providing potential links between metabolic activity and specific function.

Proposed Course: To continue above studies in progress.

Part B Included: Yes.

Serial No. NINDB-3(c)

PHS - NIH
Individual Project Report
Calendar Year 1959

Part B:

Publications:

1. McKhann, G. M. and Tower, D. B.
Gamma-aminobutyric acid: a substrate for oxidative metabolism of cerebral cortex.
Am. J. Physiol. 196: 36 (1959).

2. McKhann, G. M., Albers, R. W., Sokoloff, L., Mickelsen, O. and Tower, D. B.
The quantitative significance of the gamma-aminobutyric acid pathway in cerebral oxidative metabolism.
In Roberts, E. (Ed.) Inhibition in the Nervous System and γ-Aminobutyric Acid.
London, Pergamon, In press.

3. Tower, D. B.
Some neurochemical aspects of cortical neurobiology.
In Schadé, J. P. and Tower, D. B. (Eds.) Structure and Function of the Cerebral Cortex (2nd Int[illegible] Meet. Neurobiol.) Amsterdam, Elsevier, In press.

Honors and Awards: None.

Serial No. NINDB-4(c)
1. Medical Neurology
2. Clinical Neurochemistry
3. Bethesda, Md.
4. Same as 58-NINDB-23(c)

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A:

Project Title: Clinical Evaluation of Various Amino Acids and Related Compounds in Control of Seizures, including Studies of their Metabolism *in Vivo*.

Principal Investigator: Dr. Donald B. Tower

Other Investigators: Dr. Guy McKhann, Dr. Bushnell Smith, Dr. John Wherrett, Dr. C. Ajmone-Marsan, Mr. E. L. Peters, Dr. G. M. Shy

Cooperating Units: C. C. Pharmacy.

Man Years: Patient Days: 125

Total: 1.0
Professional: 0.5
Other: 0.5

Project Description:

Objectives: To assess the effectiveness of various amino acids and related compounds of the glutamate-aspartate group for control of clinical seizures and to complement these studies with investigations of the *in vivo* metabolism of the various compounds in use.

Methods: See previous reports on this project.

Patient Material: Admissions to NINDB wards specifically for this project, plus follow-up studies, and use of patients admitted to NINDB for other purposes.

Major Findings:

(1) All patients previously carried on oral L-asparagine therapy have been discontinued or switched to γ-aminobutyric acid. The asparagine phase of the project is completed. Reports from cooperating clinics [See 57-NINDB-5(c)] have finally been received as of 1 September 1959 so that this study can be written up in the near future.

(2) Limited trials with oral γ-aminobutyric acid have continued, utilizing a total of 10 patients. Overall results are similar to those reported in 1958, about half improved (two essentially seizure-free) and the rest not benefitted.

EEG studies on one of the seizure-free patients indicated a very dramatic improvement of the EEG abnormalities while on γ-aminobutyric acid, but other patients have failed to exhibit similar responses. From the blood, urine, and cerebrospinal fluid levels of γ-aminobutyric acid obtained during 1958 and 1959 it seems evident that one factor in variability of clinical response is blood-brain barrier permeability to γ-aminobutyric acid. Animal studies recently reported by Van Gelder and Elliott indicate that normally penetrability is minimal, but other studies on seizure subjects (Cf. Lending, Slobody and Mestern; Purpura et al.) indicate modification of barrier permeability during seizures or concomitant with chronic seizure states. Variability in such modification undoubtedly occurs, clinically and is in part reflected by CSF levels of γ-amino-butyric acid and clinical responses of our patients.

(3) One patient with "essential" hypertension was specifically studied for effects of γ-aminobutyric acid on the hypertension, following the recent report from Japan by Tsukada et al. A significant, reasonably sustained reduction of systolic and to lesser extent of diastolic pressure was observed during oral γ-aminobutyric acid. (Such a response was not obtained with various other agents tried during a previous NINDB admission). No sustained effect on seizure frequency was obtained during the same therapeutic trial, a result consistent with but not proof of peripheral, systemic action of γ-aminobutyric acid. Data recently reported by Elliott and Hobbiger for experimental animals definitely support such actions for this compound.

Significance of Project: This project is part of a long term study of the biochemical basis of seizures. Clinical applications of promising leads developed in the experimental phases of the study are important both as potential complimentary clinical validation of experimental findings and also as potential new and more rationally based therapies.

Proposed Course: Present results indicate that little further information is to be expected from patient trials now in progress. It is contemplated that this project will be suspended sometime in calendar 1960, unless appropriate new developments warrant otherwise.

Part B Included: Yes.

Serial No. NINDB-4(c)

PHS - NIH
Individual Project Report
Calendar Year 1959

Part B:
Publications:

1. Tower, D. B.
Neurochemistry of Epilepsy: Seizure Mechanisms and Management. Springfield, Thomas, 1959.

2. Tower, D. B.
The administration of gamma-aminobutyric acid to man: systemic effects and anticonvulsant action.
In Roberts, E. (Ed.) Inhibition in the Nervous System and γ-Aminobutyric Acid, London, Pergamon, In press.

Honors and Awards: None.

Serial No. NIMB-5(.)
1. Medical Neurology
2. Clinical Neurochemistry
3. Bethesda, Md.
4. New

PHS - NIH
Individual Project Report
Calendar Year 1959.

Part A:

Project Title: Anticonvulsant Drugs and Cerebral Electrolyte Metabolism.

Principal Investigator: Dr. Bushnell Smith

Other Investigators: Dr. Donald B. Tower, Mr. Edmund L. Peters

Cooperating Units: None.

Man Years: Patient Days: 0

Total: 0.6

Professional: 0.6

Other: 0

Project Description:

Objectives: To study by detailed in vitro methods effects of anticonvulsant drugs, reported to affect cerebral electrolytes, on the electrolyte metabolism of normal and epileptogenic brain samples.

Methods: Standard procedures of experimental animal preparation, brain sampling, slice incubation and analyses for tissue and incubation medium potassium, sodium and chloride will be used, based on procedures already developed and in use in this laboratory. Initial studies will utilize soluble preparation of acetazoleamide (Diamox) and diphenylhydantoin (Dilantin). In the former case, additional studies on the effect of this drug on the carbonic anhydrase activity of incubated slices will be carried out by standard procedures already established in the literature.

Patient Material: None.

Major Findings: This project has been activated only 4 months during calendar 1959. Base line studies on normal cat cortex slices have been carried out and a preliminary survey of Diamox concentrations added to the incubation medium of such slices. A slight effect in reducing the non-chloride space sodium concentration during incubation with a Diamox concentration of 3μM/L. (approximately the concentration known to effectively inhibit blood carbonic anhydrase). Other concentrations of Diamox are now under study. In addition some effect of these low Diamox concentrations in expanding the non-chloride space of tissue slices is so far apparent.

Significance of Proposal: Various types of evidence suggest that many [illegible] and anticonvulsants may act physico-chemically at the excitable membranes of conducting cells, presumably by altering ion fluxes across such membranes. For two anticonvulsants more detailed mechanisms have been suggested. It has been proposed that Diamox acts via its inhibition of carbonic anhydrase, resulting in intracellular accumulation of carbon dioxide and bicarbonate ions derived from it, normally removed from the cell by carbonic anhydrase. Accumulated intracellular bicarbonate is generally thought to exclude sodium entry and hence reduce excitability. Conversely it has been proposed that Dilantin increases sodium efflux from the intracellular space by mechanisms as yet unknown and hence also reduces excitability. The *in vitro* demonstration of these effects would help establish the validity of these concepts and permit more detailed studies of the mechanisms involved. Furthermore availability of means for manipulation of slice electrolyte metabolism would immeasurably increase possibilities for elucidating many of the metabolic facets and mechanisms related directly or indirectly to the electrolyte transport systems themselves. These studies bear on three important aspects of neural function: seizures, anticonvulsant drug action, and the neurochemical mechanisms subserving excitation and conduction.

Proposed Course: To continue the types of studies already initiated or planned.

Part I Included: No.

2. Clinical Neurochemistry
3. Bethesda, Md.
4. Same as 53-NINDB-26(c)

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A:

Project Title: Comparative Biochemistry of Smooth Muscle and Striated Muscle.

Principal Investigator: Dr. Beni Horvath

Other Investigators: Mr. Joseph B. Proctor

Cooperating Units: Dr. K. Laki, LPB, NIAMD

Man Years:
Total: 0.2
Professional: 0
Other: 0.2

Patient Days: 0

Project Description:

Objectives: To characterize the actomyosin of smooth (uterine) and striated muscle in physico-chemical terms preliminary to study of actomyosin synthesis in muscle.

Methods: See previous reports on this project.

Patient Material: None.

Major Findings: Work on this project was temporarily suspended during calendar 1959 while immunochemical procedures and findings for other projects in this laboratory [see 59-NINDB-7 (c)] could be evaluated for application to the basic problems of this project The finding [project 59-NINDB-7 (c)] that antibodies to clam tropomyosin A cross react with cat myosin with a 50% crossover indicates as relationship of the two proteins despite the very remote relationship of the two species. Such data have an obvious bearing on the problem of comparative biochemistry of muscle, but more investigation is required before full scale applications can be made here. Meanwhile the "dystrophic" mouse colony is being maintained to provide one type of source material for such studies. The application of immuno-chemical techniques to these source materials may be expected to assist greatly in the analytical problems inherent therein.

Significance of Project: Studies on formation of actomyosin may have an important bearing on the locus of disease in muscular dystrophy and other myopathies. Development of suitable micromethods and animal preparations is a necessary prelude to extension of experimental animal data to human specimens.

Proposed Course: To extend these and related studies to include mice in various stages of their disease in order to obtain information on the dynamics of muscle degeneration. Such information will subsequently be applicable to projected studies of muscle protein formation (synthesis) in uterine muscle under hormonal stimulation.

Part B Included: No.

2. Clinical Neurology
3. Bethesda, Md.
4. Same as 53-NINDB-35(c)

PHS - NIH
Individual Project Report
Calendar Year, 1959

Part A:

Project Title: Distribution of Actin and Tropomyosin in Normal and Diseased Muscle.

Principal Investigator: Dr. Beni Horvath

Other Investigators: Dr. Igor Klatzo; Mr. Joseph B. Proctor; Dr. J. Miquel.

Cooperating Units: Dr. K. Laki, LPB, NIAMD

Man Years: Patient Days: 0

Total: 1.4
Professional: 0.3
Other: 0.6

Project Description:

Objectives: To obtain additional information on the molecular architecture of muscle, to study the distributio of functionally important proteins in normal and diseased muscle, and to establish immunological properties of functionally important muscle proteins.

Methods: See previous reports on this project and under major findings.

Patient Material: None.

Major Findings: Previous studies have indicated that structural muscle proteins may have unusual immunological properties and that immunochemical methods may be used to advantage in combination with other methods for elucidation of the structural interrelationships of these proteins. The following represent progress in these approaches: using primarily clam tropomyosin A.

(1) Tests were developed and standardized for quantitative determination of immune precipitins:

(a) an adaptation of the Kabat method where immune-precipitates were washed with saline and nitrogen content determined by direct Nesslerization;

(b) a chromatographic method developed by Dr. Miquel in which antibody and antigen solutions are applied together on a paper strip and developed with buffer, which removes soluble muscle proteins while the immune-precipitate remains at site of application. The latter can then be stained with bromphenol blue, eluted and determined spectrophotometrically. On a variety of systems results by the two methods gave close correspondence.

(2) Both above methods provided information on quantity of circulating antibody and on stoichiometry of the antigen-antibody reactions. With the clam tropomyosin A system it was found that at the equivalence point 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 between 200 and 500 μg. antibody N/ml. were found after the first series of immunizing injections and rose as high as 2000 μg./ml. after a second set of injections two months later. Circulating antibodies were identified by paper electrophoresis γ-globulins. The antibody titers fell within about a month after immunization to about 10% of maximal levels, and booster shots given at this time indicated that 3-10 days are required to reach maximal titer and that drop in titer begins almost immediately thereafter.

(3) For extension of these studies a more rapid test was needed. It was found that the passive hemogglutination test of Vorlaender (using tannic acid treated sheep red cells) could be adapted. Immediately after tannic acid treatment, the cells bind tropomyosin A and are agglutinated by high dilations of anti-clam tropomyosin A sera, while remaining intact with normal sera or in absence of tropomyosin A. This test does not have the accuracy of quantitative precipitin reactions, but it is very much faster. Within an hour of bleeding, an estimate of the amount of circulating antibodies may be obtained, whereas the precipitin reaction requires a week to reach completion. The hemagglutination test is being further developed in terms of specificity and standardization at this time.

(4) In contrast to the ephemeral nature of circulating antibodies, skin tests demonstrate persistence of changed reactivity several months after immunization.

(5) Diffusion agar plates have been used for testing the homogeneity of the antibodies. After the first set of immunizing injections, only single precipitin lines appeared. From tests with various dilutions of antigen, it could be estimated that impurities in test preparations could not have exceeded 3%. Similar tests after a second set of immunizing injections revealed multiple precipitin lines. The causes of this are currently under study.

(6) Experiments with cat muscle myosin indicate that this antigen is considerably more complex than clam tropomyosin A. Agar plates show several precipitin lines and the quantitative precipitin reactions fail to give clearcut optima. Both observations are consistent with the concept that myosin possesses several determinant antigenic groups and that the subunit structure of myosin

may consist of a complex of tropomyosin and actin. A study of the cross reaction of cat myosin and clam tropomyosin revealed a 50% crossover, i.e., half the antibodies to clam tropomyosin was removed from the immune serum by cat myosin. This is a very respectable figure in comparison to those in the literature, and, considering the remote relation between the two species, indicates a close relationship of the two proteins.

Significance of Project: The immunological findings are consistent with present concepts of the Myosin molecule as consisting of subunits = Tropomyosin A, Tropomyosin B, and Actin. The latter can be prepared in a higher state of purity than myosin itself, so that they are more suitable for investigational purposes. Since these proteins are iso-antigenic, immunological responses of the organism may be important in conditions where destruction of muscle could permit these proteins to escape from the usual confines of the muscle and enter the general body circulation.

Proposed Course. To extend and confirm these findings by quantitative immunochemical methods. And to evaluate the significance of immune reactions in patients with neuromuscular diseases by skin tests and by quantitative precipitin reactions of their sera.

Part B Included: Yes.

Part B.

Publications:

1. Horvath, B., Miquel, J., Klatzo, I. and Proctor, J.B.
Immunochemical Studies on Muscle Proteins.
Neurology: in press.

2. Miquel, J., Horvath, B. and Klatzo, I.
A Chromatographic Technique for the Quantitative Study of the Precipitin Reaction.
J. Immunol.: In press.

Honors and Awards: None.

Ser [illegible] NINDS [illegible]
1. Medical Neurology
2. Clinical Neurochemistry
3. Bethesda, Md
4. Same as 83-NINDS-[illegible]

YES - NIH
Individual Project Report
Calendar Year 1985

Part A:
Project Title: Determinants of Actomyosin Contile strength and of Muscle Proteins in Neuromuscular Diseases
Principal Investigator: Dr. Geal Horvath
Other Investigators: Dr. T. M. Shy, Dr. Igor Mazzo; Dr. W. K. Engel; Dr. Joseph B. Proctor
Cooperating Units: None
Man Years: Patient Days: 0
Total: 0.4
Professional: 0.2
Other: 0.2

Project Description:
Objectives: To compare normal and diseased human muscle biopsy specimens for actomyosin content, tensile strength of contractile threads prepared from such biopsies, and for physico-chemical and immunochemical characteristics of protein extracts.

Methods: Sodium dodecyl sulphate and polyacrylamide gel electrophoresis; [illegible]

Major Findings: Muscle biopsy specimens were received from [illegible] patients admitted to other protocols.

Future Plans: [illegible] approaches outlined in project [illegible] have been applied to protocol studies on the problems of neuromuscular diseases as follows:

(1) Investigation of rabbits with muscle protein prepared from rabbit muscle are underway. Old rabbits and rabbits could be immunized with rabbit actin. So far, new myosins have been prepared from rabbit actin [illegible]. In the case of myosin from rabbit actin prepared by Saunders's procedure [illegible] pure by the criteria of polymerization, also evidence [illegible] of the immunities were prepared to be accompanied by [illegible] myosins were prepared from rabbit actin. [illegible] rabbits [illegible]

Serial No. NINDB-(c)

PHS - NIH
Individual Project Report
Calendar Year 1959

Part B:
Publications:

1. Horvath, B. and Proctor, J. B.
Quantitative Studies on the Composition of Dystrophic Muscle
Proc. Assoc. Res. Nerv. Ment. Dis.: in press.

2. Klatzo, I., Horvath, B. and Engle, W. K.
Observations on Myosin in the Abnormal Muscle, using Fluorescent Antibody Technique.
Proc. Assoc. Res. Nerv. Ment. Dis. in press.

Serial No. NINDB-9(c)
1. Medical Neurology
2. Clinical Neurochemistry
3. Bethesda, Md.
4. Same as 58-NINDB-33(c)

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A:

Project Title: The Surface-Chemical Behavior of Urine in Relation to its Surface-Active Macromolecular Constituents.

Principal Investigator: Dr. William C. Curtis

Other Investigators: Mr. L. Kenerson

Cooperating Units: None

Man Years: Patient Days: 0

Total: 0

Professional: 0

Other: 0

Project Description:

Objectives: To develop suitable physico-chemical methods for isolation, identification and characterization and of macromolecules, such as polypeptides, pyrogens and the like, which occur in urine and other biological fluids but whose chemical individuality, physiological significance, and mechanisms of action remain obscure.

Methods: See previous reports on this project.

Patient Material: Obtained from NINDB patients admitted for other purposes.

Major Findings: None. Owing to resignation of the principal investigator during calendar year 1959, no significant advances in this project were made.

Significance of Project: Many of the complex polypeptide macromolecules which normally are excreted in the urine are considered to be by-products of *in vivo* degradation of proteins. In pathological states, notably neurological and neuromuscular diseases, urinary output of these materials increases markedly. The increase may be due to substances characteristically associated with a particular disease state. The lack of any successful attempt to characterize these substances makes this project of importance in this regard.

Proposed Course: Since this project was conceived and conducted in its entirety by the principal investigator, his resignation necessitates termination of the project.

Part B Included: No.

Serial No. NINDB-10(c)
1. Medical Neurology
2. Clinical Neurochemistry
3. Bethesda, Md.
4. Same as 58-NINDB-32(c)

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A:

Project Title: Physico-Chemical Studies of Human Cerebrospinal Fluid.

Principal Investigator: Dr. William C. Curtis

Other Investigators: Mr. Lamar Kenerson

Cooperating Units: None

Man Years: Patient Days: 0

Total: 1.0

Professional: 0.5

Other: 0.5

Project Description:

Objectives: To determine whether specific substances liberated or produced in association with primary or secondary demyelinating processes in the central nervous system give rise to alterations in cerebrospinal fluid composition and characteristics which can be demonstrated by physico-chemical techniques.

Methods: See Major Findings.

Patient Material: Obtained from NINDB patients admitted for other purposes.

Major Findings: (1) Prior to the resignation of the principal investigator in mid-1959, some progress in fractionation of cerebrospinal fluid by physico-chemical procedures had been effected. By bubbling benzene, under controlled conditions of rate and drop size, up through a column of CSF complete removal of lipids appeared to occur and subsequent chromatography of the lipid containing benzene on silicic acid impregnated paper demonstrated the presence of lecithin, cephalins, inositides, sphingomyelin, cholesterol and cerebrosides. Preliminary studies indicated elevation of some, especially the latter three, in CSF samples from multiple sclerosis patients. No quantitative data were possible by this method, but the lipid "profile" appeared consistent with the recent report by Tourtellotte. The benzene-bubble lipid extraction procedure and the previously developed carbon-dioxide foam extraction procedure

for proteins and peptides [58-NINDB-32(c)] would appear to be promising approaches to isolation of these fractions when present in minimal concentrations in CSF and similar biological fluids.

(2) Complementary studies on the model protein, guinea pig serum asparaginase, yielded considerable information. This globulin with readily assayable enzyme activity can be isolated as a crude fraction from serum by ammonium sulfate precipitation and partial purification with calcium phosphategel. As such the specific activity is much enhanced (enzyme activity per unit protein nitrogen), but the crude fraction contains two visible contaminants by electrophoresis and at least one by ultracentrifuge analysis. Previous studies indicated that the enzyme activity was associated with the fastest component in the electrophoretic analysis and with the slower component in the ultracentrifuge analysis. However, purification by these means was unsuccessful. In 1958 it was found that the modified cellulose resin developed by Peterson and Sober would completely adsorb the enzyme. Studies this year on elution of the enzyme clearly showed that under appropriate pH and ionic strength conditions, four separate protein-containing fractions could be eluted from these cellulose columns to which the crude asparaginase preparation had been adsorbed. Good separation of the fraction was obtained and only one contained asparaginase activity (of much higher specific activity than any previous preparation). This suggests that complete purification had been achieved without loss of enzyme activity, and represents, as far as we are aware, the first such isolation of an enzyme protein in this manner. Insufficient material was available from these preliminary runs to permit full physico-chemical characterization of the pure fraction, and the resignation of the principal investigator has delayed further studies in this regard.

Significance of Project: This project represents a long range effort to solve the analytical problems presented by small samples available from patients with demyelinating diseases. Traditional approaches and methods fail, so that the development of new, sensitive and specific procedures would not only be of great practical value but would also permit fresh attacks on promising leads in the pathological chemistry of demyelinating diseases in man.

Proposed Course: The CSF studies conceived and carried out entirely by the principal investigator are terminated owing to his resignation. The protein studies will be incorporated with suitable modifications into project #59-NINDB-13 (c).

Part B Included: No.

2. Clinical Neurochemistry
3. Bethesda, Md.
4. Same as 58-NINDB-29(c)

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A:

Project Title: The Relation of Pyridoxine (Vitamin B_6) to Certain Seizure States.

Principal Investigator: Dr. Guy M. McKhann

Other Investigators: Dr. Donald B. Tower, Mr. Edmund L. Peters

Cooperating Units: Dr. Olaf Mickelsen, NIAMD Lab. of Nutrition; Dr. David B. Coursin, St. Joseph's Hospital, Lancaster, Pa.

Man Years:

		Patient Days: 0
Total:	1.6	
Professional:	0.6	
Other:	1.0	

Project Description:

Objectives: To elaborate the role of pyridoxine (Vitamin B_6) in certain seizure states by both *in vivo* and *in vitro* studies.

Patient Material: Patients admitted to NINDB specifically for this project.

Methods: Pyridoxine deficiency can be produced in man and experimental animals by dietary means and by the use of pyridoxine antagonists. There also exists in man an inborn error of pyridoxine metabolism manifested as "pyridoxine dependency." A similar condition may be present in the I-strain of mice.

Regardless of the cause or method of induction of pyridoxine deficiency, the clinical end-point is generalized seizures. *In vitro* the deficiency can be evaluated by determinations of levels of cerebral amino acids (notably glutamic and γ-aminobutyric) and by evaluation of cerebral oxygen consumption in deficient and control subjects.

Location of the block of cerebral metabolism under these conditions can be determined by addition of the compound which is metabolized one step beyond the site of the block, specifically, γ-aminobutyric acid, and comparison of the effect of its addition with those after addition of the coenzyme form of the missing vitamin itself, pyridoxal phosphate.

Major Findings:

(1) Studies of the original case of "pyridoxine dependency" were completed in 1953 [see 58-NINDB-29(c)] and have now been published [see Part B]. The essential finding was decreased cerebral oxygen consumption *in vivo* during pyridoxine depletion with very rapid restoration to normal

following intravenous pyridoxine, changes closely paralleling changes in clinical state and in the EEG. Dr. Coursin, cooperatin with this project, has subsequently conducted preliminary investigations of pyridoxine metabolism by this patient and other human and animal subjects. His findings indicate that two defects of pyridoxine metabolism may occur: (1) an abnormally rapid conversion of ingested pyridoxine to 4-pyridoxic acid, the normal urinary excretion product, yielding elevated blood and urine levels of the latter; or (2) an abnormally rapid and excessive excretion of ingested pyridoxine directly into the urine, resulting in low blood levels, normal output of 4-pyridoxic acid, and very high levels of B_6 compounds themselves in the urine. The pyridoxine-dependent patient studied here exhibits the second type of abnormality, and thus represents a deficiency state secondary to ma ive urinary loss of ingested vitamin.

(2) Experimental animal studies have been carried out on weanling kittens placed on a pyridoxine-free diet (devised by Dr. Mickelsen) together with appropriate litter-mate dietary controls. Within 6 to 8 weeks deficient kittens show progressive weight loss, coarseness and thinning of hair, ataxia and ultimately seizures, followed rapidly by death. None of these effects occur in litter-mates fed the same diet supplemented only by pyridoxine, and the weight gain curves of these controls are identical to kittens on regular diets. Studies of brains from both sets of animals have shown the following:

(a) Litter-mate brain weights are identical for deficient and diet-control animals.

(b) Incubated cortex slices from deficient animals show a significant decrease of γ-aminobutyric acid compared to the rise observed in diet control and normal animals. The decrease can be reversed to control values by *in vitro* addition of pyridoxal phosphate.

(c) Incubated cortex slices from deficient animals consume oxygen at a rate only 75% of the rate for control slices, but can be corrected to control, normal values by *in vitro* addition of either pyridoxal phosphate or γ-aminobutyric acid.

These findings are consistent with previous studies which all indicate that in pyridoxine deficiency the production of γ-aminobutyric acid in brain is reversibly impaired by deficiency of the coenzyme, pyridoxal phosphate, necessary

for functioning of the synthesizing enzyme, glutamic decarboxylase. In addition the present findings clearly show, for the first time, that this metabolic block is associated with reduced oxidative metabolism, due to reduced substrate metabolism via the γ-aminobutyric acid pathway, in brain. It is significant that in seizure states due to pyridoxine deficiencies, cerebral oxygen consumption is reduced (even during seizures) in contrast to the usual finding of elevated oxygen consumption during seizures and normal consumption inter-ictally.

Significance of Project: The role of the γ-aminobutyric acid shunt pathway in cerebral oxidative metabolism is discussed in a companion project [see 59-NINDB-3 (c)] This pathway accounts for some 40% of total cerebral oxidative metabolism and an even higher proportion of neuronal oxidative metabolism. Studies on pyridoxine metabolism and function emphasize that the maintenance of this metabolic pathway is essential for normal neuronal functioning in vivo and that its derangement results in neuronal hyperactivity and seizures.

Proposed Course: The dietary study on kittens is complete and in process of preparation for publication. Further studies on pyridoxine dependency states will be conducted utilizing additional patients, as available, and the I-strain of mice.

Part B Included: Yes.

Serial No. NINDB-11(c)

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Part B:

Publications:

1. Sokoloff, L., Lassen, N. A., McKhann, G. M., Tower, D. B., and Albers, W.
   Effects of pyridoxine withdrawal on cerebral circulation and metabolism in a pyridoxine-dependent child. Nature 173: 751 (1959).

2. McKhann, G. M., Albers, R. W., Sokoloff, L., Mickelsen, O. and Tower, D. B.
   The quantitative significance of the gamma-aminobutyric acid pathway in cerebral oxidative metabolism.
   In Roberts, E. (Ed.) Inhibition in the Nervous System and γ-Aminobutyric Acid. London, Pergamon, In press

3. Tower, D. B.
   Neurochemistry of Epilepsy: Seizure Mechanisms and Management. Springfield, Thomas, 1959.

Honors and Awards: None.

NIMDB-12(c)
Serial No.
1. Medical Neurology
2. Clinical Neurochemistry
3. Bethesda, Md.
4. New

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A:

Project Title: Isolation, Fractionation, characterization and Metabolism (Synthesis, Turnover, etc.) of Ribonucleic Acids in Brain.

Principal Investigators: Dr. Michael Sporn and Dr. Wesley Dingman

Other Investigators: Mr. Edmund L. Peters

Cooperating Units: None

Man Years: Patient Days: 0

Total: 0
Professional: 0
Other: 0

Project Description:

Objectives: To carry out detailed studies of the characteristics and metabolic activity of cerebral ribonucleic acids (RNA).

Methods: Isolation, fractionation, and characterization of brain RNA will be carried out by tissue ultra-centrifugation using density gradient methods, chemical and chromatographic dissociation and fractionation procedures will then be applied to ultracentrifuge crude fractions. Behavior of brain RNA fractions under various of these procedures will be compared with similarly isolated liver RNA as an initial approach to characterization. Metabolic aspects will be studied utilizing carbon-14 labelled precursors and components of RNA to yield information on RNA synthesis and turnover. Much of the foregoing has been applied to RNA of a variety of biological materials by a number of investigators with success but little has been done on brain RNA to date.

Patient Material: None.

Major Findings: This project is to be activated in July 1960 when the principal investigators come on duty in this laboratory.

Significance of Project: Numerous studies mostly by cytological techniques have established (1) that brain RNA is very actively turning over, (2) that neuronal activity can markedly alter amounts and characteristics

of brain RNA, (3) that a variety of toxic agents affect RNA in brain primarily, and (4) that a number of vital processes in celular metabolism (notably protein synthesis) depend on brain RNA. The studies proposed here represent a beginning in a more detailed approach to the understanding of these facets of brain RNA.

Proposed Course: To implement the proposed studies.

Part B Included: No.

Serial No. NINDB-13 (c)
1. Medical Neurology
2. Clinical Neurochemistry
3. Bethesda, Md.
4. 58-NINDB-34(c)

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A:

Project Title: Cerebral Protein Metabolism and Turnover in Tissue Slices incubated *in vitro*.

Principal Investigator: Dr. John L. Wherrett (Guest Worker)

Other Investigators: Dr. Donald B. Tower, Mr. E. L. Peters

Cooperating Units: Dr. Heinrich Waelsch, New York State Psychiatric Institute

Man Years:
Total: 1.5
Professional: 1.3
Other: 0.2

Patient Days: 0

Project Description:

Objectives: To determine whether slices of cerebral tissue will incorporate labelled amino acids into the protein fractions of these tissues during incubation *in vitro* and, if so, to study rates of incorporation, turnover and factors affecting them in samples of normal mammalian cerebral tissues.

Methods: See previous report and Major Findings.

Patient Material: None.

Major Findings:

(1) The hog pancreas enzyme of Barry was found unsuitable for enzymatic hydrolysis of the isolated protein fraction and the use of mixtures of pure enzymes (trypsin, etc.) was equally unsatisfactory. However, pancreatin NF (Viokase) was found to give very satisfactory and reproducible results.

(2) After cat cortex slice incubation, free amino acids isolated in the TCA-soluble fraction gave quantitative yields comparable to previously established free pool levels. The pure protein fraction obtained averaged 8% of the wet weight of the samples, in accord with numerous previous analyses by others. Acid hydrolysis of the proteins yielded total glutamate (glutamic acid + glutamine) of 73 μM/100 mg. proteins and total amide analysis yielded 55 μM/100 mg. proteins. Analysis of the enzyme hydrolysate of the slice protein fraction yielded regularly 55-65% of the total glutamate values obtained by complete acid hydrolysis.

a yield consistent with a number of previous reports by others. Correcting these yields to the total content, the following normal values for protein bound amino acids in incubated cat cortex slices were found: glutamic acid 43, glutamine 30, and asparagine 25 μM/100 mg. protein.

(3) Incubation of slices with L-glutamine-U-C^{14} and isolation of free and protein-bound amino acids for quantitative assay and C^{14}-counting, demonstrated incorporation of C^{14} into protein-bound glutamine, the SA being about 0.5% the SA of free pool glutamine. This finding is of the same order of magnitude reported by Lajtha et al. for incorporation of various C^{14}-amino acids into cerebral proteins in vivo.

(4) Slices incubated with 40 mM malonate (which markedly elevates free glutamic acid [Cf. 59-NINDB-1 (c)]) exhibited a significant decrease of protein-bound glutamine with a comparable rise in protein-bound glutamic acid. Similar experiments with 10 mM NH_4Cl (which markedly elevates free glutamine and depresses free glutamic acid) exhibited a very striking rise of protein bound glutamine with a comparable decrease of protein-bound glutamic acid. Slices from animals with methionine sulfoximine induced seizures (where free glutamine synthesis is almost totally inhibited) exhibited normal protein-bound glutamic acid and glutamine levels. These data, together with the apparently slow turnover rate of protein glutamate shown in C^{14} studies, suggest that a portion of cortical proteins is involved in an active amino group transfer process with amino acids of the free pool, utilizing primarily carboxyl and amide groups on protein glutamic acid and glutamine respectively. The metabolic and functional implications of t these findings may have great fundamental importance.

Significance of Project: The experiments to date indicate the feasibility of studying a number of aspects of cerebral protein metabolism by the use of incubated slices in vitro. Several investigators, notably Waelsch, have proposed that cerebral proteins may participate in amino nitrogen metabolism, and Waelsch and we have jointly evolved a concept that the transfer of amide from protein glutamine to free glutamic acid may be important to neuronal activity as well as metabolism. The integration of oxidative metabolism via γ-aminobutyric acid with these aspects may provide the missing link between γ-aminobutyrate-glutamate dysfunction and seizures. And the findings with NH_4Cl have very obvious bearing on both the seizures and hepatic coma aspects of cerebral ammonia toxicity. Here the connections between structure and metabolism and between metabolism and functional activity are finally beginning to emerge, so that eventual integration of these various facets of cellular biochemistry may be possible.

Proposed Course: To continue the above studies and to extend them to aspartic acid, with the use of other C^{14}-labelled amino acids, and with the use of N^{15}-labelled glutamine and N^{15}-ammonia.

Part B Included: Yes.

Serial No. NINDB-13(c)

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Part B:

Publications:

1. Tower, D. B.
Some neurochemical aspects of cortical neurobiology.
In Schadé, J. P. and Tower, D. B. (Eds.) Structure and Function of the Cerebral Cortex (2nd Internat. Meet. Neurobiol.), Amsterdam, Elsevier, In press.

Serial No. NINDB-14(c)

1. Medical Neurology Branch
2. Section on Neurological Disorders Service
3. Bethesda, Maryland
4. Continuation of NINDB-13(c), 1958

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: The Histopathological and Chemical Investigations of Neuromuscular Disorders.

Principal Investigators: G. Milton Shy
Theodor Wanko
Gunter Haase

Man Years:
Total: 4.4
Professional: 2.8
Other: 1.6

Patient Days: 2,768

Project Description:

OBJECTIVES:

This is a continuation of the long-term study initiated at the inception of this Institute. Over the past years these studies have 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 we 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 the various neurogenic and primary muscle disorders.

An inclusive study of the primary pathology of peroneal muscular atrophy has just been concluded and the final studies of the various interrelated factors in cationic paralysis have been concluded.

METHODS EMPLOYED:

To follow regeneration of muscle, tritium labelled thymidine has been used in some 20-odd cases of different neurological disorders, and autoradiology is now underway on these sections. Proliferating cells will manufacture DNA, and in doing so incorporate the labelled thymidine. The low energy range of tritium allows precise localization of such DNA. The cationic disorders were studied in relation to thyroid metabolism, to K-42 exchange, to aldosterone by means of double isotope derivative methods, and to the relation of pharmaceutical agents such as SC-8109, a steroid-17 lactone, and 2-methyl-9-alpha-fluorhydro cortisone, which deliberately shift monovalent cations. Sodium and potassium were studied by flame photometry, and chloride by the Catlove technique.

Eighteen cases of Charcot-Marie-Tooth disease were studied after the pathology had revealed an initial impression that a primary disorder of muscle in addition to a disorder of nerve was present in this disease. This was controlled by a series of 15 cases of amyotrophic lateral sclerosis.

MAJOR FINDINGS:

Over 80 percent of cases of Charcot-Marie-Tooth disease demonstrate myopathic findings on pathological examination. No such findings were found in cases of amyotrophic lateral sclerosis. Two of this series are clinically classical, but demonstrated only myogenic changes. Eighty percent demonstrated some myopathic involvement, whereas 100 percent of the cases of amyotrophic lateral sclerosis showed only neural involvement.

In the cationic diseases, three separate entities have now been segregated which may cause sudden flaccid skeletal paralysis. These are: (1) familial periodic paralysis; (2) paramyotonia congenita; and (3) primary aldosteronism. The pathophysiology and treatment of each is different. In familial periodic paralysis there is a retention of potassium and sodium. There is a hypokalemia. Such

potassium lost from the serum enters the cell. Enough water enters the cell, however, to hold the concentration of potassium approximately the same. Sodium depletion aids in the recovery of the attack, or prevents the onset of such an attack. Sodium loading precipitates the attack. The administration of d-l aldosterone does not initiate an attack. Carbohydrate initiates the attack, as has been long known. Exactly the reverse is true for paramyotonia congenita, although also in this disorder d-l aldosterone does not precipitate an attack. As noted in the 1958 report, cationic K concentrations within the cell were confirmed by resting potentials obtained by intracellular microelectrodes.

The studies on regeneration of muscles by use of tritium labelled thymidine must await a three-months' film exposure before the autoradiographs may be read. A study of the mitachondria in familial periodic paralysis will be undertaken on appropriate sections by Dr. Wanko with the electron microscope.

PROPOSED COURSE OF THE PROJECT:

Muscle disorders will continue to occupy much of the time of the Medical Neurology Branch in that so many different parameters of investigation may be brought to bear on this readily accessible material.

SIGNIFICANCE TO NEUROLOGICAL RESEARCH:

Over the past seven years this Institute has clarified many of the problems associated with disease of striated muscle which constitutes 80 percent of the body mass. Such initial promise justifies continual research in these fields.

Part B included: Yes

Serial No. NIMDB-14(c)

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards, and Publications

Publications other than abstracts from this Project:

Haase, G., and Shy, G. M. The Pathological Basis of Peroneal Atrophy. In preparation.

Rowley, P. T., and Kliman, B. The Effect of Sodium Loading and Depletion on Muscular Strength and Aldosterone Excretion in Familial Periodic Paralysis. Am. Jnl. of Med., In Press.

Shy, G. M. Some Metabolic and Endocrinologic Aspects of Disorders of Striated Muscle. Proceedings of the Assoc. for Research in Nervous and Mental Diseases, December, 1958. In Press.

Serial No. NINDB-15(c)
1. Medical Neurology Branch
2. Section on Neurological Disorders Service
3. Bethesda, Md.
4. Continuation of NINDB-15(c)

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: The Physiology and Pharmacology in Myasthenia Gravis

Principal Investigator: G. Milton Shy

Other Investigators: Andrew Engel
Mark Lane
William Matthews

Man Years:
Total: 4.4
Professional: 2.8
Other: 1.6

Patient Days: 311

Project Description:

OBJECTIVES:

As stated in last year's Annual Report, the main objective was that of intracellular recording of resting potentials in patients with myasthenia gravis. The difficulties of this procedure were pointed out in the last Annual Report, in which a description of the percutaneous method of introducing microelectrodes blindly inside single cells was given. Approximately 18 such patients were studied in the current year, with over 200 penetrations. It has become apparent that, with our present technique at least, we will not be able to successfully carry out this procedure through the intact skin, and that such patients may have to have surgical exposure of the muscle to carry on such a study. This phase in the program, hence, has been temporarily abandoned. It is of interest that this program, like many others, has now separated into two different

components. First, the availability of agents which influence thyroid formation and TSH formation, as well as methods of influencing 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 been well worked out by Dr. Andrew Engel, and will now be recorded in a separate project.

At the beginning of this reporting year, the Institute was fortunate in having with it for a period of one month, Dr. Fritz Buchthal, and the Copenhagen method of electromyography is now being extensively used within the Branch. The recording instrumentation for the intracellular work in myasthenia gravis has been converted therefore to this particular project, which will be referred to in succeeding Annual Reports as the electrophysiological abnormalities of muscle disease.

METHODS EMPLOYED:

Multiple concentric electrodes were utilized in these studies so that synchronization and motor unit areas may be mapped. Studies at the present time include a study of the internal ocular muscles in nystagmus in cooperation with the Ophthalmological Unit, which is just now being initiated. Of interest is the fact that the characteristic response of myasthenia gravis has now been seen in over 60 percent of patients with generalized sarcoid disease. The reason for this is still not clear, but 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 are not charted is being undertaken by the Fellow assigned to this project. A direct correlation of the EMG with the muscle pathology is continually carried out on each patient admitted to a muscle project in this Institute.

COURSE OF THE PROJECT:

This will be a long-term project, continuing probably for the duration of admissions of patients with neuromuscular disorders.

SIGNIFICANCE TO THE PROGRAM:

Electromyography has fallen in disrepute in most medical centers in the United States today. This Institute is convinced, after analyzing some 110 of our own patients and the Copenhagen 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, that the EMG is of definite value when correctly done and interpreted, and has many times given us information of real importance in the understanding of our patients.

Part B Included No

Serial No. NIMDB-16(c)

1. Medical Neurology Branch
2. Section on Biophysical Applications
3. Bethesda, Maryland
4. Same as NIMDB-12(c), 1958

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Clinical Pathological Correlative Study of the Nervous System in Orthostatic Hypotension.

Principal Investigators: Glenn A. Drager
G. Milton Shy

Man Years

Total:	2.2	Patient Days:	37
Professional:	1.4		
Other:	0.8		

Project Description:

OBJECTIVES:

To study a series of patients with uncomplicated orthostatic hypotension in which no general medical disorder such as diabetes, syphilis, or other systemic disease is to be found. In such cases the nervous system is always involved. Controversy, at the present, exists as to whether the nervous system pathology is primary or secondary to the neurological findings. The presence of a given reproducible syndrome of the nervous system associated with hypotension would be an argument in favor of the nervous system disorder as the etiological agent, provided the areas of involvement were not synonymous to those induced by hypoxia, or, upon postmortem examination, reveal multiple vascular lesions.

PATIENT MATERIAL:

Four patients with an identical syndrome have now been admitted to this study, and complete serial sections have now been obtained and studied, on the case reported in the 1958 Annual Report.

METHODS EMPLOYED:

Fixation was as described in the previous Report. Representative serial sections of the central and peripheral system were prepared in the usual manner and stained or impregnated with hematoxylin and eosin, cresyl violet, and by the methods of Holzer, Holmes, Cajal's gold sublimate, Smith-Quigley, Sudan IV, Bielschowsky, and Penfield. Pathological reports from patients dying subsequent to hypoxia and chronic ischemia were reviewed and compared. In addition the primate work of the Laboratory of Anatomical Sciences on fetal hypoxia were reviewed and compared.

MAJOR FINDINGS:

Abnormal neuronal changes were found in the ventral, intermediolateral, and Clark's cell columns 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, nucleus ambiguus, the dorsal nucleus of the vagus, the medium Raphe of the tegmentum, and the lateral cuneate nucleus were found.

In the cerebellum and pons, neuronal changes were found in the Purkinje cells and 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.

In the diencephalon some changes were found in the posterior hypothalamus, and in the intercalated nucleus of the mammilary body. Changes were also found in the caudate nucleus; the cerebral cortex was normal.

It would thus appear that the lesion involves almost exclusively autonomic, basal ganglionic, and spinal motor cells, and that this lesion is symmetrical and does not correspond to ischemia or anoxia in the adult.

SIGNIFICANCE TO NEUROLOGICAL RESEARCH:

It is highly suggestive in these studies 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.

PROPOSED COURSE:

These findings are to be published.

Part B Included Yes

Serial No. NINDB-16(c)

PHS - NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards, and Publications

Publications other than abstracts from this Project:

1. Drager, G. A., and Shy, G. M. A Reproducible Neurological Syndrome Associated with Orthostatic Hypotension. To be published

Serial No. NINDB-17(c)
1. Medical Neurology
2. Clinically Applied Pharmacology
3. Bethesda, Md.
4. Continuation of NINDB-23(c)

PHS-NIH
Individual Project Reports
Calendar Year 1959

Part A.

Project Title: A study of naturally occurring choline esters.

Principal Investigator: Richard L. Irwin

Other Investigators: Jay B. Wells and Andrew G. Engel

Cooperating Units: None

Man Years: (calendar year 1959)
Total : 1.2
Professional: 0.6
Other : 0.6

Patient Days: 0 (calendar year 1959)

Project Description:

OBJECTIVES: An increasing number of choline esters are being identified as natural constituents of biological systems. The physiological significance of choline esters other than acetylcholine is largely unknown. Pharmacological data is not prevalent. The objectives of the present study are: (a) To study the action of choline esters and chemically related substance on muscle membranes. (b) To isolate and identify choline esters or other quaternary compounds which may be present in biological systems.

METHODS EMPLOYED: A traveling fluid electrode technique is used to measure depolarization of muscle membranes. The technique has the advantage of recording the differential depolarization of a large number of intact muscle fibers, and provides a convenient means to determine whether chemical substances depolarize post-synaptic muscle membranes. Although the method is not entirely quantitative, it does allow semiquantitative comparisons of the depolarizing potency of various drugs to be made. The method also permits the observation of the time course of depolarization of an intact whole muscle; thus having advantages in this respect over a microelectrode technique.

Paper and column chromatography are used for isolation and identification of choline esters and other quaternary compounds.

The heart of the clam venous mercenaria is used for identification and assay of acetylcholine. This method is being adapted to the search for other active compounds.

MAJOR FINDINGS: Comparisons of the natural occurring choline esters and other depolarizing compounds have been made using frog muscle and the fluid electrode technique. It was found that accurate concentration-response relationships could not be obtained since one depolarization reduced the sensitivity of subsequent tests and test muscles varied considerably in their response. By selection of a narrow range of depolarization to be produced and the use of one muscle for each test a comparison of the potency was possible. Butyrylcholine, imadazoleacrylylcholine and dihydro-acrylylcholine are nearly equal to succinylcholine, (a neuromuscular blocking compound) in depolarizing strength. This appears to indicate that if choline esters which are not hydrolysed by muscle cholinesterase, as is acetylcholine, were present in muscle in abnormal amounts pronounced effects on muscle function could occur. The identification of such a substance in diseased muscle would require the use of small muscle samples. Recent experiments indicate that acetylcholine can be assayed from muscle samples as small as one gram.

Experiments in which excess acetylcholine was added showed recovery of the excess. It thus may be possible to identify other less labile choline esters from muscle if they were present providing adequate tests can be developed. Present experiments appear to indicate that low concentrations of active choline esters affect the response of the venous mercenaria heart to acetylcholine. This may offer a new approach to the identification of choline esters in physiological systems.

SIGNIFICANCE TO THE PROGRAM OF THE INSTITUTE: Information concerning the biological activity of substances which occur in animal tissue greatly enhances our knowledge of normal and pathological physiology.

PROPOSED COURSE OF PROJECT: To continue to study the biological effects of choline esters and to attempt to isolate and identify these compounds from tissue.

Part B included: No.

Serial No. NINDB-18(c)
1. Medical Neurology
2. Clinically Applied Pharmacology
3. Bethesda, Md.
4. Continuation of NINDB-22(c)

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Study of muscle cholinesterase and its inhibitors.

Principal Investigator: Richard L. Irwin

Other Investigators: Henry J. Smith, III

Cooperating Units: Laboratory of Chemistry of Natural Products, National Heart Institute.

Man Years: (calendar year 1959)
Total : 1.2
Professional: 0.6
Other : 0.6

Patient Days: 0 (calendar year 1959

Project Description:

OBJECTIVES: The cholinesterase content of muscle is low and not uniformly distributed throughout the tissue. Muscle has therefore not been adequately studied in respect to either the type of cholinesterase it contains or as to substrate and inhibitor specificity. As Augustinsson has recently pointed out (Method in Biochemical Analysis, 1957), the results obtained with inhibitors and substrates are dependent upon the enzyme preparation used, both species and organ specificity being of importance. In view of these considerations, one of the objectives of this project is to adequately characterize this important muscle enzyme as to substrate specificity. Another objective is to examine the activity of the enzyme in the presence of the well-known inhibitors which are in wide use

clinically and to correlate this activity with their usefulness. This would form a basis for testing newer compounds having a potential in the treatment of myasthenia gravis. A further objective is to determine the inhibitory and depolarizing activity of compounds either used or proposed as useful agents in the treatment of myasthenia.

METHODS EMPLOYED: The standard Warburg manometric technique is used for determination of muscle cholinesterase activity. The depolarizing properties of cholinesterase inhibitors are determined by use of the traveling fluid electrode system used by Fatt, J. of Physiol., 111:408.

MAJOR FINDINGS: In the course of this project the clinically useful therapeutic agents used in the treatment of myasthenia gravis have been studied to determine whether a correlation exist between clinical efficacy, inhibition of muscle cholinesterase and depolarization of muscle membranes. Ambenonium was found to have the greatest inhibitory activity on muscle cholinesterase of any of the compounds tested. Neostigmin has greater activity in this respect than pyridostigmin and also has muscle membrane depolarizing effects which pyridostigmin lacks. Thus, since pyridostigmin does not depolarize muscle membranes appreciably and has a lower inhibitory potency than other useful compounds, no direct correlation exist between clinical use, muscle cholinesterase inhibition and depolarization of muscle membranes.

A major part of the work this project has centered around the testing, both in vivo and in vitro, of compounds which seemed likely to have a potential use in the treatment of myasthenia gravis and possibly other neurological disorders. Galanthamine is an alkaloid that has recently been isolated from the bulbs of Amaryllidaceae plants in Russia, Japan and in this country by the Laboratory of Chemistry of Natural

Products of the National Heart Institute. Lycoramine is an alkaloid with a chemical structure closely related to that of galanthamine. We have investigated these compounds and several related derivatives as to their muscle potentiating and acetylcholinesterase inhibiting properties. These compounds are derivatives of phenanthrene and are of interest because chemically they differ markedly from the 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, deoxy-demethyl-lycoramine methyl iodide, neopine and neopine methyl iodide.

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 or edrophonium. Deoxy-demethyl-lycoramine methyl iodide was the most active compound found. This compound produces cholinesterase inhibition equal to that of neostigmin or physostigmine. The in vivo muscle potentiating activity of the compounds is well correlated with the in vitro muscle cholinesterase inhibiting activity. Plasma, red blood cell and brain cholinesterase is also inhibited. The potency of these compounds suggest the possibility that they may be of value in the treatment of myasthenia gravis. Whether these compounds will offer advantages over the therapeutic agents now in use for the treatment of myasthenia will depend on toxicity studies and clinical trial. Galanthamine hydrobromide has received such trial in the USSR and more recently in Sweden. The biological activity and use of lycoramine and deoxy-demethyl lycoramine has not been previously described. A recent monograph from Russia describes the use of galanthamine hydrobromide in a neurological disorder. "Cerebral Paresis in Children and Its Treatment", M. B. Eydinova and E. N. Pravdina. Publisher: Academy of Pedagogical Society of the RSFSR, Institute of Defectology, Moscow, 1959.

SIGNIFICANCE TO THE PROGRAM OF THE INSTITUTE: This project relates to the development and testing of clinically useful drugs for the treatment of myasthenia gravis.

PROPOSED COURSE OF PROJECT: To perform toxicity studies on the most active compounds of the group of alkaloids mentioned above to determine whether clinical testing is feasible or desirable. Other active compounds are being considered for pharmacological testing.

A rapid, sensitive and graphic method is being developed for determination of muscle cholinesterase in order to facilitate the study of its inhibition. A more purified muscle esterase preparation will be studied as to substrate specificity and compared to a purified acetylcholinesterase obtained from other tissues.

Part B. Included: Yes

Serial No. NINDB-18(c)

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

In press: "Cholinesterase Inhibition by Galanthamine and Lycoramine". Richard L. Irwin and Henry J. Smith, III. Biochemical Pharmacology.

Honors and Awards relating to this project: None

Serial No. NINDB-19(c)
1. Medical Neurology
2. Clinically Applied Pharmacology
3. Bethesda, Md.
4. Continuation of NINDB-24(c)

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: A study to determine the effects of depolarizing drugs on muscle enzymes.

Principal Investigator: Richard L. Irwin

Other Investigators: Henry J. Smith, III

Cooperating Units: None

Man Years: (calendar year 1959)
Total : 0.8
Professional: 0.4
Other : 0.4

Patient Days: 0 (calendar year 1959)

Project Description:

OBJECTIVES: To determine whether depolarization of muscle membranes effect the efflux of enzymes from muscle.

METHODS EMPLOYED: Standard biochemical procedures are used to estimate enzyme activity in plasma and muscle.

Mammalian in vivo experiments using pairs of muscles are utilized in both experimental and control procedures.

A traveling fluid electrode procedure is used in isolated muscle experiments to determine that muscle membranes are intact during experimentation.

MAJOR FINDINGS: Muscle loses aldolase when kept in a solution containing depolarizing concentrations of potassium. It has not been determined whether the depolarization of cell membrane is the causative factor, or whether the loss of enzyme is due to the activity of potassium at another site. Even less is known about the efflux of other enzymes from muscle, although it has been observed that certain of them, phosphohexoisomerase, transaminase and lactic acid dehydrogenase, are present in the plasma in increased concentration in muscle dystrophies. Plasma concentrations of these enzymes, and aldolase, are not increased in muscle atrophy of neural origin. Laboratory, and particularly in vivo experiments in this area, are notably few.

To date we have attempted to determine whether aldolase leaves the muscle under the influence of succinylcholine, a compound which depolarizes frog muscle membranes rapidly at a concentration of 3×10^{-6}M. Experiments were done on pairs of rat muscles removed prior to and after the administration of the drug. Changes in the aldolase content of the experimental muscles were variable and not pronounced at the end of brief periods of depolarization. Additional experiments are needed to determine if significant changes in the aldolase content of muscle occurs during drug depolarization.

We have found that substances which depolarize muscle membranes also inhibit muscle cholinesterase. Inhibitory concentrations are much greater than those necessary for depolarization, and thus esterase inhibition by these compounds appear to be unrelated to their depolarizing activity.

SIGNIFICANCE TO THE PROGRAM OF THE INSTITUTE: The efflux of enzymes from muscle due to the action of a drug would be a new finding in drug action which may aid in understanding abnormal states in muscle.

PROPOSED COURSE OF PROJECT: To continue to investigate the conditions which influence the movement of enzymes out of muscle.

Part B. included: No

Serial No. NINDB-20(c)
1. Medical Neurology
2. Clinically Applied Pharmacology
3. Bethesda, Md.
4. New

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: The localization of muscle fibers in a single motor unit.

Principal Investigator: Richard L. Irwin

Other Investigators: Forbes H. Norris, Jr.

Cooperating Units: None

Man Years: (calendar year 1959)
Total : 0.8
Professional: 0.4
Other : 0.4

Patient Days: 0 (calendar year 1959)

Project Description:

OBJECTIVES: The interpretation of muscle action potentials obtained by electromyographic recording is handicapped by a lack of knowledge as to the exact anatomical distribution of fibers of a single motor unit. The objective of this project is to develop suitable methods and to study the anatomical distribution of the fibers of a single motor unit and its electrical activity.

METHODS EMPLOYED: The project is in an early stage and changes in the experimental procedure may be necessary. Rat preparations are in use; the study will be extended to cat. Single motor unit preparations have been achieved in acute experiments by many workers; chronic single unit procedures should also be possible. The work of Eccles & Sherrington in 1930 establishes the anatomical basis

for this study. Anterior rami of fused roots are isolated for several lumbar segments. By microdissection, the middle ramus is pregressively divided until only one, or a very few fibers remain. The rami above and below are resected. Histological studies of roots, nerve and muscle will be undertaken in order to chart the extent of subsequent degeneration. Evoked electrical activity will be recorded by conventional methods to determine anatomical electrical relationships.

MAJOR FINDINGS: None

SIGNIFICANCE TO THE PROGRAM OF THE INSTITUTE: This study, admittedly facing a difficult technical problem, if successful will provide a direct correlation between anatomical and electrophysiological areas of the motor unit. It might thus have some bearing on clinical electromyographic studies.

PROPOSED COURSE OF PROJECT: See Methods

Part B included: No

Serial No. NINDB-21(c)
1. Medical Neurology
2. Clinically Applied Pharmacology
3. Bethesda, Md.
4. Continuation of NINDB-21(c)

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: The action of neuromuscular blocking drugs on directly stimulated innervated and denervated muscle.

Principal Investigator: Richard L. Irwin

Other Investigators: Jay B. Wells and Henry J. Smith, III

Cooperating Units: None

Man Years: (calendar year 1959)
Total : 0
Professional: 0
Other : 0

Patient Days: 0 (calendar year 1959)

Project Description:

OBJECTIVES: To study the decrease in contractile response of directly stimulated skeletal muscle which follows the administration of certain quaternary ions.

METHODS EMPLOYED: Stated in Annual Report, NINDB-21(c), 1958.

MAJOR FINDINGS: Details of the major findings of this project are stated in the Annual Report of 1958. In general, this project has shown that the clinically useful neuromuscular blocking agents decrease muscle function by additional processes as well as by interruption of transmission of impulses between nerve and muscle.

SIGNIFICANCE TO THE PROGRAM OF THE INSTITUTE: The drugs investigated in the course of this project are in common clinical use. They also serve as valuable research tools in clarifying physiological processes related to transmission of impulses between nerve and muscle.

This project contributes to an increased understanding of the physiological processes related to myasthenia gravis.

PROPOSED COURSE OF PROJECT: Terminated.

Part B included: Yes

Serial No. NINDB-21(c)

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards, and Publications:

Publications other than abstracts from this project:

Published in 1959: "The Contractile Response of Directly Stimulated Muscle after Administration of Neuromuscular Blocking Compounds". The Journal of Pharmacology and Experimental Therapeutics, 125: (2) Page 159, February, 1959.

"The Effect of Certain Neuromuscular Blocking Compounds on Directly Stimulated Muscle". Communication in Curare and Curare-Like Agents, Elsevier Publishing Company, Amsterdam, 1959, Page 395.

Honors and Awards relating to this project:

U. S. Government Special Service Award, Jay B. Wells.

Serial No. NINDB-22(c)
1. Medical Neurology
2. Clinically Applied Pharmacology
3. Bethesda, Md.
4. Continuation of NINDB-20(c)

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Blood and tissue cholinesterases in neuromuscular blockade.

Principal Investigator: Richard L. Irwin

Other Investigators: Jay B. Wells and Henry J. Smith, III

Cooperating Units: None

Man Years: (calendar year 1959)
Total : 0
Professional: 0
Other : 0

Patient Days: 0 (calendar year 1959)

Project Description:

OBJECTIVES: To determine the function of specific and non-specific cholinesterase in relation to the blocking of transmission between nerve and muscle.

METHODS EMPLOYED: Stated in Annual Report, NINDB-20(c), 1958.

MAJOR FINDINGS: Stated in Annual Report, NINDB-20(c) 1958.

SIGNIFICANCE TO THE PROGRAM OF THE INSTITUTE: This project has provided information on the metabolism of chemically useful cholinesterase inhibitors and neuromuscular blocking compounds. In addition, it has increased the understanding of the physiological processes related to myasthenia gravis and its treatment by cholinesterase inhibitors.

PROPOSED COURSE OF PROJECT: Terminated.

Part B included: Yes

Serial No. NINDB-22(c)

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

"The Effects of Selective Inhibition of Muscle and Plasma Cholinesterase on Neuromuscular Block". Proceeding of the Second (1959) International Symposium on Myasthenia Gravis.
Accepted for publication and in press by the Arthur H. Thomas Publishing Company, Richard L. Irwin, Jay B. Wells, and Henry J. Smith, III.

Honors and Awards relating to this project: None

[illegible] - NIH
[illegible] Project Report
[illegible] Year 1959

[illegible]:

[illegible] Title: Skull Changes in [illegible] Cases of Dystrophia Myotonica

[illegible] Investigators: Giovanni [illegible]
John Eg[illegible]

[illegible]

[illegible]

[illegible] (December [illegible] 1959)
[illegible]: 0.6
Professional: 0.3
Other: 0.3

Project Description:

Objectives: [illegible] have reported on skull changes in dystrophia myotonica. These changes [illegible] one of the variable features of the disease.

Methods Employed: The skull X-rays of [illegible] dystrophia myotonica have been reviewed.

Major Findings: A high percentage (75 per cent) of cases showed "hyperostotic" changes of the vault. These have been grouped in four different types. The degree of the skull changes is [illegible]. The longer the duration of disease the more marked are the "hyperostotic" changes. In eight cases with proof of hypogonadism the "hyperostosis" was most advanced.

Significance to Individual Research Project: Changes in skulls of patients with dystrophia myotonica [illegible] the changes found may be physiologically [illegible] increased circulating growth hormone.

[illegible] [illegible] [illegible] No

PHS - NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards and Publications

Publications other than abstracts from this project:

Di Chiro, Giovanni and Caughey, John Egerton: Skull Changes in Eighteen Cases of Dystrophia Myotonica. (Accepted for publication in Acta Radiologica; to be published in the near future).

Honors and Awards Relating to this Project:

None

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A:

Project Title: RISA (radio-iodinated serum albumin) Encephalography and Conventional Neuroradiological Methods

Principal Investigators: Giovanni Di Chiro, M. D.

Other Investigators: None

Cooperating Units: None

Man Years: (Calendar year 1959) Patient Days: None
Total: 0.2
Professional: 0.1
Other: 0.1

Project Description.

Objectives: To assess the reliability of a comparatively new method as the "brain scanning" in comparison to the related radiological methods. Emphasis has been laid on the intracranial space-occupying lesions. To establish the advantages and the pitfalls of the two respective techniques. To establish the relative value of the "brain scanning" technique and of the neuroradiological methods in the different types and in the differently located lesions.

Methods Employed: The verified cases of intracranial space-occupying lesions which have been diagnostically worked up both with "brain scanning" and different neuroradiological methods have been compared.

Major Findings: The final screening of the collected data is being done at present.

Significance to Neurological Research: It is hoped that the present contribution will better elucidate the advantages, the limitations and the future possibilities of the brain scanning technique.

Serial No. [illegible]
1. Medical Neurology
2. Neuroradiology
3. Bethesda, Maryland
4. New

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A:

Project Title: Combined Laminagraphy and Fractional Encephalography

Principal Investigators: Giovanni Di Chiro, M. D.

Other Investigators: None

Cooperating Units: Diagnostic X-Ray Department, CC

Man Years: (calendar year 1959) Patient Days: None
Total: 0.2
Professional: 0.1
Other: 0.1

Project Description:

Objectives: The superimposition of different anatomical structures often makes the interpretation of encephalographic findings difficult. This is especially true in the posterior fossa. Laminagraphy may be a method for better visualization of the different structures bordering the pathways of the cerebrospinal fluid. Comparatively small structures not demonstrated before may be visualized with this new technique.

Methods Employed: Laminagraphic "cuts" of the different sections of the air filled cerebrospinal fluid pathways have been taken. The laminagraphic study has generally been done "dynamically" that is during the passage of air through the different sections of the ventricular system and subarachnoidal spaces according to the fractional encephalographic technique ad modum Lindgren. Laminagraphic cuts in different projections and in the two ortogonal planes have been taken. With the patient examined in the sitting position different technical problems concerning the associated laminagraphic and encephalographic techniques have been solved. The number of laminagraphic cuts carried out has varied. Up to now about thirty patients have been examined with this technique. Our experience is limited to cases without intracranial space-occupying lesions.

Major Findings: The visualization of the different structures demonstrated by fractional encephalography has been markedly improved.

Significance to Neurological Research: Fractional encephalography combined with laminagraphy is a technically difficult procedure. The improvement of the visualization of the different structures will however pay its rewards.

0.2

0.1

PHS - [illegible]
Individual Project Report
Calendar Year 1959

Part I:

Project Title: The Sella and the Pituitary in the Coronal Plane

Principal Investigators: Giovanni Di Chiro, M. D. and Joseph Morel, R. T.

Other Investigators: None

Cooperating Units: Diagnostic X-Ray Department, C[illegible] and Pathological Anatomy Department, [illegible]

Man Years: (calendar year 1959) Patient Days: [illegible]
Total: 0.2
Professional: 0.1
Other: 0.1

Project Description:

Objectives: Surgery of the pituitary is now a [illegible] problem of neurology. Hypophysectomy is carried out in [illegible] conditions. Pituitary adenomae have been lately [illegible] removed in ten cases by Norlén. Intrasellar implantation of Yttrium 90 seems to be a highly promising technique to be used in a wide variety of conditions (British, Italian, and [illegible] authors). To approach the intrasellar region for the [illegible] purposes the trans-sphenoidal route is preferred by [illegible] authors. The preoperative knowledge of the lateral [illegible] of the sella and of the pituitary is of utmost importance for trans-sphenoidal surgery. Furthermore the assessment of the exact three-dimensional size of the sella is a problem which has not yet been solved. At present we have only the possibility of measuring [illegible] depth of the sella and its [illegible]

Purpose of the present [illegible] and roentgenographic study is to establish [illegible] the [illegible] of the sella turcica and [illegible] pituitary, and to assess the three-dimensional size of the sella.

Methods Employed: Bases of skull with the sella, the pituitary, and the parasellar vessels will be [illegible]

with different roentgenographic techniques including laminagraphy. Metallic wires and X-ray opaque fluids are used to outline the different border lines of the sella and of the pituitary and the adjacent structures. The obtained results are compered with standard X-ray films of normal cases and of cases with intra- and parasellar pathology. Volumetric measurements are being made both in the specimens and in the X-ray films. Due account is of course given to the roentgenographic magnification which is tested in comparable conditions.

Significance to Neurological Research: It is hoped that this study will increase our knowledge of the anatomical substratum of the pathology of the sellar region.

Part B included: Yes No [X]

Serial No. NINDB-28
1. Medical Neurology
2. Neuroradiology
3. Bethesda, Maryland
4. New

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A:

Project Title: A Bilateral Angiographic Evaluation of the Superficial Veins and Sinuses of the Brain

Principal Investigators: Giovanni Di Chiro, M. D.

Other Investigators: None

Cooperating Units: None

Man Years: (calendar year 1959) Patient Days: None
Total: 0.2
Professional: 0.1
Other: 0.1

Project Description:

Objectives: The venous "drainage" of the brain through the superficial veins and the major sinuses of the convexity has previously been studied with different techniques (anatomical, with radioactive isotopes). No systematic angiographic study has been done on this subject. In particular no bilateral angiographic comparison of the problem of the superficial venous drainage of the brain has ever been done. Such a study is considered by the present author important especially in regard to possible anatomical fundaments for the predominancy of one of the hemispheres.

Methods Employed: At the NINDB we have a comparatively large material of bilateral carotid angiographies in patients with no intracranial space-occupying lesions and with no gross abnormality. This represents an ideal material to study the problem of superficial venous "drainage" of the brain. In addition every angiography is carried out by us seriographically with a minimum of six films on each plane. In this way the different phases of the superficial venous drainage may be angiographically demonstrated. The seriographic "rhythm" has been in different occasions changed to arrive to an "optimum" for studying the venous discharge. In a certain number of patients who have received bilateral carotid angiography, tests are done (in the neurosurgical department) to assess which hemisphere is the

predominant (Wada-test). This will of course make possible a comparison of the bilateral angiographic findings with reliable determination of the functional predominancy of one of the hemispheres.

Major Findings: In a significant number of cases the venous drainage takes place through different vascular channels in the two sides of the same patient. While the main drainage venous trunk on one side may for instance be the Trolard vein, on the opposite side the Labbe's or the superficial Sylvian veins may be the main discharge pathways. A statistical study of the drainage of the two hemispheres through the right or left transverse sinuses is part of the project.

Significance to Neurological Research: It is hoped that the present study will be a contribution to establish whether an anatomical vascular fundament exists in relation to the predominancy of one of the hemispheres.

Part B included: Yes No [X]

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A:

Project Title: Encephalographic Changes in Cases of Temporal Lobe Epilepsy

Principal Investigators: Giovanni Di Chiro, M. D.

Other Investigators: None

Cooperating Units: None

Man Years: (calendar year 1959) Patient Days: None
Total: 0.2
Professional: 0.1
Other: 0.1

Project Description:

Objectives: To assess the frequency, the type, and the entity of encephalographic changes in cases of temporal lobe epilepsy (t.l.e.) using a standard and reliable encephalographic technique.

Methods Employed: While reviewing the available encephalographic material of t.l.e. (in particular cases with associated angiomatous malformations and cases with bitemporal foci) the present author arrived to the conclusion that a standardized encephalographic technique to give a demonstration of the real anatomical conditions of the temporal horns and the surrounding structures is necessary. For this reason every case of t.l.e. is now studied encephalographically in such a way that reliable comparison of the two temporal horns is obtained in every case. Each temporal horn is filled separately and after examined in the two ortogonal planes. The technique of filling the temporal horns has been simplified and standardized so that the X-ray technician will be able to carry out the necessary maneuvers.

Major Findings: About twenty patients with temporal lobe epilepsy have up to now been studied with this encephalographic technique. Further experience will be accumulated in the future.

Significance to Neurological Research: The value of encephalography in establishing the anatomical changes in the temporal regions in cases of t.l.e. will be definitely assessed.

Part B included: Yes No [X]

Serial No. NINDB-
1. Medical Neurology
2. Neuroradiology
3. Bethesda, Maryland
4. New

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A:

Project Title: A Radiographic Study of the Soft Tissues in the Different Muscle Diseases

Principal Investigators: Giovanni Di Chiro, M. D.

Other Investigators: None

Cooperating Units: Diagnostic X-Ray Department, CC

Man Years: (calendar year 1959) Patient Days: None
Total: 0.2
Professional: 0.1
Other: 0.1

Project Description:

Objectives: This project is a long term study of the roentgenographic changes of the soft tissues in cases of pathological muscle conditions. No large and systematic study of this type has been carried out before.

Methods Employed: Several technical problems are involved in the "optimal" roentgenographic visualization of the soft tissues. Different methods and techniques have been suggested and used. Long exposures with scarcely penetrating X-rays, particular type of screens, injection of X-ray opaque fluids, injection of gas, and color radiography have been some of the technical methods suggested to obtain a good demonstration of the soft tissues. It is our opinion, however, that at present the best available method is a combination of a good X-ray technique, particularly suitable for soft tissues, and a good reproduction technique. The "Logetron" equipment has been chosen for the reproductions. The amount of contrast and detail obtainable with this equipment is unsurpassed. Control studies of normal cases and possibly of autopsy specimens will be carried out.

Major Findings: None yet.

Significance to Neurological Research: The different muscle diseases may possibly show different patterns of roentgenographic soft tissues changes.

Part B included: Yes No [X]

Serial No. NINDB-31(c)
1. Medical Neurology Branch
2. Section of Neurological Disorders Service
3. Bethesda, Md.
4. New

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Thyroid function and neuromuscular disease

Principal Investigator: Andrew G. Engel

Other Investigators:

Cooperating Units:

Man Years: (calendar year 1959) Patient Days: 570
Total :1.0
Professional:1.0
Other :0.0

Project Description:

OBJECTIVES: (a) 5% to 10% of patients with myasthenia gravis are also hyperthyroid. Where the two diseases coexist the prognosis appears grave. Some observers reported an inverse relation between the two disorders; this view has been challenged by others. The present study was designed to define the interaction of the two disorders and to evaluate the respective roles of pituitary TSH and of the thyroid gland in whatever this interaction might be.

(b) 10% to 30% of patients with periodic paralysis are also thyrotoxic. Such cases are usually non-familial; they are precipitated by the thyrotoxic state and abate when the latter is treated. In 1926 Shinoshaki reported worsening of the periodic paralysis by thyroid extract in 7 non-familial cases, 4 of whom had goiters. In 1943 Wolf reported amelioration of the familial and non-toxic form by the administration of thyroid extract. In the present study

the effects of thyroid substance and TSH in a euthyroid patient with the familial form of the disease were observed.

METHODS EMPLOYED: Five myasthenic patients were studied. Antimyasthenic medications were held constant during the study. The clinical evaluation consisted of daily measurements of: Hand grip, arm elevation time, manual muscle testing, diplopia measurement in prism diopters by the Lancaster red and green test, or measurement of the limits of unioccular ductions to the nearest degree on a projection perimeter with a target that required macular perception. 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 radio-activity, corrected for physical decay, as described by Goldsmith, Greer, Solomon and Benua.

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 the concurrent administration of NaI to offset the thyroid hormone release accelerating effect of TSH.

5. Prolonged administration of Tapazole until depletion of thyroidal hormone stores resulted in hypometabolism with concurrently high pituitary TSH secretion.

The patient with familial periodic paralysis was carried on a constant Na, K and carbohydrate diet. Daily Na, K balances, and frequent serum K determinations were obtained. Clinical evaluations were carried out at 3 a.m. and 8 a.m. Thyroid function

evaluations were as above. Steps 1 and 2 were as for the myasthenic patients. Step 3 consisted of the administration of graded doses of TSH until the patient became hypermetabolic.

MAJOR FINDINGS: In the myasthenic study the following were noted:

Therapy	TSH Secretion	Thyroid Secretion	BMR	No. of Studies	Effect on Myasthenia
T3, <100 mcg	-	-	0	3	None
T3, >100 mcg	-	-	++	3	Worse
TSH, Tapazole	-	++	++	2	Worse
TSH, Tapazole, NaI	-	(+)	(+)	3	Slightly worse or no change
Tapazole *	++	-	-	1	Better *

*Delayed effect

In all patients who became thyrotoxic, the myasthenia became worse. 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 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 with NaI the effect on the disease was also blocked, but only to the extent that the rise in the BMR was blocked. It thus appeared that TSH per se had no significant extrathyroidal effect on the myasthenia.

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; this was relieved by TSH administration. TSH withdrawal again resulted in an exacerbation. Repeat TSH study could not be performed as the patient became resistant to TSH. Thus, there may be a further difference between the thyrotoxic nonfamilial and the familial nonthyrotoxic forms of the disease, namely the response to abnormal levels of thyroid function.

SIGNIFICANCE TO THE PROGRAM OF THE INSTITUTE:
The thyroid function study on myasthenia gravis has therapeutic implications for thyrotoxic myasthenics and, possibly, for euthyroid patients as well. It may also offer cues for further studies on the biochemical lesion in myasthenia gravis. The study on periodic paralysis may contribute to further differentiation of the familial from the non-familial-thyrotoxic form of the disease.

PROPOSED COURSE OF PROJECT:

Part B included: No.

Serial No. NINDB-32(c)
1. Medical Neurology Branch
2. Section on Neurological Disorders Services
3. Bethesda, Maryland
4. New Project

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Studies of the Chromosomal Constitution of:
1) Human subjects with various disorders.
2) Local tissue abnormalities.

Principal Investigators: Paul H. Altrocchi, M.D.
Robert Krooth, M.D.

Other Investigator: G. Milton Shy, M.D.

Cooperating Units:

Man Years (calendar year 1959):
Total: 0
Professional: 0
Other: 0

Patient Days (calendar year 1959): 0

Project Description:

OBJECTIVES:

To further document the reported chromosomal aberrations in patients with Klinefelter's syndrome, Turner's syndrome, and mongolism.

To explore the possibility of quantitative chromosomal abnormalities in patients with mental deficiency, spinal deformities, multiple congenital anomalies, and various neurological disorders.

To investigate the micro-genetics of local tissue abnormalities ---e.g., café au lait spots, vitiliginous areas, nevi.

To study quantitatively the chromosomal constitution of neoplasms of the nervous system, both benign and malignant.

If the techniques become sufficiently refined, to analyze individual chromosomes more minutely for qualitative changes in relation to the above disorders.

METHODS EMPLOYED:

Fresh bone marrow aspirates will be incubated overnight, treated with colchicine, and then fixed and stained by modifications of the methods described by Ford et al., Polani, Tjio et al., and Furguson-Smith. Squash preparations will be searched systematically for cells in the metaphase of mitosis which will then be analyzed both by direct microscopy and by micro-photography 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 mentioned above, for chromosomal analysis.

MAJOR FINDINGS:

No major findings have been reported as yet.

SIGNIFICANCE TO NEUROLOGICAL RESEARCH:

It is hoped that micro-genetic studies may help clarify the nature of certain disorders of the nervous system. Since many neurological diseases are hereditary, such studies form one aspect of an eventual many-pronged attack on the precise gene-enzyme-biochemical defects involved.

PROPOSED COURSE OF THE PROJECT:

Bone marrow specimens and other tissue samples will be obtained from patients on the wards of the Clinical Center. They will then be cultured and suitably prepared for chromosomal analysis.

Part B included NO

Project No. NINDB-3[illegible](c)
1. Medical Neurology Branch
2. Neurological Disorders
3. Bethesda, Maryland
4. Continuation of NINDB-14(c)

PHS NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Pathological Study of Intramuscular Motor and Sensory Nerve Endings in the Normal and in Neuromuscular Diseases.

Principal Investigator: G. S. Haase, M.D.

Other Investigator: None

Cooperating Units: None

Man Years
Total: 3.0
Professional: 1.0
Other: 2.0

Patient Days: 0

Project Description:

Objective: The study is concerned with an investigation into the histological structure of the motor and sensory nerve endings in normal muscle and with changes occurring in these structures in various diseases affecting the neuromuscular system. Morphological changes have been described, in particular, by Cöers (Cöers and Wolff: The Innervation of Muscle - Thomas 1959) in a variety of diseases affecting the lower motor neuron and including primary neuropathies as well as myopathies. The objectives of the present study are to determine whether such changes can be observed in the [illegible]ich biopsy material available at this institution. It was further hoped to obtain information about morphological changes of the muscle spindle in a variety of diseases.

Method Employed:
1. Injection of methylene blue at the time of biopsy as indicated by Cöers.

2. Acetyl cholinesterase stain as modified by Cöers.

3. Silver stains, in particular, the modifications of Bielschowsky and Winkelmann.

Patient Material: The material is usually obtained in the course of routine biopsies, and only slight variations are imposed by the intravital injection of methylene blue. In all these specimens, routine staining methods are also employed by the Section of Neuropathology.

A total of fifty-five biopsies have been obtained in the manner described. The patient material includes predominantly patients with a neuromuscular disorder. Several patients with involuntary movements were also biopsied. In addition, material has been obtained at the time of autopsy from patients dying of diseases other than disorders of the neuromuscular system. Finally, various muscles from various laboratory animals have been obtained.

Major Findings: The concentration of the motor end-plates in a narrow zone at about the center of each individual muscle fiber has been, in general, confirmed, at least in the rat. Recently, several muscles such as the sternocleidomastoids and the biceps have been stained with the acetyl cholinesterase stain in muscles obtained from rabbits and cats. Here, there appears to be either distribution in several bands or in a fairly random manner. Since the slides provide only a two-dimensional aspect, it will be necessary to reconstruct the position of the motor end-plates in all three dimensions of the muscle.

Changes in the terminal innervation and in the motor end-plate of patients suffering from muscular diseases and diseases of the lower motor neuron have been found, such as bulbous enlargement of the axons, rich axonal sprouting and enlargement of the motor end-plate in the case of neuropathies. Marked alterations of the motor end-plates in myotonic dystrophy have been found in two patients. Since the inception of the study, this has also been reported by Cöers and Wolff. These same authors report changes in the motor end-plate in cases of myasthenia gravis. So far, only two patients with this disorder have been studied in the manner described and these changes have not been found. Marked ramification of the terminal innervation and multiple end-plates on single fibers have been found in two cases of Huntington's

Chorea, who originally were biopsied for control purposes. Some cases of Parkinson's disease showed, on orthodox stains, evidence of mild myopathy, and these were reflected by changes suggesting degeneration of the motor end-plate by methylene blue stains.

With regard to the morphological changes in muscle spindles, it is thought that this method does not promise to bear fruit. The muscle spindles even in the normal apparently show great variation and it is believed impossible to clearly state pathological changes in these structures.

Recently, the extraocular muscles of a patient dying of non-neurological disease were obtained and it is intended to study the eye muscles in a number of cases. During the last month, small specimens of muscle from the zone of innervation have been forwarded to Dr. T. Wanko for electromicroscopic investigation. Results of these examinations are not yet available.

Proposed Course of Project: Because of the anticipated departure of the investigator from N.I.H., the study will be closed at this institution but is intended to continue similar investigations in the future.

Significance to Neurological Research: These studies contribute towards understanding of the normal innervation patterns in muscle and the changes produced by various pathological conditions.

Part F included [ ] Yes [X] No

Serial No. NIMDB-34(c)
1. Medical Neurology Branch
2. Section on Neurological Disorders Services
3. Bethesda, Maryland
4. Continuation of NIMDB-11(c)

PHS-NIH
Individual Project Report
Calendar Year 1959

PART A:

Project Title: The Use of a Monoamine Oxidase Inhibitor (JB-516) as an Anti-convulsant Medication for Centrencephalic Seizures

Principal Investigators: Darwin Prockup, M.D.
Bushnell Smith, M.D.

Other Investigators: Andrew Engel, M.D.
Mark Lane, M.D.

Cooperating Units: Kristof Abraham, Electroencephalography Branch
John Oates, National Heart Institute

Man Years (calendar year 1959):
Total: 0.5
Professional: 0.5
Other: 0

Patient Days (calendar year 1959): 250

Project Description:

OBJECTIVES:

The objective of the present study is to evaluate the effectiveness of JB-516 ((phenyl isopropyl) - hydrazine) as an anti-convulsant in persons with centrencephalic seizures.

METHODS EMPLOYED:

Patients were admitted and anti-convulsant medications were reduced to a minimum whenever possible to do so without danger to the patient. After a short baseline period of observation, patients were given a placebo or JB-516 daily for four to six weeks in addition to other medications they had been taking. This was given in a form unknown to the investigator. 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 6th and 7th day of medication.

MAJOR FINDINGS:

Two patients received JB-516. One developed drowsiness followed by generalized convulsions after reduction of barbiturate medications. Before reduction the seizures were fully controlled. One patient became hypotensive and had syncopal attacks after four weeks of medication. During the 4th week the patient's seizures were fully controlled. Four patients received placebos - one patient showed improvement.

SIGNIFICANCE TO NEUROLOGICAL RESEARCH:

JB-516 shows slight signs of control of centrencephalic seizures.

PROPOSED COURSE OF PROJECT:

The study of other monoamine oxidase inhibitors with more specificity for the central system will be undertaken. The present project is discontinued.

PART B included NO

Serial No. NINDB-35(c)
1. Medical Neurology Branch
2. Section on Neurological Disorders Services
3. Bethesda, Maryland
4. Continuation of NINDB-16(c)

PHS-NIH
Individual Project Report
Calendar Year 1959

PART A:

Project Title: A Study of Progressive Parenchymatous Degeneration of the Central Nervous System

Principal Investigators: Bushnell Smith, M.D.
Glenn Drager, M.D.

Other Investigators:

Cooperating Units: Surgical Neurology Branch
Clinical Neuropathology Branch

Man Years (calendar year 1959):
Total: 1.0
Professional: 1.0
Other: 0.0

Patient Days (calendar year 1959): 2

Project Description:

OBJECTIVES:

Microscopic study of the brain and spinal cord of a deceased 51 year-old white female with a clinical diagnosis of progressive parenchymatous degeneration of the central nervous system. Upon completion of this study, correlation of the findings will be made with the clinical data, pertinent literature and findings of brain biopsy made during the third month of the illness. (See NINDB-16-c)

METHODS EMPLOYED:

The spinal cord, brain stem, cerebellum, and cerebrum sectioned and stained with the routine techniques for demonstrating morphological changes in the nervous system. Special stains were used for the demonstration of iron, inclusion bodies, and lipids.

MAJOR FINDINGS:

There is widespread diffuse loss of neurons in the brain. The occipital cortex is marked by status spongiosus and astrocytic proliferation. The cerebellum shows loss of Purkinje's cells and granule cells. The lateral columns (pyramidal tracts) show degeneration.

The nodules of acute inflammatory cells with foci of necrosis scattered throughout the brain and spinal cord are probably due to the terminal infection. (See NINDB-16-c)

SIGNIFICANCE TO NEUROLOGICAL RESEARCH:

This is a relatively rare, progressive, degenerative disease of the central nervous system. The etiology is quite obscure and the pathological process from beginning to end has been well documented. A cortical biopsy was obtained during the third month of the disease. The pathological autopsy material obtained six months after the onset of the condition should reveal information concerning the progress of the disorder. Special histological studies may be helpful in establishing the etiology. It is also of importance to correlate the clinical picture with the pathological findings.

PROPOSED COURSE OF PROJECT:

The proposed course of the project is to complete the microscopic study undertaken and correlate with the clinical findings.

PART B included NO

BRANCH OF ELECTROENCEPHALOGRAPHY AND CLINICAL NEUROPHYSIOLOGY

SUMMARY

The activity of the Branch is divided between routine diagnostic service for the entire Clinical Center and research. In a large portion of the patient population of NIMDB, as well as of other Institutes, the examinations are also utilized for research purposes, as in the previous years.

In the eleven months included between the last report (prepared November 30, 1958) and the present one (prepared November 1, 1959) a total of 1579 electroencephalographic examinations have been carried out with the following distribution among patients of the various Institutes:

NCI. . . .	291
NHI. . . .	48
NIAMD. . .	53
NMI. . . .	73
NIMH . . .	104
NIMDB. . .	1100
Total	1579

It seems worth emphasizing that this total represents the highest number of examinations carried out during the last six years. Thus the monthly average number of examinations has risen from 60.5 in 1954, and 125 in 1958 to 143.5 in 1959. Although the majority of referrals still come from our Institute, it is interesting to note that during the last four years about one-third of patients have been referred from other Institutes, among which the NCI accounts for over 60%. A considerable number of EEG examinations were performed not as a simple diagnostic procedure but as part of research projects originating outside of our Institute.

During the same period of time, in collaboration with the Branch of Neurological Surgery, 24 electrocorticographic studies were performed on the occasion of cortical exposure during surgical treatment of epileptic patients or during operations carried out for hypophysectomy in cases of carcinoma. Extensive EEG studies in resting conditions and during seizures and electrical stimulation were also carried out on a certain number of epileptic subjects in which electrodes had been chronically implanted within subcortical structures for diagnostic localizing purposes as well as for investigation.

Serial EEG examinations with qualitative and quantitative analysis of epileptiform abnormalities were also carried out to help in a project of the Branch of Clinical Neurology, designed for the purpose of assessing the value of new medical treatments of seizures.

From the Branch of Electroencephalography and Clinical Neurophysiology a total of 9 research projects are in progress or have been completed within 1959. Of these, 4 are continuations of projects previously outlined in other reports (36C, 37C, 38C, 39C) and 5 are new (40C, 41C, 42C, 43C, 46C). Three of these projects are carried out in strict collaboration with the Branch of Neurological Surgery and the detailed description of two of them will be found in the report of that Branch.

Projects 36C, 37C, 38C and 43C are related to clinico-electroencephalographic problems in the field of epilepsy. Project 46C deals with the effects of hypothermia and blood pressure on the electrical activity as recordable from exposed normal human cortex. Projects 39C, 41C and 42C deal with experimental investigation of thalamo-cortical and cortico-thalamic relationship, while project 40C represents an experimental approach to the problem of seizures.

Project 36C (continuation of 1C, 1958) has been completed. It deals with the study of close relationship between clinical events and electrographic patterns taking place during the course of epileptic seizures. The results have been collected in a monograph in the form of an Atlas which has been accepted for publication and will soon appear as a supplement to the Journal of Electroencephalography and Clinical Neurophysiology. The Atlas includes a wealth of photographic documentation correlated with EEG tracings of 43 different seizures selected out of over 250 ictal episodes. Although its investigative part may be considered accomplished, this study is continued for practical purposes as an important adjunct in the routine screening of possible candidates for surgical management among the epileptic patients admitted by the Branch of Neurological Surgery.

Data pertaining to project 37C (continuation of 2C, 1958) and project 38C (continuation of 3C, 1958) are still in the process of being elaborated and analyzed. Both investigations are part of a long range research dealing with diagnostic and physiopathogenetic aspects of focal epilepsy and, in particular, of temporal lobe epilepsy. Cases suitable for chronical electrode implantation must be selected with great care; they are of necessity rather rare, and additional cases are needed before attempting any reliable evaluation and interpretation of the numerous interesting findings provided by each single case. Also the number of patients in which a complete and satisfactory electrocorticographic examination may be carried out from the exposed cortex on the occasion of surgical interventions is still inadequate to allow any definite conclusion. In addition, a long

post-[illegible] ... case to properly evaluate the various da[illegible] ... pathogenetic and etiopathogenetic aspects [illegible].

Project 390 [illegible] has been completed. This study deals with [illegible] the evoked potential elicitable on the [illegible] following stimulation of the optic radiation. By analysis of the [illegible] behavior of the various unitary elements activated within the visual cortex and underlying white matter and by their temporal correlation with the different components of the gross surface response, it has been possible to differentiate pre- from postsynaptic elements and to obtain compelling evidence on the anatomo-functional substrate of the characteristic visual response. The results have been collected in a paper which has recently been submitted and accepted for publication.

Project 40[illegible] is a research project carried out by a guest worker [illegible] Brazil. It is an experimental study of the epileptic seizure and in particular, it [illegible] investigation of the mechanism subserving the [illegible] which is produced following repetitive electrical stimulation of the neocortex. The results [illegible] described in detail in the [illegible] these findings would suggest that during repetitive electrical stimulation a progressive depolarization of the cellular membrane takes place manifesting itself with repetitive firing and progressive increase in amplitude of the unitary spikes. When this depolarization [illegible] a critical value, the spike firing stops and when this stage is reached, cell contraction and activity will follow. The latter would correspond to a progressive repolarization process and is characterized by high frequency low voltage spikes which progressively increase in their amplitude and decrease in their rate of firing. This repetitive activity eventually tops when the spikes have reached the pre-stimulation level of amplitude. It would appear from these experiments that one of the characteristics in seizures of the "epileptic" neuronal pool is actually this slow, long-lasting process of repolarization. This study has been completed and a paper has been submitted and accepted for publication.

Project 44[illegible] is a new project somewhat related to Project 390. It deals with [illegible] and specifically, with centrifugal influences in sensory mechanisms. In this study an attempt was made to investigate whether and with which modalities, a primary receiving cortical area or a nearby cortical association region may control the arrival of sensory messages at the [illegible] relay thalamic nucleus. Interesting reciprocal inhibitory and facilitatory effects were found to take place, at the level of the lateral geniculate nucleus, upon unitary spikes elicited by either photic or optic tract stimulation as well as by cortical stimulation. This project has been terminated and the various results are now in the process of being analyzed; they should be ready for publication in the near future.

Another purely experimental study of Project 42C . This also deals with thalamocortical mechanisms. It involves a systematic cortical microelectrode study of the three main systems (specific, association and diffuse or non-specific), and has the purpose of analyzing and emphasizing the main functional differences existing among them. The large number of data collected out of more than 50 cats employed for this project are presently being in the process of elaboration and though it is not yet possible to outline here any definite conclusions, the analysis of the results should be ready before the end of the year.

A small project (8C, 1958) is not mentioned among those undertaken in 1959. This concerns the preparation of a complete bibliographical list of papers dealing with electroencephalography and Clinical Neurophysiology for the last ten years (1949-1958). This project was, however, continued in cooperation with Dr. C. Henry of the Institute of Living (Hartford, Conn. in spare time; it progresses slowly but should be completed during the next year.

The Chief of the Branch took part in the colloquium on "Teaching methods in Electroencephalography" organized by the Committee on Education and Training of the American EEG Society in Atlantic City, June 1959. A paper was presented on the "Method of interpretation of the EEG of adults", as well as a second brief paper (invited discussion on "EEG Instrumentation and Technique"). Following an official invitation, a lecture was also given at the Montreal Neurological Institute in October 1959 on the subject "In Neurology this interests me most".

The following papers related to research projects outlined and considered completed in previous annual reports have appeared in their final form in this calendar year:

1. Enomoto, T. F. and Ajmone Marsan, C.: Epileptic Activation of Single Cortical Neurons and their Relationship with Electroencephalographic Discharges. EEG Clin. Neurophysiol. 1959, 11: 199-218.

2. Enomoto, T. F.: Unilateral activation of the non-specific thalamic system and bilateral cortical responses. EEG Clin. Neurophysiol. 1959, 11: 219-232.

3. Long, R. G.: Modification of sensory mechanisms by subcortical structures. J. Neurophysiol. 1959, 22: 412-427.

As indicated by the names of the investigators in the various research projects, a considerable contribution to the activity and research of the Branch has been provided by scientists temporarily affiliated with the Branch for training purposes or for active cooperation or original investigation. During 1959 one visiting scientist, one guest worker, one research

associate and three clinical associates were the appreciated collaborators in either routine diagnostic service of the Branch or its research activity. Their enthusiastic participation and the outstanding contribution of some of these investigators deserves more than simple acknowledgement. It is through them as well as through the anonymous but quite efficient daily help of the technical and secretarial staff that the functioning of the Branch was made possible.

As in the previous years the Clinical Director has provided continuous constructive cooperation, useful suggestions and invaluable help wherever requested; for this, the Chief of the Branch wishes to express his most sincere appreciation.

Serial No. NINDB-36(C)
1. Electroencephalography and Clin. Neurophysiology
2. EEG
3. Bethesda, Maryland
4. Continuation of 80C, 1956, 78C, 1957 and 1C, 1958

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: A Seizure Atlas (Clinical-Electroencephalographic Correlations).

Principal Investigator: Cosimo Ajmone Marsan

Other Investigators: Kristof Abraham

Cooperating Units: None

Man Years (calendar year 1959):
Total: 2.0
Professional: 0.4
Other: 1.6

Project Description:

Objectives: Outlined in the title and described in previous reports (78C, 1957).

Methods employed: Described in previous reports (1C, 1958).

Major findings: Out of over 250 epileptic seizures of different types observed from beginning to end and recorded photographically and by continuous dictation on tape, 43 have been selected and mounted in an Atlas form. Each seizure consists of from one up to four large plates and the Atlas contains a total of 91 plates. In each plate EEG tracings and synchronized corresponding photographies appear, correlated by a detailed description and comments of both clinical and electroencephalographic features. All the various clinical signs were analyzed in detail and separated into different groups. A number of interesting observations are discussed. These include coexistence and synchrony of both clinical and electrographic changes; presence of clinical changes in the absence of EEG modifications; definite EEG activation in the absence of visible clinical features; various possible EEG patterns accompanying loss of consciousness; focal movements of

Part B included Yes [x] No [ ]

body. [illegible] various types of [illegible] electrographic changes; several seizures observed in the [illegible] electrographic correlates when using chronically implanted electrodes, etc.

Significance to the program of the Institute: The project is related to, and is an important part of the general study of diagnostic, pathogenetic and therapeutical aspects of epilepsy, and in particular of temporal lobe seizures. It fulfills, in addition, the need for a clear monographic demonstration of the various clinical patterns which the different epileptic attacks originating in different cortical regions may assume. It is, finally, an attempt to correlate the modifications of the electrical activity of the brain with the numerous, multiform motor aspects of clinical behavior and a complement to the monograph published in 1957 by one of the authors on the localizing significance of the various clinical manifestations in the different types of epilepsy (see project 780, 1957).

Proposed course of the project: The project in itself is over. The monograph has been accepted for publication and will appear shortly as Supplement #15 of the Journal of Electroencephalography and Clinical Neurophysiology. The study is, however, carried on for practical purposes (in cooperation with the Branch of Neurological Surgery) as an adjunct of the various screening and diagnostic procedures in cases of patients considered as possible candidates for surgical treatment of seizures.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Ajmone Marsan, C. and Abraham, K.: A Seizure Atlas (Clinical-Electroencephalographic Correlations). Supplement #15, EEG Clin. Neurophysiol. In press.

Honors and Awards relating to this project:

None

ORP-2

Serial No. NINDB-37(C)
1. Electroencephalography and Clin. Neurophysiology
2. EEG
3. Bethesda, Maryland
4. Continuation of 81C, 1956 79C, 1957 and 2C, 1958

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Depth electrography in epileptic patients.

Principal Investigator: Cosimo Ajmone Marsan

Other Investigators: John Van Buren

Cooperating Units: Branch of Neurological Surgery

Man Years (calendar year 1959):

Total:	1.8
Professional:	0.2
Other:	1.6

Project Description:

Objectives: See previous reports.

Major findings and proposed course of the project: New data collected since the last report are still in the process of analysis and elaboration. In the meantime collection of further cases is contemplated in view of the fact that patients suitable for this type of study are rather rare.

Part B included Yes ☐ No ☒

Serial No. NINDB-38(C)
1. Electroencephalography and Clin. Neurophysiology
2. EEG
3. Bethesda, Maryland
4. Continuation of 79C, 1956, 84C, 1957, 3C, 1958

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Electrocorticographic studies in temporal lobe epilepsy and in focal cerebral seizures.

Principal Investigator: Cosimo Ajmone Marsan

Other Investigators: Maitland Baldrin

Cooperating Units: Branch of Neurological Surgery

Man Years (calendar year 1959):
Total: 0
Professional: 0
Other: 0

Project Description:

Objectives: See previous report (84C, 1957).

Major findings and proposed course of the project: New data collected since the last report have still to be analyzed. The project continues with addition of new cases.

Part B included Yes ☐ No ☒

ORP-2

Serial No. NINDB-39(C)
1. Electroencephalography and Clin. Neurophysiolo
2. EEG
3. Bethesda, Maryland
4. Continuation of 5C, 195

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Unitary analysis of the response elicited in the visual cortex of cat.

Principal Investigators: Lennart Widen and Cosimo Ajmone Marsan

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1959):

Total:	3.7
Professional:	0.7
Other:	3.0

Project Description:

Objectives: To study temporal and spatial distribution of unit activity within the visual cortex and underlying white matter during the course of the evoked surface response elicited by stimulation of the optic radiations, and to analyze the functional characteristics of the unitary elements in relation to the various phases of the surface response. The purpose is to understand better the nature of the various components of the 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 and it is a useful tool lending itself to the analysis of basic properties of the specific projection systems.

Part B included Yes [x] No [ ]

Methods employed: Described in project 5C, 1958.

Major findings:

1. The majority of spikes recorded, discharge during the course of the specific evoked surface response and may be temporally related to its various components.

2. On the basis of latency and of their behavior when tested with double shocks and repetitive stimulation, all spikes may be divided into two groups: "presynaptic" and "postsynaptic".

3. All spikes temporally related to the first wave, and almost all spikes firing during wave 2 are recorded from white matter and all have presynaptic characteristics. Those temporally related to wave 3 have the most extensive spatial distribution being picked up in both cortex and white matter: some behave as presynaptic, others as postsynaptic events. The spikes related to wave 4 and the rare ones related to wave 5 and all of the postsynaptic type and most of them are only found within the cortex.

4. A positive correlation exists between presence of spike and amplitude of the surface response. This can be expressed as follows: first or second wave spikes appear when waves 1 or 2 have reached a critical size. The spikes then remain stable responding to every stimulus. A third wave spike of a presynaptic type behaves similarly. Spikes of postsynaptic type related to waves 3, 4 or 5 also may appear when the first wave has reached a certain size; above this threshold value, however, they are "unstable" responding irregularly to stimuli of constant strength. Their probability of discharge is greater the larger the amplitude of the late surface wave.

5. These findings provide further confirmation of some of the current concepts of the nature of the various components of the "visual" response. They also offer an explanation of the difference of opinion still existing in regard to the nature of wave 3, which is considered as presynaptic by some, and postsynaptic by other investigators. On the basis of our results, both pre- and postsynaptic elements are present in wave 3.

Significance to the program of the Institute: This project emphasizes the relationships between "slow" cortical events and single cell activity. In particular, it deals with the specific projection system between thalamus and cortex which are of unquestionable interest, and in view of the fact that far-reaching conclusions concerning their organization has been built upon the functional substrate of the evoked response, the above-described investigation appears more than justified.

Proposed course of the project: This project has been terminated: preliminary results were presented at the March meeting of the Eastern Association of Electroencephalographers. The final paper has been sent and accepted for publication.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Widen, L. and Ajmone Marsan, C.: Unitary analysis of the response elicited in the visual cortex of cat. Archives Ital. Biol. 1960. In press.

ORP-2

Serial No. NIMDB-40(C)
1. Electroencephalography and Clin. Neurophys.
2. EEG
3. Bethesda, Maryland
4. New

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Microelectrode investigations of the mechanisms of the electrically induced epileptiform seizure ("afterdischarge").

Principal Investigator: Paul Gerin

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1959):
Total: 1.0
Professional: 1.0
Other: 0

Project Description:

Objectives: To explore systematically by means of microelectrodes the cellular phenomena characterizing the onset, development and end of the electrical afterdischarge elicitable by repetitive stimulation of the cortical surface and to attempt an interpretation of the mechanisms at the basis of this type of self-sustained activity.

Methods employed: Acute experiments were performed on cats. Electrical stimulation was applied on the cortex of the suprasylvian gyrus with variable parameters. Pick-up cortical electrodes were silver-silver chloride for the slow surface events and tungsten microelectrodes, the movement of the latter being controlled by a hydraulic micromanipulator. Recording was carried out during the stimulation and following it. A total of 2500 afterdischarges were elicited and of these 600 were recorded on film with a detailed analysis of 80 different units activated during their development.

Part B included Yes [X] No [ ]

Major findings:

1. Repetitive stimulation, capable of eliciting afterdischarge, is accompanied by progressive developing and characteristic changes in the spikes activated by each electric pulse. Units tend to fire repetitively and the amplitude of each successive spike decreases until they eventually might disappear.

2. In the absence of such changes, the development of an afterdischarge is highly improbable.

3. During the EEG afterdischarge, unit spikes generally absent at the very beginning, progressively appear: their voltage is at first low and their frequency high, then the rate of firing slows down and their amplitude reaches the pre-stimulation value at which point the afterdischarge ends. The spike changes have been interpreted as the expression of different membrane polarization levels, the characteristic ones consisting of an excess of depolarization and of longlasting repolarization process.

Significance to the program of the Institute: This is an experimental approach to the problem of epilepsy. The electrical afterdischarge elicitable with repetitive stimulation is morphologically indistinguishable from a spontaneous electrographic attack occurring in humans affected by epilepsy and recorded from their exposed cortical surface. The understanding of the intimate mechanism at the basis of this particular type of self-sustained activity is an important step for the understanding of the physiopathology of epilepsy.

Proposed course of the project: This project has been completed. Results were presented in June at the Atlantic City meeting of the American EEG Society. The final paper has been sent and accepted for publication.

Serial No. NIMDB-40(C)

PHS-431H
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Gerard, Paul: Microelectrode investigations on the mechanisms of the electrically induced epileptiform seizure ("afterdischarge"). Archives Ital. Biol. 1960, in press.

Honors and Awards relating to this project.

NONE

ORP-2

Serial No. MINDB-41(C)
1. Electroencephalography and Clin. Neurophysiology
2. EEG
3. Bethesda, Maryland
4. New

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Effects of corticofugal and corticopetal impulses upon single elements of the lateral geniculate nucleus.

Principal Investigator: Lennart Widen and Cosimo Ajmone Marsan

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1959):
Total: 1.7
Professional: 0.7
Other: 1.0

Project Description:

Objectives: This study was undertaken in an attempt to investigate whether specific or association cortical areas may exert any effect upon the incoming sensory messages at a thalamic level and specifically upon units activated within a sensory relay nucleus. Visual and suprasylvian (association) cortex and lateral geniculate nucleus were the regions respectively selected for this investigation. The interest in this project was aroused by anatomical work, stating that in the visual cortex about 3/4ths of the fibers are corticofugal, although their actual destination is presently unknown.

Methods employed: The experiments were performed on unanesthetized "cerveau isole" preparation) cats. Tungsten microelectrodes were

Part B included Yes [ ] No [x]

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 ipsilateral optic tract and of various points of the visual and suprasylvian cortex was carried out. Photic stimulation was also used (single flashes of light of progressively increasing intensity).

Major findings: These are still in the process of being analyzed in detail. A fairly large percentage of about 200 unitary spikes recorded from the region of the lateral geniculate nucleus 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. It was found that different types of effect could be obtained. In general, cortical stimulation tends to inhibit the spikes elicited by optic tract stimulation and stimulation of the optic tract tends to facilitate the spikes elicitable by cortical stimulation. Examples with opposite effects (facilitatory action of cortex and inhibitory action of optic tracts) were found, as well as rare instances in which both stimuli would have a reciprocal facilitatory or inhibitory effect. Some of the cortical effects are very likely interpretable as the result of antidromic stimulation, however, the extremely long latency of many spikes elicited by cortical stimulation strongly suggests that these be ortodromically activated elements.

Significance to the program of the Institute: This project deals with the investigation of the mechanisms underlying sensory perception. Recently there has been a large number of experimental results indicating the existence of important corticofugal influences upon the non-specific reticular formation and emphasizing the role of the formation in exerting a control on the different afferent messages at that level therefore providing some indirect information to our understanding of problems related to arousal and consciousness in general. It is, however, in the neurophysiological study of the reciprocal relationships between cortex and specific thalamic nuclei which one must look for the integrating mechanisms of the perceptual data which form the unity of the actual vigilant consciousness.

Proposed course of the project: The experimental part has been completed and we are now in the process of analyzing the various data and preparing the presentation of results in a publishable form.

ORP-2

Serial No. NINDB-42(C)
1. Electroencephalography and Clin. Neurophysiology
2. EEG
3. Bethesda, Maryland
4. New

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Thalamocortical mechanisms. A comparison between specific, association and non-specific systems.

Principal Investigator: Arturo Morillo

Other Investigators: Cosimo Ajmone Marsan

Cooperating Units: None

Man Years (calendar year 1959):
Total: 1.0
Professional: 1.0
Other: 0

Project Description:

Objectives: Some of the neurophysiological characteristics of these three functionally different systems are relatively well known. The purpose of this project is to extend the knowledge we already possess and gather new information about the differential behavior in the three thalamocortical sectors, by means of unitary analysis within the cerebral cortex upon stimulation of different thalamic nuclei. Possible functional interrelationship between these three systems were also investigated.

Methods employed: Experiments were performed on cats partly under barbiturate anesthesia and partly unanesthetized (cervoum isole" preparation). The studies were carried out on the suprasylvian (association) cortex, as well as on the primary visual cortex. Within the thalamus the following nuclei were specifically stimulated: lateral geniculate nucleus, nucleus lateralis posterior, and nuclei belonging to the non-specific system (centralis lateralis, centre medianum, ventralis

Part B included Yes [7] No [X]

anterior, etc.). Systematic survey through the cortical depth of the two abovementioned areas was carried out with tungsten microelectrodes controlled by a hydraulic micromanipulator. Surface cortical recording was also employed to monitor the gross surface (evoked) response.

Major findings: The various findings obtained in over 50 cats are still in the process of being analyzed in detail. Observations concerning differences in latency, variability in latency and changes of unit activation by different stimuli, inter-effects between the different stimulations upon the same unitary element, etc., were made and from a preliminary analysis the results appear rather interesting.

Proposed course of the project: Completing the analysis and carrying out, if necessary, further experiments before drawing final conclusions.

Significance to the program of the Institute: This type of investigation could be useful not only for elucidating the intimate mechanisms of thalamocortical relay systems and problems related to perception and control of cortical activity, as well as for general problems connected with arousal, attention and sensory elaborations but could also provide some clue for the understanding of certain types of "focal" epileptogenic lesions which may actually involve sectors, including both cortex and thalamus ("arecthalamic" concept of Gastaut).

Annual Report
Surgical Neurology Branch
National Institute of Neurological
Diseases and Blindness

November, 1959

SUMMARY

During the period of this report, 231 patients were studied on the wards, while in the Outpatient Department, 27[illegible] patients were examined in a total of 394 scheduled visits. In the neurosurgical operating room, 108 major procedures were completed. Of these, 50 were concerned entirely with the surgical treatment and investigation of epileptic mechanisms, 48 were performed for space-occupying lesions which came to operation through investigation of epileptic patients or patients referred from other programs. The[illegible]e 10 miscellaneous major procedures. These were performed for t[illegible]ef of pain. This report is based on information drawn from the investigations in ward and clinic, as well as relevant laboratory work. Thirty reports were prepared for publication during this year.

The majority of the patients studied suffered from temporal lobe epilepsy. Some cases of focal epilepsy originating elsewhere in the brain were studied, and a small group of centrencephalic seizures were also investigated. Twelve patients referred from the National Cancer Institute were studied and eventually treated by either hypophysectomy or stalk section. This small group of patients provided a source of considerable information on relevant endocrine problems, as well as physiology of the orbital frontal cortex.

The etiology of temporal lobe epilepsy has been further studied. In this year, several patients with temporal lobe epilepsy came to operative treatment without clear evidence for the precipitating cause of the epileptogenic lesions. In these cases, a bony deformity of the middle fossa was discovered. This was not apparent on pre-operative or retrospective postoperative examination of the plain films. The deformity consisted essentially of a stalactite formation of bone. Several spicules protruded through the dura into the brain. The peculiar combination of bony mass, dural defect, and cortical lesion was related to electrographic abnormality, as well as pathological changes in the underlying brain. The cause of the bony deformity is not clear. In some cases, there was a history of injury to the head, but this did not appear significant. In others, there was no reliable clue as to cause-effect relationships. The bony spicules are not metastatic and do not constitute evidence of osteoma. Nor was there evidence of acute or chronic inflammatory change. In these cases no other etiological factor was evident. These cases may be compared to those mentioned in the previous Annual Report, in each of which a vascular malformation was discovered at operation, being undisclosed by pre-operative investigations which included arteriographic contrast studies.

On the ward, the ictal, interictal, and postictal behavior of the epileptic patients formed the basis for the majority of observations.

In addition, several problems of intractable pain were investigated and the application of urea to abnormal intracranial pressure was continued. Several cases of congenital defects of the brain were studied, and all the patients concerned form the basis of social and psychological studies within the framework of the ward environment.

Automatism was once more a principal target of investigation. The seizure observation program developed by Miss Pirnie and carried on by Mrs. Thompson, has provided some 2,000 photographs of patients as they appeared during this peculiar fractionation of consciousness. Each patient is being subjected to a series of stimuli during the period of his automatism, while it is photographed and noted. His response to pain, pressure, heat, cold, touch, noise, and light was cataloged. The relationship of his movements to the spatial characteristics of the environment and to these stimuli is also noted. These patients do respond to pain by withdrawal or by combination of withdrawal and semi-purposeful movement. Pain will interrupt the repetitive pattern of movements which seems to characterize the outward expression of this peculiar state. That interruption is short-lived, however, and the state cannot be interrupted by touch, ordinary pressure, heat, or cold. The patient will turn his head towards a sharp noise and his pupils dilate at the sound. His pupils do respond to light individually and consensually. Insofar as examination is possible, the reflexes have been tested occasionally during these automatic states. Apparently, no abnormal reflexes have been elicited and the deep tendon reflexes are not markedly different in response during this state. Since it has been known for some time that automatism and speech occasionally coincide, samples of such speech have been recorded and the patient, in turn, has been subjected to stereotyped language stimuli. The response to this complex stimulus pattern is variable and apparently irrelevant. Dr. Adamkiewicz has begun a systematic study of this fascinating condition. His study begins with a photographic comparison between the actual clinical state as recorded from a patient, and the imitation of this state as portrayed photographically by one of the medical staff quite familiar with its characteristics. All observers agree that they can tell the occurrence of such a state by 'looking at' the subject's face, but no observer has been able to say what facial characteristics indicate either the beginning or the continuation. Conversely, all observers agree that they can tell when the condition is over by observing the patient's face, and yet there is no agreement as to the relevant facial changes. These changes are the object of a search continuing in the present. In addition, Dr. Adamkiewicz is studying changes in vital signs and skin resistance, the observation of which may support or otherwise relate to the systematic study of the autonomic concomitants of automatism conducted by Dr. John Van Buren. Dr. Laskowski is engaged in the study of the relationship of handedness to the occurrence of automatism and its particular characteristics in the individual case. He is studying lateralization of speech and relevant cerebral dominance in these patients by means of the Wada test. Dr. Otenasek is assisting him with this study. In addition, Dr. Adamkiewicz is studying the problem of unusual bleeding in patients coming to craniotomy, while Dr. Meecubbin has begun an intensive review of the results of treatment

of temporal lobe epilepsy during the past 5 years. In addition to the statistical and systematic aspects of this study, he is studying the relationship between area of ablation and subsequent clinical findings, as well as between area of ablation and subsequent electrographic characteristics. He will, in turn, relate wherever possible the pathological lesion as identified in the laboratory to the clinical characteristics of the case. In addition, his study will incorporate information derived from the routine investigations of physical description, language characteristics, and studies of social interaction. Mrs. Thompson has continued the observation of physical characteristics, including photographs, the language sampling, and the basis for social studies begun by Miss Pirnie.

In the neurosurgical operating room, the studies of airborne infection continue. Miss Lewis is conducting these, in cooperation with Dr. Herman and other members of Mr. Snow's staff. Various aseptic techniques are being tested and are under study at present. The use of special clothing, masks, and development of traffic patterns more suitable for reduction of airborne infection are being developed and tested. New and more effective methods of cleaning are also under study. Many of these ideas have been incorporated by Dr. Laskowski into a report which is being studied by the Surgical Administrative Committee of NIH. Miss Lewis has completed her Procedure Manual for Neurosurgical Nurses. This embodies descriptions of the various special and general techniques, as well as instrumentation and its particular usage. Dr. Van Buren has completed the development of a stereotaxic instrument and is now preparing it for use on patients in the operating room. In cooperation with Dr. Clarence Hebert and staff, the use of urea in conjunction with hypothermia has been developed and is now a useful and successful tool in the treatment of increase of intracranial pressure during operation, as well as in the protection of the brain which must be handled during surgery.

The design problems arising from the development of the new Neurosurgical Suite have occupied many hours. The ground for the building which will house this suite has been broken and before this the final plans committed to the hands of the architects. The suite will constitute a new departure in operative design for neurosurgical procedures.

There was some progress in the laboratory, as well. A study which effectively bridged the artificial gap between laboratory and clinic was Dr. Van Buren's analysis of autonomic concomitants of automatism. This study, based on the ward, was conducted in part in the Laboratory of Electroencephalography in cooperation with Dr. Marsan. In addition, and with much the same approach, these investigators and Dr. Allen Mirsky studied the effect of centrencephalic discharge on the patient's response to the standard test situation, as well as the autonomic changes which occurred during the discharge.

In Neurosurgical Anatomy, Dr. Van Buren has studied the connections of the human temporal lobe with Dr. Paul Yakovlev, as well as the

pathological anatomy subsequent to a tumor in the parietal region, a suprasellar meningioma, and temporal destruction due to an extracerebral neoplasm. These studies were controlled by comparison with 5 "normal" brains subjected to similar processes of imbedding and sectioning. His studies have provided physiological, as well as anatomical information. He has studied the electrographic activity of the cooled human frontal lobe and its response to hypotension, as well as the autonomic responses subsequent to electrical stimulation of the orbital frontal cortex. These observations were made during the course of hypophysectomy or stalk section for metastatic cancer. These operations provided anatomical material which permits a quantitative evaluation of hypothalamic and hypophysial tissue. They also provided opportunity for study of the endocrine effects of stalk section and hypophysectomy which were carried out in conjunction with the Cancer Institute. In addition, Dr. Van Buren has continued his study of the human visual system and has developed a satisfactory method for two-dimensional retinal reconstruction. Similarly, he has continued his anatomical studies which form the background for the future clinical investigation of involuntary movements.

In Neurosurgical Physiology, Dr. Li has studied the cortical intracellular potentials in epileptic cortex, as well as the response of similar potentials to a single cortical stimulus. In a further investigation of cerebral cortex, he studied inhibitory neurons and synaptic activation of nerve cells in the primary visual area. Also, in conjunction with Dr. Igor Klatzo, he is also investigating electrical activity of various cellular elements which can be studied in Dr. Klatzo's tissue culture apparatus.

In the Laboratory of Neuropathology, Dr. Klatzo has begun a study of morphological changes due to radio frequency energy. He is also studying the pinocytosis and uptake of proteins and neutral red dye in vitro. In conjunction with Dr. Engel, he has studied the histochemical and electrophysiological characteristics of muscle fibers grown in vitro. In collaboration with Dr. Jaime Miquel, he has undertaken the quantitative study of precipitin reaction. Along with Dr. Laskowski, he has studied the effects of hypothermia on injured and normal brain tissue. He has also investigated the pathology of Kuru Disease and localization of myosin in human striated muscle with various investigators.

In Developmental Anatomy, Dr. Dekaban continues the study of etiology, pathology, and clinical manifestations of cerebral palsy. He has also begun a systematic study of epilepsy in childhood. The latter is, in fact, a collaborative project in which his Section and the Sections of Neurology and Electroencephalography of the Mayo Clinic are participating. He is continuing his production of congenital malformations of the central nervous system using mice as experimental animals. In the clinic, he is studying the incidence and type of central nervous system abnormalities in offspring born to diabetic mothers. Similarly, he is studying the products of abortion and their relationship to maternal condition during pregnancy and the course of birth.

The Laboratory of Clinical Psychology under the direction of Dr. Herbert Lansdell has added Mrs. Sylvia Jones (Audiologist) to the staff. Under the direction of Dr. Lansdell, the laboratory continues to analyze all test results obtained from patients suffering from epilepsy and various other neurological diseases. Dr. Lansdell has worked with Dr. Laskowski in a study of cerebral dominance and is also studying the verbal efficiency of patients with temporal lobe epilepsy.

The Laboratory of Neuroanesthesiology has been maintained through the efforts of Drs. William Pritchard, Charles Bucknam, and Shelly Chou. These investigators have continued the development of a technique by which the brain may be selectively cooled through shunting inflow blood through an appropriate apparatus. The outflow blood is similarly shunted to a warming apparatus. It is hoped that this method will be soon applicable to clinical trials and that it will provide an efficient experimental tool in the study of the effects of low temperature on primate brains in laboratory investigation. In this laboratory, Dr. Bucknam is also studying the effects of selective cooling of the cerebral spinal fluid on brain temperature and is beginning preparation for a study of selective cooling and/or heating of the hypothalamus. In conjunction with members of the Primate Neurology Laboratory, he has studied the effects of fluothane on chimpanzees with polygraphic recording devices.

In the Laboratory of Primate Neurology, Dr. Forbes Norris has been investigating the effects of nucleus amygdaliformis stimulation. He has implanted depth electrodes in the limbic system and superior brain stem. In each preparation, he averages 19 electrode placements. Action potentials were recorded from other electrodes during and after stimulation, while he made movies of the total effects, when present. The animals were awake and free of narcotic effects during this stimulation. He succeeded in evoking reactions similar to those seen in human automatism. These stereotyped reactions were concomitant with a spike-and-wave discharge recorded from the mesial temporal region. In addition, Dr. Norris has been investigating amygdalo-amygdala connections by conventional physiological techniques.

Dr. Bach, Miss Lewis, and Dr. Baldwin investigated the effects of radio frequency energy on the awake, unanesthetized rhesus monkey. Such energy developed from standard ground-to-air transmitter was conveyed to the monkey's head, which could be fixed in certain positions by a specially designed head-holder. The head and head-holder were contained in a resonating cavity of copper mesh, so designed as to permit both observation and photographic recording of the monkey's reactions. Electrographic studies were also carried out after standard placement of cerebral electrodes. Some animals were subjected to radio frequency and sacrificed immediately thereafter, or actually killed during exposure to radio frequency energy. Their brains were studied under ultraviolet light for immediate changes in blood brain barrier. The majority of animals were autopsied, after which brain and spinal

cord were subjected to conventional histological and histochemical techniques. Exposure to radio frequency energy within the range 200 to 400 Mc. at a standard output of 100 watts was undertaken. There were no clinically observable effects save at frequencies between 385 and 390 Mc. More effect was observed at 388 than at any other frequency. The clinical effects consist of drowsing, arousal, pupillary dilatation, disorganization of limb movements, vertical nystagmus, horizontal nystagmus, unilateral grimace, salivation, tachycardia, increased ventilation. In some animals there was a temperature rise as measured by rectum. This was trivial and unpredictable. Electrographic effects consist of development of a "sleep" record during radiation and return to a normal "waking" record thereafter. Application of critical frequencies will arouse an animal from deep surgical anesthesia during periods of exposure to radiation. Likewise, exposure to radiation will forestall the effects of heavy barbiturate sedation. The physical characteristics of the resonating cavity and the antennae of the target area within the cavity have been intensively studied. An experimental design consisting of a plastic phantom of known volume equating that of the monkey's head, and an amplitude signal recorder form the basis for these physical observations. Nineteen phantoms of different shapes and in various positions corresponding to the shapes and positions of the monkey's heads have been tested. There is a clear correlation between amplitude of signal, temperature rise of 5°C., and frequency of carrier wave. Carrier wave patterns from 200 to 400 Mc. tested under these conditions produce equal rises in temperature in the phantom system. Yet the clinical effects are only apparent in the narrow frequency range of 385 to 390. These results have recently been reported by Baldwin, Bach, and Lewis, and by Bach, Baldwin, and Lewis at the American Psychiatric Association meeting and the Tri-Service Microwave Conference. These reports were based on the study of 50 animals. Such reports form the basis for continuing studies based on a new (kilowatt) transmitter, physical capability for peak pulsing the energies and development of a signal generator system permitting application of radio frequency energy to single cell systems in tissue culture.

The effect of ablation on the communication pattern of the chimpanzee has been studied. Three animals have been subjected to unilateral and then bilateral ablation of the area usually described as Broca's area in the human. Such ablations were preceded by electrocorticography and a sequential stimulation of the precentral and postcentral and other relevant cortical areas. The ablations did not significantly alter the communication pattern of the subjects. In other chimpanzees, the entire cortical convexity has been exposed at a single operation. Sequential electrical stimulation of the exposure has been undertaken and the effects recorded. Then the area was surveyed with recording electrodes, and finally a frontal parasagittal penicillin focus was established. The effects of this focus on the electroencephalogram and as recorded by color photography have been noted. It may be that there is some relationship between a change in blood vessel color and the electrical spread of the seizure.

In further studies on the effects of hallucinogenic drugs, it seems that there is a substitutive tolerance between lysergic acid

and psilocin. The effects of lysergic acid on the chimpanzee decrease if the drug is administered each day for one week. The animal becomes resistant to it in the second week. If psilocin is substituted for lysergic acid in the first week, the animal is resistant to lysergic acid in the second week.

Miss Lewis has continued her observations of social hierarchy and communication in the chimpanzee colony. Dr. Bucknam and Miss Lewis have drawn blood which has been tested systematically by Dr. A. S. Wiener of the New York Medical Examiners Office. Dr. Wiener is now in the course of a study of sub-groups in these blood samples. It is hoped that these hematological investigations will lead to a further understanding of circulating blood in the chimpanzee, including clarification of their extremely efficient clotting mechanism.

Serial No. NINDB 43(c)
1. Surgical Neurology Branch
NEG Branch
2. Neuropathological Section
Primate Neurology Section
3. Bethesda, Maryland
4. NINDB-61(c) 1958

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Epileptogenic Mechanisms in the Brain of Man and Other Primates.

Principal Investigator: Maitland Baldwin, M. D.

Other Investigators: J. M. Van Buren, M. D., C. Ajamne Marsan, M. D. I. Klatzo, M. D., S. A. Lewis, R. N. and S. A. Bach, M. D.

Man Years (Calendar Year 1959):		Patient Days (Calendar Year 1959)
Total:	7.0	
Professional:	2.5	
Other:	4.5	4045

Project Description:

Objectives:

a. To study causal mechanisms of epileptic seizures in man and other primates.
b. To study the electrographic characteristics of epileptogenic activity in the brain of man and other primates.
c. To study the approved methods of surgical therapy for these lesions and develop new therapeutic methods.
d. To study brain function as it is exposed in the extravagent experiments devised by these lesions.

Methods Employed:

a. Clinical neurological examination.
b. Special radiographic examination.
c. Electrographic examination.
d. Electrocortigraphic examination.
e. Electrical stimulation of the lesion exposed at operation.
f. Selective isolation of the lesion at operation.
g. Photographic and sound recording.
h. Histological and chemical examination.

Major Findings: One hundred forty-three patients with temporal lobe seizures were studied during the past year. In those that came to operative treatment, a new etiological factor was uncovered, and a peculiar relationship between language difficulty and location of objective lesion in the affected lobe was noted. In 6 cases in which the etiology of the seizures was not clear before operation, a severe bony deformity of the middle fossa was uncovered during surgical exploration. This consisted essentially of bony spicules protruding into the overlying temporal lobe through relevant dural defects. There was a meningocortical cicatrix. This deformity could not be related to inflammation or neoplasm, although it may have been related to otherwise insignificant head injury in 2 cases. It was related to electrographic abnormality, and objective pathological changes in the relevant temporal lobe. In previous reports, the language difficulties of temporal lobe patients have been outlined and emphasized. During the past year, several cases did not exhibit such language difficulty, although it was quite clear that they suffered from temporal lobe epilepsy which was localized to one or the other temporal lobe. At operation, it was noted that in each case there was a small, well circumscribed objective lesion in or on the lateral temporal cortex. For example, in one such case, a tiny meningioma lay imbedded in the lateral temporal cortex. In this case, the man's language was direct, simple, and devoid of the characteristics associated with temporal lobe disease. On the other hand, a patient with a small congenital vascular malformation of the mesial temporal structures exhibited these language characteristics to a marked degree. He typifies an apparent relationship between location of lesion within the lobe and language difficulty. When the lesion is mesial and deep within the lobe, the language difficulty seems marked and characteristic. If the lesion is lateral and superficial in relationship to the affected lobe, the language difficulty is minimal or absent. In the case of the patient with the small meningioma, the seizures had begun 10 years before operation and continued until treatment without significant alteration in pattern or frequency. Likewise, the total duration of seizures in the patient with a mesial vascular malformation was approximately 10 to 12 years. Thus, in this small group, the duration of the lesion does not seem to significantly alter the occurrence of language difficulty.

The language of all patients with temporal lobe and other epilepsy is being studied. These studies are in addition to the formal psychological testing. Each patient must provide a 1,000-word sample of conversational speech which is recorded surreptitiously. He also provides a 1,000-word written account of himself and his home. It is hoped that these language samples, some of which are recorded on tape, can be subjected to further linguistic analysis by means of a panoramic analyzer At present, each patient is also tested by the common semantic method of object, word and action relationship. It is very difficult for a patient with temporal lobe disease to provide a rapid word association between

Major Findings (cont'd): such simple objects as chair or table. He can indicate a satisfactory response to standard aphasia tests with facility, but when he must use his language to describe relationships between space, time, and action, he usually becomes increasingly complicated in responses, and if pressed succumbs either to irritability and even rage, or unintelligible descriptions. The language difficulties are no more common in left handed than in right handed individuals, nor are they particularly relevant to ambidexterity. The cerebral dominance of these patients is being checked by use of the Wada test.

As has been previously mentioned, most patients with temporal lobe disease complain of difficulty in remembering or are labeled as having poor memories. In the past year, it has become increasingly clear that these patients have, in the most literal sense of the term, good memories. They do record and can recollect recent events. However, there is, of course, some difficulty in doing this. In the past, this has been described to a difficulty in recollection and nothing further has been said about it. Actually, it may be a difficulty in recollection, but on closer examination, it is also a difficulty in time relationships. For example, if a patient with mesial temporal lobe lesion is asked what he did yesterday at 9:00 o'clock in the morning, he finds it extremely difficult to answer with any degree of accuracy. He goes through normal association attempts and tries to "think backwards" in terms of the routine of the ward day, or in relationship to a special event of that day, such as a pneumoencephalogram or other diagnostic examination. Despite considerable effort and sincere motivation, he is usually unsuccessful in his answer. However, if he is asked about brushing his teeth or about any other routine series of actions which may have taken place around the time at question, he can build up a relatively accurate description of the morning's activities; Likewise, if he is asked about a particular object, such as a chair or table which he has seen during the past morning, he can build up a reasonably accurate account of his spatial experience during that time period. That is to say, he can describe his room as it appeared during those hours. Now, if he is asked specifically what the chair looked like during a certain time period, or if he is asked whether he brushed his teeth, ate his breakfast, etc. during this certain time interval, he does not answer accurately or he cannot answer at all.

The study of perceptual aberration in temporal lobe patients is continuing. When the lesion is mesial in the affected lobe, and particularly if its effects are bilateral, the patient has very considerable difficulty in describing the space in which he lives. He cannot relate objects within that space with any accuracy, nor can he describe its shape with any clarity. The patient who has lived in the ward room for 30 days and who has been able to undertake complicated psychological testing, read novels, newspapers, and conduct reasonable conversation throughout that period, finds it difficult to draw a simple floor plan of his room or to relate objects within it. If asked to relate the

Major Findings (cont'd): objects in space over a certain time, he [illegible] do this. However, other patients not [illegible] from temporal lobe epilepsy (for example, those whose epilepsy originates in parietal region or the so-called centrencephalic regions) can do this. As noted previously, patients whose auras are characterized by perceptual aberrations can be influenced by discussion of these auras. Thus, if the patient is asked to describe every detail of his aura to the best of his recollection and then is cross-examined on each detail, he will frequently begin an ictal pattern. Such perceptual auras are occasionally related to conceptual change, as for example in the case of the patient who began thinking about a phrase or sentence which occurred in random conversation or casual reading, and then perseverated on this thought, at the same time wondering what it meant, and then while aware of its passage through his mind lost the sense of its meaning. As he experienced this panorama of mental aberration, he was also aware that his surroundings seemed different and more sharply outlined, and then he was almost totally unaware of them save for one object--the book containing the phrase or the person who said the sentence. The book or the person was emphasized in his mind with alarming clarity and then he progressed into the pattern of his habitual ictus. Such a combination of perceptual and conceptual factors in the auras usually indicate progression to or primary involvement of the speech representations in the dominant temporal lobe.

During the past year, Miss Pirale, and more recently Mrs. Thompson have been studying the photographs of automatism sequences. There appears to be some relationship between the motor phenomena of these sequences and handedness. This is being studied in detail by Drs. Laskowski and Otenasek.

Dr. John Van Buren has studied a series of 22 seizures in 20 patients subject to seizure activity characterized by automatism and predominant temporal epileptogenic activity on EEG for possible correlations between vegetative and electrographic function. The seizures showed a remarkable consistency from patient to patient in that a given response tended to appear in a stereotyped fashion. Thus, if a change in blood pressure occurred, the blood pressure was seen to rise; if a change in pulse occurred the change was toward tachycardia with practically no exception. The two individual variations that could appear consisted in the inclusion or omission of an autonomic response or a change of sequence of appearance. In general, the results of the present examination agreed with that of the previous study (see bibliography) in that early changes were commonly esophogeal peristalsis and falls of skin resistance, with vascular changes and respiratory changes appearing somewhat later. In nearly all instances loss of response had been preceded by some autonomic change.

In correlating electrographic phenomena with these vegetative changes about half the bilaterally symmetrical "aspecific metrazol bursts" were associated with brief autonomic changes, usually tachycardia and fall of skin resistance, but were unaccompanied by any observable change in consciousness. Sudden loss of voltage with a tendency to increase in the frequency of the record (appearing over 1/5 sec. or less) was in all cases

Major Findings (cont'd): associated with overt seizure activity. usually in the form of an automatism or a tonic clonic seizure. Gradually, progressive increase in the area involved by epileptiform activity with increase in voltage posed a particular problem in that relationships to suddenly appearing autonomic changes or loss of responsiveness in most cases could not be made. Several instances were obtained of focal spike activity of sudden appearance or progressive augmentation without any demonstrable vegetative or clinical change.

A further study carried out with Dr. Allen Mirsky of NIMH was made in patients subject to three per second spike-and-wave attacks, either spontaneous or precipitated by photic stimulation or hyperventilation. The patients' level of response was determined by a continuous performance test (a motor response to certain letters presented either visually or by loud speaker). A simultaneous autonomic record was made of all these seizures. To date, some 300 spike-and-wave attacks have been fully recorded. The material is at present under analysis 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 include the infrequency of autonomic changes with the attacks and the ability of the patient to carry out a rhythmic monotonous mechanical movement during an attack when he is unable to distinguish auditory or visual symbols. The lack of relationship between autonomic features has been apparent on numerous occasions. The autonomic response of these attacks is much simpler than that of attacks of temporal lobe epilepsy. The nature of the change, however, is quite similar, affecting predominantly respiration (inhibition) and falls of skin resistance.

In the laboratory, the relationship of frontal cortex to spread of seizure discharge has been noted and further studied. A penicillin lesion has been produced on the frontal cortex of the chimpanzee and its subsequent spread recorded electrographically. Interestingly enough, the seizure spreads in an almost concentric pattern across the ipsilateral hemisphere and then seems to cross to the opposite side, spreading from mesial to lateral. Finally, electrodes record seizure activity from frontal, parietal, and occipital cortices. However, as might be expected, the discharge is greater in amplitude around the area of the lesion. Paradoxically, the animal suffered a contralateral focal motor seizure despite the bilaterality of the electrographic abnormality. (The penicillin lesion was in the intermediate frontal region and anterior to precentral cortex, which had been carefully marked out by electrical stimulation.) The animal suffered electrographically and clinically evident status epilepticus. The focal motor seizures continued as a Jacksonian march on the side opposite the lesion, while all electrodes continued to record seizure disturbance for 2 hours. Subsequently, when the wounds were closed and the craniotomy revised, the animal was conscious, but continued to have focal motor seizures. It was then necessary to give an anesthetic to control the seizure process. It is interesting that bilateral electrographic abnormality which began in one frontal cortex should result in a unilateral focal motor seizure pattern.

Significance to Neurological Research: These observations may

Significance to Neurological Research (cont'd): contribute towards further understanding of epileptic mechanisms as they occur in temporal lobes of higher pri

Proposed Course of the Project: The various clinical and experimental studies will be continued.

Part B included: Yes

Serial No. NINDB 43(c)

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Individual Project Report
Calendar Year 1959

Part B: Honors, Awards and Publications

Publications other than abstracts from this project:

Baldwin, M.: Surgery of epilepsy. In Jackson, I. J. and Thompson, R. K. (ed.): Pediatric Neurosurgery. Springfield, Chas. C. Thomas, 1959, Chap. XXI, pp. 512-529.

Edgar, R. and Baldwin, M.: Vascular malformations associated with temporal lobe epilepsy. J. Neurosurg. In press.

Pirnie, F. A. and Baldwin, M.: Observing cerebral seizures. Am. J. Nursing, 59: 366-369, 1959.

Van Buren, J. M.: Some autonomic concomitants of ictal automatism. Brain, 81: 505-528, 1958.

Honors and Awards relating to this project:

Major Findings (cont'd): obtained from postcentral, as well as precentral. As noted last year, the areas comparable to the representation described by Broca have been excised. In last year's report, the effects of unilateral excision have been described. Now it is possible to note that bilateral excision of these areas does not materially affect communication pattern. The chronic studies of effects of bilateral temporal lobe ablation are continuing. Dr. Heinrich Kluver has cooperated in these during the past year, and it is interesting that he was unable to see any of the stigma of his "syndrome" in these animals. There are now 6 chimpanzees whose bi-temporal ablations have been studied for 5 years. All of these animals have continued to compete in hierarchy and tribal structure, and their development appears within the norms established by Yerkes, Nissen, and Kohler. There seems to have been some weight gain in the past year in 2 of these animals. This weight gain is out of proportion to that of their peers who have not been subjected to such operations.

12 unilateral temporal lobectomies have been performed on patients. The general effects of such unilateral lobectomy in the human are still not clear. There is some change in mood and language. There may be change in libido and a progressive weight gain.

As noted elsewhere, Dr. Norris is studying the effects of mesial temporal depth electrodes. He has succeeded in reproducing behavior which is reminiscent of human automatism, along with or besides a panorama of other responses.

Miss Lewis and Dr. Baldwin are continuing the study of hallucinogenic substances. Psilocin and various isomers of lysergic acid are being tested. In addition, these drugs are being tested, one against the other. Such administration has revealed an apparent similarity between psilocin and LSD-25. Administration of LSD-25 daily for one week provides a considerable resistance to further administration. Likewise, daily administration of psilocin for 7 days provides a considerable resistance to further administration of lysergic acid.

Significance to Neurological Research: This project provides opportunity for study of the functional representations of the temporal lobe, as well as its structural relationship.

Proposed Course of the Project: This project is developing because of information derived from electrical, surgical, anatomical and biochemical studies. It will continue development from this wide base.

Part B included: Yes

Serial No. NINDB 44(c)

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards and Publications

Publications other than abstracts from this project:

Baldwin, M., Lewis, S. A., and Bach, S. A.: The effects of lysergic acid after cerebral ablation. Neurology, 9, No. 7: 469-474, 1959.

Honors and Awards relating to this project:

Serial No. NINDB 45(c)
1. Surgical Neurology Branch
2. Primate Neurology Section
3. Bethesda, Maryland
4. New

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Effect of Radio Frequency Energy on Primate Brain Mechanisms.

Principal Investigator: Maitland Baldwin, M. D.

Other Investigators: Sven A. Bach, M. D., Shirley A. Lewis, R. N., Igor Klatzo, M. D.

Man Years (Calendar Year 1959)		Patient Days (Calendar Year 1959)
Total:	7.0	
Professional:	2.5	
Other:	4.5	0

Project Description:

Objectives: To study the effects of radio frequency energy as applied to the heads of living monkeys and chimpanzees.

Methods Employed:

1. Electronic

a. Development of transmitter series of physical capabilities for applying the energy to the target areas.

b. Further research and development of electronic devices so as to produce pulsing and modulating which will permit rapid application of peak power free of carrier wave dependence.

c. Development of panoramic analyzers sufficient for frequency analysis and record of other wave characteristics.

d. Development of signal generator and connections capable of introducing critical frequencies without minimal thermal energies into tissue culture medium.

e. Development of remote recording devices capable of telemetering electrographic outputs activated in an experimental animal by photic driving.

Methods Employed (cont'd):

f. Orthodox electrographic recording of the effects of radio frequency.

2. Photographic recording of clinical effects.

3. Histological and chemical examination techniques.

Major Findings: 50 Macaca rhesus monkeys have been exposed to radio frequency energies in the range of 200 to 400 Mc. These energies were transmitted to the head from a standard ground-to-air transmitter through the means of an antenna placed in the roof of a cylindrical copper mesh resonating cavity. The antenna was adjusted so as 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. The physical characteristics of this array have been monitored directly and studied indirectly. Nineteen phantoms containing Elliot's solution, gelatin, Ringer's saline and other suitable test media were positioned so as to imitate critical head positions. The amplitude of the signal received at the head area was monitored by a probe, and the signal at a given frequency was continued long enough to raise the temperature of the test medium 5°C. Frequencies from 200 to 400 Mc. were used. The results indicate that there is a uniform thermal response in this test medium which occurs some time after the signal has begun. However, only those frequencies between 380 and 390 Mc. produce clinically discernible effects. These effects consist of sinusoidal alteration in awareness in which periods of seeming alertness alternate with periods of drowsiness. During this time, the animal does not respond to pain, but will respond to light and noise in the usual manner. None of these animals were anesthetized or narcotized in any way in the basic experiments. However, some were anesthetized with pentobarbital to surgical levels so as to test the effects of radio frequency on this depressed state. At the critical frequencies, the animal arouses from the anesthesia while the radio frequency is on and then returns to the anesthetic coma when the radio frequency is turned off. This appears as an "on-off" relationship. If the radio frequency is continued at critical frequencies (380-390 Mc.) the period of sinusoidal awareness, which is brief, is followed by alteration in pupillary response, paradoxical pupillary response, disorganization of eye movements, horizontal and vertical nystagmus, grimacing, autonomic changes, and finally a generalized seizure which usually begins with eye blinking and head nodding. If the animal is carried to this stage of reaction, recovery is unlikely and he usually dies. However, if the animal is exposed to radio frequency energies so as to produce any of the preceding signs or all of them, recovery is prompt and usual. Some animals develop focal neurological signs following long exposures at critical frequencies. A long exposure is

Major Findings (cont'd): here defined as an exposure longer than 3 minutes. The focal signs consisted of a facial paralysis of the central type, loss of pain and touch in the areas supplied by the first and second divisions of the right fifth nerve, weakness of one leg or of one arm, unilateral or bilateral plantar extension, trunkal ataxia sometimes accompanied by a continuation of previously developed nystagmus. These focal signs were transient and the animals recovered in periods varying from 4 hours to 48 hours after exposure.

It is possible to introduce radio frequency energy into a tissue culture bath by means of a signal generator and appropriate connections. The prototype for such a design has been developed and will be applied in the laboratory of Neuropathology. It will be used in order to test frequencies and specific frequency effects on unicellular elements as they are maintained in the now standard techniques available for this purpose. These elements will be photographed and subjected to various corollary tests. There is promise that radio frequency energies in these ranges has effects on the chromatin patterns. Some such effects have already been observed by Heller in the paramecia.

The histological and histochemical analysis of brains derived at postmortem examination is being conducted in the Neuropathology Laboratory and has been described in another portion of this Annual Report.

It is worth mentioning that the orthodox electrographic response to radio frequency energies in what we have called the critical frequencies is the production of a "sleep" record. When the radio frequency energy is turned off, the record reverts to a normal "alerted" tracing. There are autonomic changes, such as tachycardia, hyperpnea, and probably hypertension. No attempt has been made to record these on the polygraph in a systematic manner. Some of them have been recorded at random.

In summary, the major findings in this project are that frequencies within the range of 380 to 390 Mc., when applied to the head of a living, waking monkey, seem to cause alterations in awareness and other changes which may be related to stimulation or activation of brain stem mechanisms. The effects of this remote stimulation are transient when it is properly controlled. It has a paradoxical effect upon surgical anesthesia in that it seems to arouse the anesthetized animal.

Significance to Neurological Research: These findings suggest that there may be a frequency-specific effect when radio frequency energy of certain characteristics is applied to the primate brain. It is thought that radio frequency energies in this narrow band of the spectrum may alter neuromolecular structures within the individual neuron or glia so as to produce transient changes in the living animal. Further application of this new experimental tool may indicate its

Significance to Neurological Research (cont'd):

usefulness in the study of unicellular preparations. The results obtained indicate that it can affect the arousal systems and other brain stem mechanisms and yet leave the test animal apparently unharmed.

Proposed Course of the Project: The electronic capabilities will be enlarged. A kilowatt transmitter with capability for pulsing and modulation has been obtained and is being modified. The output of this transmitter will be calibrated in terms of the physiological test models previously used and then its output will be applied to the heads of monkeys and finally chimpanzees. If the effects observed and noted above are not species-linked and can be transferred to another species, it may be that the application of these frequencies will be more universal and effective. They will be applied to unicellular elements in the tissue culture system as noted above. The effects will be monitored wherever possible by orthodox recording techniques and a new telemetering and recording technique is now under development.

Part B included: Yes

Serial No. NINDB 45(c)

PSH-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards and Publications

Publications other than abstracts from this project:

Bach, S. A., Baldwin, M. and Lewis, S. A.: Some effects of ultra-high frequency energy on primate cerebral activity. Proceedings of Third Tri-Service Conference on Biological Effects of Microwave Energy. In press.

Baldwin, M., Bach, S. A., and Lewis, S. A.: The effects of certain radio frequency energy on primate cerebral activity. Neurology. In press.

Honors and Awards relating to this project:

Serial No. NINDB 46(c)
1. Surgical Neurology Branch
2.
3. Bethesda, Maryland
4. NINDB 63 (c) 1958

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project title: Effect of Destructive Lesions upon the Function and Structure of the Human Central Nervous System

Principal Investigator: J. M. Van Buren, M. D.

Other Investigators: None

Cooperating Units:

Man Years (Calendar Year 1959):

Total:	1.2
Professional:	0.4
Other:	0.8

Patient Days (Calendar Year 1959): 351

Project Description:

Objective: This study has a dual aim: (1) to carry out physiological-anatomical correlations in man, (2) to evaluate the effects of new methods of treatment of tumors of the central nervous system. In the present program attempt will be made to utilize the intrusion of disease upon the central nervous system of man as an "experimental" lesion.

Method Employed:

Specifically, the material is used in three major ways: (1) physiological observations can be made during surgery, (2) the effects of surgery itself can be evaluated, (3) post mortem material may, in certain cases, prove valuable for anatomical studies.

Major Findings:

A. Ablative pituitary surgery in man.

1. Clinical effects of hypophyseal stalk section in man.

The program of ablative pituitary surgery in conjunction with the Branch of Endocrinology, NCI, has been continued. A

total of eight pituitary stalk sections were carried out for palliation of metastatic breast carcinoma. Clinical experience with this operation has been discouraging. The patients have shown a considerable morbidity and at the present time, continuation of this series is not contemplated. Specifically, the patients demonstrated marked sensitivity to water intoxication with irregular and sporadic water outputs not well related to intake. In the first two cases where routine intravenous fluids were given (1500 to 2000 cc. daily) the patients became unconscious and both had convulsions apparently due to water intoxication. Following this experience, all parenteral fluids were stopped following the surgery and the patients allowed to take only what they desired by mouth. Despite this precaution, several patients between the fourth and seventh day became increasingly drowsy, poorly oriented and several hallucinated. Although this disturbance of water metabolism proved eventually reversible in all cases, the comparison of clinical course between these patients and those subjected to pituitary curettage as well as stalk section was a striking feature. In the latter case, the patient's recovery from operation was rapid. The evaluation of hormonal and tumoral response to hypophyseal stalk section as opposed to pituitary removal is still pending.

2. Quantitative evaluation of hypothalamic changes following hypophyseal stalk section in man.

Collection of post mortem material from the hypophysectomy cases continues and work has been undertaken to devise a suitable means for quantitative anatomical evaluation. Total cell estimations (cell counts) of the supraoptic nucleus may be easily carried out (in one case of hypophysectomy the left supraoptic nucleus contained 11,500 cells, the right 10,500 cells with a normal between 60,000 and 90,000 cells, according to Rasmussen). Quantitative estimations of cell population in the para ventricular nucleus, however, has to date not been satisfactory due to the confusion caused by a mixture of large cells from both the perifornical magnocellular nucleus and the adjacent large cells in the lateral hypothalamic area. Likewise, the characteristic cell form of the neurons seen in the supraoptic nucleus seems to be less well marked than the para ventricular nucleus. Further reconstruction work must be done in this direction and it is possible that a delimitation of this nucleus adequate for counting may be achieved by combined cytological and topographic relationships. Similarly, difficulty has been encountered in evaluating neurosecretion in the human post mortem hypothalamus and further experience is necessary to determine whether reliable results can be obtained by histochemical methods.

B. Physiological studies of the human fronto-orbital surface.

1. Autonomic responses.

In the past year, eight cases subjected to pituitary surgery were subjected to exploration of the orbital surface, adjacent temporal lobe and ventral aspect of the hypothalamus by electrical stimulation. Records were made at this time of respiration, blood pressure, pulse rate, esophogeal and gastric motility and plethysmogram. Since all patients were carried under general anesthesia, the influence of this upon the changes in the above functions were investigated. Fluothane anesthesia, even though apparently light, seemed to be attended by a large number of negative stimulations. More responses were obtained with light barbiturate, (thiopental anesthesia) and the majority of the examinations were carried out with this. The anesthetic appeared to abolish all gastric contractions and plethysmographic recordings have been unsatisfactory. The effect of stimulation at a given perimeter was most marked on the mesial temporal region when the stimulator was inserted backward along the medial aspect of the temporal lobe, so that it lay near the uncus. The characteristic response in this position was prolonged expiratory apnea which might last a total of two minutes. Bradycardia was also a common feature. Blood pressure changes were usually of slight degree, though frequently a fall was seen with bradycardia. In a few instances, an apparent motor response was seen with esophogeal peristalsis and in one case, the onset of a few gastric contractions. Curiously enough, no autonomic response has yet been seen from direct stimulation of the floor of the hypothalamus either anterior to or lateral to the hypophyseal stalk.

2. The electrographic activity of the cooled human frontal lobe and its response to hypotension.

In a study carried out with the aid of Doctors F. Norris, K. Hall, and C. Ajmone-Marsan, in twelve patients undergoing hypophysectomy with light anesthesia and artificial ventilation producing hypocapnia, the resting and post-stimulation electrocorticograms were recorded before and after reduction of the blood pressure with Arfonad (R). Nine of the 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 electrical stimulation while after-discharge with the same parameters appeared in one quarter of the stimulations at normothermic levels. In nearly

all patients, both hypothermic and normothermic, 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 Léão. In one case with miliary cortical carcinomatosis, spontaneous epileptiform activity was recorded which was potentiated by stimulation despite the presence of hypothermia. With light anesthesia, hypotension in both the normothermic and hypothermic cases induced a fall in the voltage of both the slow and fast activities, although the effect was of relatively minor degree even with systolic blood pressures in the range of 30 mm. Hg. In two cases the addition of Fluothane (R) resulted in greater depression of electrocorticographic activity with progression to intermittent activity and in one case, to electrical silence. In the latter case, recovery was uneventful. The depressant effect of hypotension upon cortical activity appeared separable from that induced by cortical stimulation and potentiated by deeper anesthesia.

C. Anatomical studies.

1. The connections of the human temporal lobe.

Two contrasting lesions of the human temporal lobe were studied by the use of whole brain serial sections and the template reconstruction method with Professor Paul Yakovlev at Harvard University.

The first case was a posterior lesion, an infarction resulting in destruction of the cortex of the posterior, superior temporal region and the area about the temporo-parieto-occipital junction which extended in depth to the mid-superior portion of the internal sagittal stratum. The second case was an anterior lesion resulting from a surgical amputation of the anterior temporal lobe.

The posterior lesion resulted in degeneration of the posterior portion of nucleus lateralis posterior and the nucleus medialis dorsalis. In the lateral geniculate body, the posterior lesion caused degeneration in the lateral half. In the medial geniculate body, the posterior lesion resulted in degeneration of the rostro-lateral portion of the nucleus while the anterior lesion caused degeneration in the caudomedial portion. Degeneration in the pulvinar from both lesions appeared to overlap in the medial and lateral subdivisions in the nucleus. With the anterior temporal lesion, degeneration was more pronounced in inferior parts of the lateral and the medial portions of the pulvinar and there was nearly complete destruction of the inferior portion of the pulvinar. With

the posterior lesion, there was greater degeneration in the medial nucleus of the pulvinar which extended farther superiorly than in the lateral portion. In this case, the inferior subdivisions of the pulvinar was spared.

Contrasting tract degeneration was seen in the tapetum. The anterior lesion causing degeneration in fibers passing anteriorly on the ventricular aspect of the splenium of the corpus callosum while the posterior lesion caused degeneration in the posterior portion of the splenium. Degeneration in the temporo-parieto-pontine tract was evident in both cases, although the myelin loss was more distinct in the case of the posterior lesion. The ventro-geniculate component in both cases but the tectal component passing to the brachium of the inferior colliculus was degenerated only in the anterior temporal lesion. As expected, the anterior temporal lesion caused degeneration of the inferior portion of the geniculo-calcarine radiation while the posterior lesion caused degeneration in the superior portion.

Tract degeneration peculiar to the anterior lesion included degeneration in the anterior commissure, uncinate fasciculus, ventral thalamic peduncle, and fimbria-fornix. The retention of cortico-medial portion of the amygdala was associated preservation of the stria terminalis despite near complete destruction of the baso-lateral portion of the amygdala.

2. Material collected for anatomical purposes.

Useful anatomical material for other studies has been obtained from a surgical excision of a metastatic tumor in the high parietal region with postoperative survival of six months, a suprasellar meningioma, and a local temporal destruction from an extracerebral tumor. In this conjunction, five "normal" human brains have been obtained and are in the process of embedding and sectioning in various planes for use as reference.

Significance to Neurological Research:

The present study with patients undergoing pituitary ablative surgery has provided further insight into the effects of derangements and deficiencies in this system.

The nearly unique opportunity afforded by hypophysectomy in exposing a normal human brain for physiological study is pointed out.

The importance of this project in providing valuable anatomical material for further human neuroanatomical study should be emphasized.

Proposed Course of Project:

Making use of experience gained to date, regarding quantitative estimation of damage in the hypothalamus following pituitary ablative surgery, quantitative correlations between function and structure are planned with the cooperation of Dr. Mortimer Lipsett of the Branch of Endocrinology, NCI.

The orbital surface stimulation study will continue to attain a wider case experience. In addition, the "activating" effect upon the EEG (Kaada) of stimulating the autonomically active areas on the basal surface of the brain will be studied (only two cases up to present report).

Work will be continued in the hypothalamic-anatomical studies to develop better technique for quantitative estimation.

Part B. Honors, Awards and Publications

Publications other than abstracts from this project:

Van Buren, J. M. and Yakovlev, P. I. Connections of the Temporal Lobe in Man. In press (Acta Anatomica).

Van Buren, J. M. and Bergenstal, D. M. An Evaluation of Graded Hypophysectomy in Man - A Quantitative Anatomical and Functional Study. In press (Acta Anatomica).

Van Buren, J. M., Norris, F. H., Hall, K. D. and Ajmone-Marsan, C. The Electrographic Activity of Cooled Human Frontal Lobe and its Response to Hypotension. In press (J. Neurosurgery).

Serial No. NINDB 47(c)
1. Surgical Neurology Branch
2.
3. Bethesda, Maryland
4. NINDB 64 (c) 1958

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project title: A Study of the Functional Anatomy and Pathology of the Human Visual System

Principal Investigator: J. M. Van Buren, M. D.

Other Investigators: None

Cooperating Units:

Man Years (Calendar Year 1959):

Total: 0.6
Professional: 0.2
Other: 0.4

Patient Days (Calendar Year 1959):

Project Description:

Objective: This study is directed toward a better understanding of the correlation of form and function within the human visual system. It is intended to carry out these studies correlating clinical physiology with anatomy using the best quantitative methods available. At the present time, the study has been divided into three parts:

(a) Retina

(1) Study of the normal human and primate retina using the Golgi and chromatic methods.

(2) Study of the effects of the lesions of the optic pathways upon the retina.

(b) Study of the effects of lesions of the optic pathways upon the lateral geniculate body.

(c) Study of the visual field defects following temporal lobectomy.

 Serial No. NINDB 47(c)

Method Employed:

The methods employed have been given in detail in report for Calendar Year 1957.

Major Findings:

The preliminary findings given in the report for the Calendar Year 1957 had indicated the practicality of undertaking these studies. Due to a still inadequate quantity of material, effort has been directed only toward collecting human material suitable for this research.

After considerable trial and error, a satisfactory method for two dimensional retinal reconstruction has finally been devised which is both simple and permits quantitative study. This entails the initial 15X photographic enlargement of the specimen. On this enlarged photograph, areas of degeneration can be marked in detail from microscopic examination. Following this, the original specimen can then be projected at 40 diameter magnification and although the cellular areas cannot be seen in detail at this power, the precise areas of degeneration could be marked from a noted photograph by comparison of general landmarks. Since the circumference of the closed segment of retina now (at 40X) measures something over a meter, tracings of this can be measured with a relatively small percentage error.

Significance to Neurological Research:

The general aims have been previously given in the 1957 calendar report. In brief, it is considered desirable to re-examine the visual system in man by anatomical reconstruction studies, which may be correlated with the clinical examination of the visual field.

Proposed Course of Project:

The primary aim continues to be that of collection of material.

Serial No. NINDB 48(c)
1. Surgical Neurology Branch
2.
3. Bethesda, Maryland
4. NINDB 65(c) 1958

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project title: Studies of Involuntary Movements

Principal Investigator: J. M. Van Buren, M. D.

Other Investigators: None

Cooperating Units:

Man Years (Calendar Year 1959):

Total: 1.2
Professional: 0.4
Other: 0.8

Patient Days (Calendar Year 1959):

Project Description:

Objective: At the present time, there is no adequate explanation of the cause of mechanism of production of involuntary movements. It is hoped by careful correlation of clinical findings, recording of the electrical activity of deeper structures at operation and study of the anatomical material may provide new information on this subject.

In order that recordings can be made from the basal ganglia and coagulation carried out here if indicated, a stereotaxic instrument is needed to guide the electrode. Since the available designs seemed inadequate in some respects, development of a new instrument has been undertaken.

Method Employed:

I. Clinical observation.
II. Photographic techniques.
III. Analysis by illustration of movement phases.

The possible use of accelerimeters for the graphic demonstration of the directional phases of involuntary movements is being investigated. Whether this will prove to be a practical recording technique remains to be determined.

Major Findings:

Over the past two years, it has been possible to obtain the brains of twelve cadavers. Studies in these cases have indicated that the changes made in the tilting mechanism for alignment of the horizontal axis of the apparatus with the anterior posterior line is effective and prevented the errors previously introduced.

After the previous year's experience (see report for Calendar Year 1958) all brains were fixed in 15% formalin without perfusion. Studies on weight and displacement changes over a two week period failed to show any marked variations. This was encouraging in that it indicated we were dealing with a brain little changed from its size in vivo. Subsequent discovery of other work on this matter has confirmed our opinion in this regard. All brains have been sectioned after agar embedding and photographed under a grid aligned with the anterior-posterior commissure line. Thus, 24 hemispheres are now available for comparison and form in each instance material from which precise measurements may be taken. Inquiry regarding individual variations in the relationship between surgically important structures and the anterior commissure has likewise been encouraging in these twelve specimens, since these variations have proved small when viewed in comparison to the magnitude of the therapeutic lesion. Two of the 24 hemispheres have been embedded for serial section in the sagittal plane in celloidin after marking of the transverse anterior and posterior commissural axis (as is done in the specimens embedded for section at 2 mm. intervals in agar). This material will permit better identification of tract and nuclear areas and will be available for comparison with the opposite hemisphere sectioned in agar (no shrinkage with latter embedding).

Design and construction in the past year has centered principally upon apparatus for depth stimulation and production of lesions. The difficulty of finding a thermostable insulating substance capable of withstanding sterilization has occasioned considerable loss of time and effort and as yet a satisfactory substance has not been found. Work in this regard will continue.

Visits have been made to clinics both in this country and abroad to observe both the therapeutic indications and operative techniques.

Significance to Neurological Research:

The present work has served simply to acquaint the principal investigator with the mechanical proficiency of the stereotaxic instrument and familiarize [illegible] with technical details. The ultimate course of the project is to study those diseases in which

stereotaxic intervention is indicated on therapeutic grounds. This material would fall largely in the group of involuntary movements.

Proposed Course of Project:

Photographic records both by moving picture and by multiple flash stroboscopic photographs, although providing a record, the patient's movements are bulky to handle and are difficult to analyze. It is hoped that with the use of accelerimeters a means may be found for simple graphic recording of the movement which could be correlated on the same time base as other features (EEG, autonomic, motor, etc.). During the course of therapy, studies will be made of the areas to be destroyed by depth electrode recording and stimulation.

Serial No. NINDB 48(c)

Part B. Honors, Awards and Publications

Publications other than abstracts from this project:

Van Buren, J. M. A Projection Apparatus for Human Neuroanatomical Reconstruction. In press (Anatomical Record).

Serial No. NINDB-49(c)
1. Surgical Neurology Branch
2. Primate Neurology
3. Bethesda, Maryland
4. New

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Some Aspects of Amygdalo-Amygdala Connections.

Principal Investigator: Dr. Forbes H. Morris, Jr.

Other Investigators: Harold Lemaire, M.S., Igor Klatzo, M.D.

Cooperating Units: None

Man Years (calendar year 1959):
Total: 0.5
Professional: 0.5
Other: 0.0

Patient Days (Calendar Year 1959)
0

Project Description:

Objectives: To study, by convential electrophysiological methods, some properties of amygdalo-amygdala projections, evidence for which was presented by a recent article from this laboratory (Frost et al 1958), as well as in another current project.

Methods Employed: Awake cats and monkeys with chronically implanted subcortical depth electrodes were studied. One electrode was stimulated, while others were recorded, until all possible combinations had been studied, including simultaneous or sequential stimulation of two electrodes. In addition to routine study of latencies, fatiguability, and thresholds, the effects of anesthesia and generalized hypothermia (24 to 26°C.) were studied.

Major Findings: Analysis of the data from cat has not been completed. The use of monkeys began only recently.

Significance to Neurological Research: This study attempts to localize amygdalo-amygdala properties to specific subdivisions of nucleus amygdaliformis. It also attempts to elucidate whether these connections are direct, mono-, or multi-synaptic and to provide an electrophysiological

confirmation of a finding in a parallel project, that facilitation and inhibition exist in this system. Such information might be of use in the problem of human temporal lobe epilepsy.

Proposed Course of the Project: Initial study of the data obtained in cat seems to support the assumptions made in originating this project. Analysis of this data will be completed, while actual experimentation continues with monkeys.

Part B included: Yes ☐ No ☒

Serial No. NINDB-50(c)
1. Surgical Neurology Branch
2. Primate Neurology
3. Bethesda, Maryland
4. New

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A:

Project Title: Effects of Nucleus Amygdaliformis Stimulation

Principal Investigator: Forbes H. Norris, Jr., M. D.

Other Investigators: Maitland Baldwin, M. D., Harold Lanauze, M.S., Igor Klatzo., M. D.

Cooperating Unit: None

Man Years (calendar year 1959):
Total: 0.5
Professional: 0.5
Other: 0.0

Patient days (Calendar Year 1959)
0

Project Description:

Objective: The first objective was to develop a technique in this laboratory for subcortical depth electrode implantation. Such implantation had to be adaptable for use in chimpanzee, thus presenting an engineering problem. Second, it seemed wise to re-examine previous reports of the effect of amygdala stimulation. Third, since an earlier article from this laboratory (Frost et al 1958) had described bilateral amygdala activity after unilateral stimulation, the effects of simultaneous, bilateral stimulation, or bilateral sequential stimulation, were studied.

Methods Employed: Subcortical depth electrodes were implanted stereotactically under moderate to deep hypothermia in the limbic system and superior brain stem. As many as 19 placements were made per preparation. Inexpensive, commercially-available materials were used. Cats were studied at first; recently, monkeys have been prepared. Action potentials were recorded from other electrodes during and after stimulation, while somatic effects were studied from cinematographic records.

Major Findings: Stimulation of several amygdala sites resulted in a stereotyped behavioral automatism.

Concomitant with the automatism there was a characteristic spike-and-wave after-discharge, recorded both ipsilaterally and (after only a few msec. or less delay) contralaterally. An early cat preparation, which incurred a temporal lobe lesion during implantation, had such automatisms spontaneously. These automatisms and after-discharges were very sensitive to anesthesia.

It seemed clear that bilateral sequential or simultaneous amygdala stimulation sometimes enhanced or (in some cases) depressed the effect which would have been expected on the basis of algebraic summation. The interval between stimuli which gave the greatest change was five to six msec., which is the interval found by Sherrington for optimal facilitation of spinal reflexes. The anatomical localization of such points is presently incomplete.

Significance to Neurological Research: This study has provided evidence that nucleus amygdaliformis may be an exciting locus for automatisms. Thus, it bears on the problem of human temporal lobe epilepsy. From the bilateral stimulation studies, it might be concluded that both these nuclei drive the same (? mesencephalic) area; however, this evidence is not yet substantial.

Proposed Course of the Project: The results in cat are being analyzed. Actual experimentation will continue in monkey and chimpanzee.

Part B included: Yes ☐ No ☒

Serial No NINDB - 5[illegible] (c
1. Surgical Neurology Branch
2. Primate Neurology
3. Bethesda, Maryland
4. Refer to NINDB 67(c)-73(c[illegible], 1958

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: The Recruiting Responses

Principal Investigator: Choh-luh Li

Other Investigators: Shelley N. Chou and Joseph Miller

Cooperating Units: None

Man Years (calendar year 1959):	
Total	0.4
Professional	0.1
Other	0.3

Patient Days (calendar year 1959): None

Project Description:

Objective: To investigate the intracellular potentials of the cerebral cortex in response to stimulation of the "intralaminar system" which is supposed to be directly related to the underlying activity of the cortex.

Major Findings: Depolarization with repetitive discharges and hyperpolarization with cessation of spike discharge in response to thalamic stimulation were found.

Significance: This is the first attempt to record recruiting responses with intracellular electrodes. The effect of recruiting responses on sensory responses was studied with extracellular microelectrodes in this laboratory in 1956. The information of the present investigation will direct our interest into an inquiry of the effect on the cell membranes.

Proposed Course: Further study of the effect of the thalamic intralaminar influence upon cortical cell responses.

Part B included Yes ☐ No ☒

Serial No. NINDB 52(c)
1. Surgical Neurology Branch
2. Primate Neurology
3. Bethesda, Maryland
4. Refer to NINDB 67(c)-73(c),1958

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Cortical Intracellular Potentials in Epileptic Cortex.

Principal Investigator: Choh-luh Li

Other Investigators: Shelley N. Chou and Joseph Miller

Cooperating Unit: None

Man Years (calendar year 1959):		Patient Days (calendar year 1959):
Total:	0.8	
Professional:	0.2	None
Other:	0.6	

Project Description:

Objective: To study the mechanism of epileptic discharge in single cortical cells.

Method Employed: An epileptic focus in the cortex was produced by repeated stimulation and the epileptic after-discharges were recorded with intracellular electrodes and surface electrodes.

Major Findings: In the pre-ictal period when the surface electrode recorded electrical inactivity, microelectrodes detected asynchronous discharges of cortical units in high frequency. When the so-called "epileptic after-discharges" occurs depolarization and restoration of resting potential periodically take place. And if depolarization continued to grow spike action potentials disappeared. There is also an accentuation of oscillation of potentials when spike activity is absent.

Serial No. NINDB 52(c)

Significance of program to Institute: The mechanism of epileptic discharge has been studied with intracellular electrodes under the effect of strychnine. This study further confirms the previous observations. In addition it is suggested that the surface electrical record, at times, provides no information as to how the nerve cells underneath act.

Proposed Course of Project: Further study in connection with thalamic stimulation is to be made.

Part B included Yes [X] No [ ]

Serial No. NINDB - 52(c)

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards and Publications

Publication: Li, Choh-luh. Cortical Intracellular Potentials and their responses to strychnine. J. Neurophysiol. 1959, 22: 436-450.

Serial No. NINDB - 53(c)
1. Surgical Neurology Branch
2. Primate Neurology
3. Bethesda, Maryland
4. Refer to NINDB 67(c)-73(c),1958

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Cortical intracellular potentials in response to a single cortical stimulus.

Principal Investigator: Shelley N. Chou

Other Investigators: Joseph Miller and Choh-luh Li

Cooperating Units: None

Man Years (calendar year 1959):
Total: 2.0
Professional: 2.0
Other: 0.0

Patient Days (calendar year 1959):
None

Project Description:

Objective: To re-evaluate the early observations of E. D. Adrian with intracellular recordings from cortical cells.

Methods employed: The somatosensory cortex of the cat was chosen. With the micropipette in the interior of the cells, single shocks were applied to the surface of the cortex not more than 5 mm away from the recording electrode tip. Simultaneous recording of the surface responses were also made.

Major Findings: It was said that the initial surface-negative potential in response to weak stimulus was the result of activation of superficial elements, probably dendrites; and the surface-positive potential in response to strong stimulus was produced by deep neurones in the cortex. The present investigation shows that this is not necessarily so. The refractory period of the cells under direct stimulation and synaptic activation was also determined in this study.

Significance of program to Institute: The observation of Adrian has been duplicated by many psysiologists and his interpretation accepted since his study 24 years ago. His concept has been also used as a basis for many hypothesis concerning the electrical activity recorded from the surface of the cerebral cortex. The result of the present study is not a challenge to Dr. Adrian's theory but provides evidences that may be useful in the interpretation of cortical surface records.

Proposed Course of Project: Further study of direct cortical stimulation with conditioned volleys initiated from other structures of the central nervous system is to be made.

Part B included: Yes [x] No [ ]

Serial No. NINDB 53(c)

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards and Publications

Publication: Li, Choh-luh. Synchronization of Unit Activity in the Cerebral Cortex. Science, 1959, 129: 783-784.

Serial No. NINDB - 54(c)
1. Surgical Neurology Branch
2. Private Neurology
3. Bethesda, Maryland
4. Refer to NINDB 67(c)-73(c), 1956

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Study of Inhibitory Interneurones of the Cerebral Cortex

Principal Investigator: Choh-luh Li

Other Investigators: Shelley N. Chou and Armando Ortiz

Cooperating Units: None

Man Years (calendar year 1959):
Total: 0.4
Professional: 0.1
Other: 0.3

Patient Days (calendar year 1959):
None

Project Description:

Objective: To investigate the intracellular responses with hyperpolarization recorded from different cortical areas of the cerebral cortex and evoked by volleys initiated from different sources.

Methods Employed: The cells in the somatosensory and visual cortex were impaled with micropipette electrodes and a peripheral nerve, lateral geniculate body or a corresponding point in the opposite hemisphere was stimulated.

Major Findings: Hyperpolarization of many cells were recorded, most often from the visual cortex. The size and duration were similar In a series of experiments the lateral geniculate body was poisoned with gamma-amino butyric acid and the responses recorded from the surface of the visual cortex were found to be enhanced, suggesting that this chemical agent might have suppressed the inhibitory interneurones.

Significance of research in the institution: This study is the beginning of an investigation of the action of gamma-amino butyric acid on the central nervous system. The preliminary observation seems to indicate that under certain circumstances this chemical agent does not inhibit the excitatory neurones but inhibit the inhibitory neurones.

Proposed course of procedure: Further study of the action of gamma-amino butyric acid and allied drugs is to be made.

Part B included Yes ☐ No ☐

Project No. NINDB [illegible]

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards and Publications

Publications: Li, Chch-luh and Chou, Shelley N. Inhibitory Interneurones in the Neocortex. To appear in book titled "Conference on Inhibition of Central Nervous System and gamma-amino butyric acid," sponsored by the Air Force Office of Scientific Research, U. S. Air Force, Washington, D. C.

Serial No. NINDB 55(c)
1. Surgical Neurology Branch
2. Primate Neurology
3. Bethesda, Maryland
4. Refer to NINDB 67(c)-73(c),1958

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Synaptic activation of nerve cells in the primary visual cortex.

Principal Investigator: Choh-luh Li

Other Investigator: Shelley N. Chou

Cooperating Units: None

Man Years (calendar year 1959):		Patient Days (Calendar year 1959):
Total:	0.4	
Professional:	0.1	None
Other:	0.3	

Project Description:

Objective: To study the intracellular responses of visual cortical nerve cells.

Methods employed: The cells in the visual cortex of cats were impaled with micropipette electrodes and the lateral geniculate body was stimulated.

Major findings: The outstanding feature of the responses is hyperpolarization. In most instances hyperpolarization follows an initial excitation. The initial excitation may be even suppressed by the arrival of inhibitory impulses.

Significance of program to Institute: The above observation is in contrast of the responses obtained from the somatosensory cortex and suggests that inhibitory process is very important in visual function.

Proposed project: This study is to be extended to test the influence of the thalamic activity on the visual cortical cells in terms of intracellular responses.

Part B included Yes ☐ No ☒

Serial No. NINDB - 95(c)
1. Surgical Neurology Branch
2. Primate Neurology
3. Bethesda, Maryland
4. Refer to NINDB 67(c)-73(c),195

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Synaptic activation of cortical nerve cells.

Principal Investigator: Choh-luh Li

Other Investigator: Shelley N. Chou

Cooperating Units: None

Man Years (calendar year 1959):		Patient Days (calendar year 1959):
Total:	0.8	
Professional:	0.2	None
Other	0.6	

Project Description:

Objective: Synaptic excitation of nerve cells in the cerebral cortex has been made and thoroughly investigated with intracellular electrodes. This study is an attempt to investigate the responses recorded from the interior of the individual nerve cells in the cerebral cortex.

Methods employed: The study was carried out in cats. The cells in the somatosensory cortex were impaled with micropipette electrodes while a contralateral sensory nerve was stimulated.

Major findings: The results of this study may be summarized as follows:

(1) In about 1 msec. after the onset of the initial surface positive potential recorded from the cortical surface, cortical neurones would respond with growing depolarization and spike responses were initiated from a level of 10 mV.

(2) Subthreshold depolarization was found to be composed of small potentials. The small potentials can be considered to be units of postsynaptic potentials; a synchronous discharge of these units give rise to a depolarization of critical size, from which spike potential is generated.
(3) Oscillating potentials are rhythmic and graded, probably responsible for the rhythmicity of spike activity. The oscillating potentials do not require a continuous presynaptic activity and may be considered as an intrinsic factor with which the excitability of the nerve cells is maintained.
(4) Tubo-curarine does not impede synaptic transmission in some elements of the nervous system.

Significance of program to Institute: The above observations have not been reported in the literature and may be of some importance in the understanding of the function of the cerebral cortex.

Proposed course of project: The study is to be extended concerning integration function of the cortex. Thus the interaction of the thalamic and sensory volleys can be studied in terms of intracellular responses.

Part B included Yes [x] No [ ]

Serial No. NINDB - 56(c)

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards and Publications

Publications: Li, Choh-luh. Some Properties of Pyramidal Neurones in Motor Cortex with Particular Reference to Sensory Stimulation.
J. Neurophysiol., 1959, 22: 385-394.

Serial No. NINDB - 57(c)
1. Surgical Neurology Branch
2. Primate Neurology
3. Bethesda, Maryland
4. Refer to NINDB 67(c)-73(c),1958

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Electrical activity of individual glia cells in tissue culture material.

Principal Investigators: Choh-luh Li and Igor Klatzo

Other Investigators: Dr. Shelley N. Chou and Joseph Miller

Cooperating Units: None

Man Years (calendar year 1959):		Patient Days (calendar year 1959):
Total:	0.4	
Professional:	0.1	None
Other:	0.3	

Project Description:

Objective: To study the membrane properties in response to convulsive and anti-convulsive agents.

Methods Employed: Similar to the project study with spinal dorsal ganglion cells in tissue culture.

Major Findings: Preliminary and not conclusive.

Significance of program to Institute: The glia elements have recently been considered to be extremely important in central nervous function. Thus it has been said that glia cells also participate in "memory mechanism". It is our belief that before a fair understanding of the physiological properties of these cells is obtained the validity

of any hypothesis concerning the role these elements play in questionable. The significance of this study is, therefore, obvious; and this study may provide evidence from which a new concept of nervous function may be derived.

Proposed course of project: The obstacles of this research is again the method of culturing these cells in a proper medium so as to facilitate the entry of the micropipette into the interior of these cells. Further effort is to be made along this line.

Part B included Yes ☐ No ☒

Serial No. NINDB 58(c)
1. Surgical Neurology Branch
2. Primate Neurology
3. Bethesda, Maryland
4. Refer to NINDB 67(c)-73(c)

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Electrical activity of individual neuronal elements in tissue culture.

Principal Investigators: Choh-luh Li and Igor Klatzo

Other Investigators: Shelley N. Chou and Joseph Miller

Cooperating Units: Neuropathology Section

Man Years (calendar year 1959):		Patient Days (calendar year 1959):
Total:	0.8	
Professional:	0.2	None
Other:	0.6	

Project Description:

Objective: To investigate the individual nerve cells in tissue culture in response to epileptic drugs, such as metrozol, strychnine, acetylcholine, etc. and in response to anti-convulsive agents.

Methods Employed: The spinal dorsal ganglion cells from chick embryos were used. These cells were explanted in either collagen or plasma clot media on Maximow's glass cover slips. The cells multiplied and on the 7th day the cells were subjected to investigation with micropipette electrodes in a chamber under a binocular microscope. The membrane resting and action potentials of the individual ganglion cells were recorded. Into the bath of the cultured chamber was added various chemical agents of appropriate concentration. Electrical measurements of the cells were made again. The excitability of the cells was then studied.

Major Findings: The resting electrical activity of the cultured nerve cells was found to be comparable to that obtained from nerve cells in vivo. For example: The resting potentials of the ganglion cells in tissue culture measured 70-80 mV and action potentials as large as 90 mV. The only difference was, perhaps, the duration of the action potentials which was found to be relatively prolonged. The responses of the isolated cultured cells are under study and the observation can only be considered preliminary. Conclusive statements can, therefore, be made at a later date.

Significance of the Program to the Institute: This study may throw some light on the mechanism of epilepsy and of the drug action on this disease. On theoretical grounds this study may also provide an understanding of the effect of an active nerve cell upon a neighboring resting neurone in the absence of synaptic contracts.

Proposed course of project: This investigation depends largely on the technique of culturing the nerve cells in a special cultured medium which will facilitate the impalement of the cells with micro-electrodes. A great deal of time has been spent on the improvement of the method and at the present no satisfactory solution has been made. It is hoped that when the technique is perfected valuable data can be obtained in a minimum length of time concerning not only the problem of epileptic activity but electrical activity of brain tumor cells removed from human patients.

Part B included Yes [x] No [ ]

Serial No. NINDB - 58(c)

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B. Honors, Awards and Publications.

Publication: Li, Choh-luh, Engel, K. and Klatzo, Igor. Some Properties of Cultured Chick Skeletal Muscle with Particular Reference to Fibrillation Potential. J. Comp. Cell Physiol., 1959, 55: 000-000 (June issue).

Serial No. NINDB 59 (C)
1. Surgical Neurology Branch
2. Clinical Neuropathology Section
3. Bethesda, Maryland
4. New

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Study on Morphological Changes due to Exposure to Radio-frequency Energy.

Principal Investigator: Igor Klatzo, M. D.

Other Investigators: M. Baldwin, M. D., E. J. Laskowski, M. D., S. A. Bach, M. D. and S. A. Lewis, R. N.

Man Years (calendar year 1959):
Total: 4.0
Professional: 1.5
Other: 2.5

Patient Days (calendar year 1959) 0

Project Description:

Objective: This project is designed to provide the data on the morphological changes produced by exposure of primates to certain radio-frequency energy. It constitutes one of the aspects of the research Project # N-1-527 conducted in The Surgical Neurology Branch which is concerned with various problems connected with the effects of this type of energy.

Methods Employed: Primates are exposed to various ultra-high frequencies within micro-wave ranges as described in Project # N-1-527. Sodium fluorescein is used for study of the permeability of the blood-brain-barrier (BBB). Various histochemical and histological procedures are employed to assess the changes in the central nervous system of the primates which are sacrificed at various time intervals after exposures.

Major Findings: Preliminary findings indicate the following: in acute experiments, where the animals are sacrificed within a few minutes after exposure in the "low position" the changes in the permeability of the BBB can be found in the pons, medulla and extending through the white matter into the cerebellar hemispheres. Sometimes, the BBB changes are localized lower and

Major Findings (cont'd):

involve the dorsal areas of the cervical spinal cord. The histological changes in the acute experiments are confined to the striking tygrolysis in the large tegmental neurons of the pons, medulla and the dentate nucleus of the cerebellum. No neurofibrillary changes can be observed in these neurons.

In the chronic experiments only a few animals showed changes in the BBB and they were localized in the dorsal portions of the cervical cord. The morphological changes were found in the majority of the animals and were seen mainly in the dorsal areas of the cervical spinal cord. They consisted in severe tissue destruction, mainly in the posterior columns. In these areas small cystic cavities could be seen transversed by greatly proliferated blood vessels with abundant reticulin. Changes in the medulla and pons found in a few animals showed similar cystic cavities with well preserved neurons in the vicinity.

Proposed course of the project: It is proposed to correlate precisely the morphological changes observed with the dose and frequencies of the exposures as well as with the head positions of the animals. Similarly, correlations will be attempted between the morphological changes observed and the clinical effects of exposure. It is intended to study the biological effects of this radiation on the cells of the central nervous system grown in tissue culture.

Part B included: Yes ☐ No ☒

Serial No. NINDB 60 (C)
1. Surgical Neurology Branch
2. Clinical Neuropathology Section
3. Bethesda, Maryland
4. Continuation of NINDB 74 (C) - 1958

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Study on Pinocytosis and Uptake of Proteins and Neutral Red Dye in Vitro.

Principal Investigator: Igor Klatzo, M. D.

Other Investigators: J. Miquel, Ph. D.

Man Years (calendar year 1959):
Total: 4.0
Professional: 1.5
Other: 2.5

Patient Days (calendar year 1959): 0

Project Description:

Objective: The main purpose of this project is to get an insight into some basic features of permeability and metabolism of various cell types grown in tissue culutre. For this purpose, uptake of non-toxic neutral red (NR) dye and several proteins (albumin, globulin and fibrinogen) labelled with fluorescein isothiocyanate according to Coons' technique is being studied on various experimental conditions.

Methods Employed: Nervous and muscle tissue is grown in vitro. Cultures of living cells are incubated with various solutions of NR or labelled proteins. Observations are made at time intervals; the cultures being subjected to various experimental conditions. Cells are observed in bright-field, phase and fluorescence microscope as well as photographed in the time-lapse cine camera.

Major Findings: The uptake of NR takes place only in living cells in good physiological condition. NR disappears from the dying cells and is not taken up by the dead cells. There are marked differences in the ratio of uptake between various cell types. Macrophages are filled with granular inclusions of NR in a short time. Astrocytes show conspicuous granules of the dye in the processes and perikaryon after an interval of time.

Major Findings (cont'd):

Nerve cells and muscle fibers take up NR in a limited way and only at the terminal expansions which are provided with undulating membranes.

The uptake of various proteins resembles in many ways that of NR. There is a similarly different ratio of uptake among the various cell types. The striking difference is the disappearance of proteins within one hour from the macrophages, whereas the astrocytes preserve the proteins throughout the time of observation (24 hours). The ratio of uptake between various proteins is correlated generally with the size of molecule and is lowest for fibrinogen and highest for albumin.

The effect of low temperature (4 °C) has been studied on the uptake of NR and labelled proteins. There is a conspicuous reduction of the uptake of NR. With regard to the proteins it is interesting to note that 6 hours after feeding the hypothermic (4 °C) cultures show macrophages filled with fluorescent proteins whereas there are only very few proteins inclusions in the astrocytes. This picture corresponds to the appearance of the normothermic (37 °C) cultures 30 minutes after feeding with protein. It is attempted to correlate these observations on the uptake of NR and proteins with the phenomenon of "pinocytosis". The pinocytosis ("drinking by the cells") has been observed as a formation and disappearance of clear vacuoles in the undulating membranes of various cell types, particularly macrophages and astrocytes; it is suggestive that initial uptake of NR and proteins takes place by pinocytosis with consequent concentration of the substances under study.

Proposed course of the project: It is proposed to continue this investigation in order to investigate further the various aspects of this basic physiological mechanism of uptake of various substances in the cells grown in vitro. Especially, attempts will be made to correlate the ratio of uptake and metabolism with that of pinocytosis and influence this ratio by various chemical and physiological conditions.

Part B included: Yes ☐ No ☒

Serial No. NINDB 61 (C)

1. Surgical Neurology Branch
2. Clinical Neuropathology Section
3. Bethesda, Maryland
4. Continuation of NINDB 77 (C) - 1958

PHS-NIH'
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Study of Regeneration in the Central Nervous System.

Principal Investigator: Armando Ortiz-Galvan, M. D. (Now at the National University o Mexico General Hospital, Mexico City, Mexico).

Other Investigators: Edward J. Laskowski, M. D. and Igor Klatzo, M. D.

Man Years (calendar year 1959):
Total: 0
Professional: 0
Other: 0

Patient Days (calendar year 1959): 0

Project Description:

Objective: Same as NINDB 77 (C) - 1958

Methods Employed: Same as NINDB 77 (C) - 1958

Major Findings:

Proposed course of the project: The project has been discontinued since the Principal Investigator has returned to Mexico.

Part B included: Yes [ ] No [x]

Serial No. NINDB - 62 (C)

1. Surgical Neurology Branch
2. Clinical Neuropathology Section
3. Bethesda, Maryland
4. Continuation of NINDB 78 (C) - 1958

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Histochemical and Electrophysiological Observations on the Muscle Fibers Grown in Vitro.

Principal Investigator: W. K. Engel, M. D. (Now at Queen's Square, London, England).

Other Investigators: Choh Lu Li, M. D. and Igor Klatzo, M.D.

Man Years (calendar year 1959):
Total: 0
Professional: 0
Other: 0

Patient Days (calendar year 1959): 0

Project Description:

Objective: Same as NINDB 78 (C) - 1958

Methods Employed: Same as NINDB 78 (C) - 1958

Major Findings: Same as NINDB 78 (C) - 1958

Proposed course of the project: The part of the project concerned with the electrical activity has been completed. The remaining studies will be resumed upon the return of the Principal Investigator from abroad.

Part B included: Yes [x] No [ ]

Serial No. NINDB 62 (C)

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B. Honors, Awards and Publication

Publication other than abstracts from this project:

Engel, W. K. : Cytological Localization in Cultured Skeletal Muscle.

In Preparation.

Honors and Awards relating to this project:

Serial No. NINDB 63 (C)
1. Surgical Neurology Branch
2. Clinical Neuropathology Section
3. Bethesda, Maryland
4. Continuation of NINDB 79 (C) - 1958

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: A New Method for Quantitative Study of Precipitin Reaction.

Principal Investigator: Jaime Miquel, Ph. D.

Other Investigators: B. Horvath, M. D. and Igor Klatzo, M. D.

Man Years (calendar year 1959):
Total: 0
Professional: 0
Other: 0

Patient Days (calendar year 1959) 0

Project Description:

Objective: Same as NINDB 79 (C) - 1958

Methods Employed: Same as NINDB 79 (C) - 1958

Major Findings: Same as NINDB 79 (C) - 1958

Proposed course of the project: This project is completed.

Part B included: Yes [x] No [ ]

Serial No. NINDB 63 (C)

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B. Honors, Awards and Publicat on

Publication other than abstracts from this project:

Miquel, J., Horvath, B., and Klatzo, I. :
" A Chromatographic Technic for the Quantitative Study of the Precipitin Reaction".

Accepted for Publication in The Journal of Immunology.

Honors and Awards relating to this project:

Serial No. NINDB 64 (C)
1. Surgical Neurology Branch
2. Clinical Neuropathology Section
3. Bethesda, Maryland
4. Continuation of NINDB 81 (C) -1958

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Study of the Effects of Hypothermia on Injured and Normal Brain Tissue.

Principal Investigator: Edward J. Laskowski, M. D.

Other Investigators: Igor Klatzo, M. D.

Man Years (calendar year 1959):
Total: 1.0
Professional: 1.0
Other: 0.0

Patient Days (calendar year 1959): 0

Project Description:

Objective: The continued use of hypothermia as an adjunct in neurosurgery has prompted this investigation to further assess the effects of lowered body temperatures on the various aspects of brain injury. The responses of the various elements of brain edema and the blood-brain-barrier were too be established and then correlated with responses of the same tissue elements under varied conditions of hypothermia.

Methods Employed: The assessment of the effects of hypothermia is based on a study of our standardized cold lesion as described in Project NINDB 57 (C) - 1956 in normothermic animals. A comparative study of normothermic and hypothermic animals in response to this cold lesion is further described in Project NINDB 31 (C) - 1957. Brain lesions were made in groups of cats under normothermic conditions. Six hours later the temperatures were lowered to 26 °C and maintained there for the remainder of 48 hours at which time they were sacrificed. Minor variations of this same general procedure were introduced.

Major Findings: A most striking difference between the normothermic and hypothermic animals has been revealed in the behavior of the blood-brain-barrier. At 24 hours, when edema is maximla in the normothermic group, the area of altered blood-brain-barrier is diminished in those treated by hypothermia. The astroglial reactions are diminished at comparable periods in the hypothermic groups as compared with the normothermic. The degree of involvement increases however, and is maximal at 48 hours after which time both groups follow a comparable course. In the current group the degree of change in all elements is markedly less than in the normothermic group at that time and is similar to the picture seen at 6 hours in the normothermic group. This suggests the measure of benefit which may be derived from the use of hypothermia in the treatment of severe head trauma with brain edema.

Proposed course of the project: This phase of the project is complete. Further evaluation of the responses of brain edema to hypothermia applied at various time intervals is indicated however, and studies will be continued.

Part B included: Yes [X] No [ ]

Serial No. NINDB 64 (C)

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B. Honors, Awards and Publication

Publication other than abstracts from this project:

Laskowski, E. J.: Observations on the Effect of Hypothermia on Experimental Brain Lesions. The American College of Surgeons, "Surgical Forum". Vol. IX,: 714-717, 1959.

Laskowski, E. J., Klatzo, I., and Baldwin, M.: Experimental Study of the Effects of Hypothermia on Local Brain Injury.

Accepted for Publication in Neurology.

Honors and Awards relating to this project:

Addendum to NINDB 75 (C) -1958

1. Surgical Neurology Branch
2. Clinical Neuropathology Section
3. Bethesda, Maryland
4.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: The Localization of Myosin in Human Striated Muscle by Fluorescent Antibody.

Principal Investigator: Igor Klatzo, M. D.

Other Investigators: B. Horvath, M. D. and W. K. Engel, M. D.

Part B: Honors, Awards and Publication

Publication other than abstracts from this project:

Klatzo, Igor, Horvath, Beni, and Engel, W. K. "Observations on Myosin in the Abnormal Muscle using Fluorescent Antibody Technic".

Accepted for Publication in the 1958 issue of the Volume of The Association for Research in Nervous and Mental Diseases.

Honors and Awards relating to this project:

Addendum to NINDB 76 (C) - 1958

1. Surgical Neurology Branch
2. Clinical Neuropathology Section
3. Bethesda, Maryland
4.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Study of Pathology of Kuru Disease.

Principal Investigator: Igor Klatzo, M. D.

Other Investigators: D. C. Gajdusek, M. D. and V. Zigas, M. D.

Part B. Honors, Awards and Publication

Publication other than abstracts from this project:

Klatzo, I., Gajdusek, D. C., and Zigas, V. : "Evaluation of Pathological Findings in Twelve Cases of Kuru".

Accepted for Publication in the Volume of the International Symposium of Actual Encephalitides, Antwerp, Belgium.

Honors and Awards relating to this project:

Addendum (Publication on which there is no current project).

1. Surgical Neurology Branch
2. Clinical Neuropathology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B. Honors, Awards and Publication

Publication other than abstracts from this project:

Engel, W. K., Kurland, L. T., and Klatzo, I.: An Inherited Disease Similar to Amyotrophic Lateral Sclerosis with a Pattern of Posterior Column Involvement. An Intermediate Form? Brain. 82: Part II, 203-220, 1959.

Honors and Awards relating to this project:

2. Developmental Neurology
3. Bethesda, Maryland
4. Same as NINDB-82 (c)

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Investigation of the Etiology, Pathology and Clinical Manifestations of Cerebral Palsy and Allied Conditions; Also of the Epilepsy During Childhood.

Principal Investigator: Anatole S. Dekaban, M.D.

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1959):		Patient Days (calendar year 1959)
Total:	0.6	
Professional:	0.2	899
Other:	0.4	

Project Description:

Objectives: Comprehensive clinical, laboratory and genetical investigation of a selected group of children suffering from organic brain syndromes and epilepsy and correlation of thus obtained data with the findings in pneumoencephalogram. The main objectives are: 1 - Correlation of the clinical features of cerebral dysfunction with the site, size and character of the cerebral lesion. 2 - Classification of a larger group of children suffering from organic brain condition according to the etiological factors whenever these were established. 3 - Preparation of publications based on smaller groups of patients presenting particularly important aspects in relation to pathology, pathogenesis or response to a new type of treatment.

Methods Employed:

1. Genetic investigation.
2. Detailed neurological examination, including developmental testing and electroencephalogram.
3. Pneumoencephalogram.
4. Other special tests as indicated.

Patient Material:

		No.	Aver. Stay in Days
Admissions:	Children Male	17	21
	Children Female	18	29

Clinical Project

Major Findings:

During 1959 35 patients were studied in great detail as in-patients and 35 on the out-patient basis. In addition, out of 160 patients under special study on epilepsy during infancy (in cooperation with Mayo Clinic), 65 were re-examined clinically and with special laboratory tests.

Material from a considerable proportion of the in-patients formed the basis of communications already published as listed below and the part of the material is currently evaluated. Our main purpose is to document hitherto unknown factors responsible for congenital malformations or brain damage - as it was done in our studies on offspring of diabetic mothers - or to contribute to clarification of clinical and pathological aspects of cerebral palsy, epilepsy and mental deficiency.

Out of 35 in-patients, in 16 we were able to establish etiological diagnosis. Of the remaining 18, in whom only descriptive diagnosis was possible, the site of pathological lesion was determined in 10 patients. The latter finding is of importance because it narrows the possibilities in differential diagnosis; also in the future some of these patients may become subjects for revealing clinico-pathological correlations.

Results of studies on a number of patients studied are reported in the publications listed below. Final analysis of the material has to await accumulation of more patients.

Significance to Neurological Research: In a majority of cases the etiology of cerebral palsy and allied conditions is poorly understood. Better knowledge of hereditary factors, clinical manifestations, as well as the location and extent of the lesion may further our insight into the diverse etiology of these conditions. Full understanding of pathology and etiology in larger groups of children with brain damage will suggest eventually better directed preventive and therapeutic measures.

Proposed Course of the Project: Accumulation of more data is needed and the organization of this project remains unchanged.

Part B included Yes [x] No [ ]

Part B: Honors, Awards and Publications

Dekaban, Anatole and Drager, Glenn: Metastases of the retinoblastoma to the central nervous system. Advisability of a combined intraorbital and intracranial removal of the affected optic nerve. AMA Archives of Ophth. 66: 239-245, 1959.

Dekaban, Anatole: Book: Neurology of Infancy. 1959, Williams & Wilkins, Baltimore. pp. 16 + 388 = 404.

Honors and Awards Relating to this Project:

1. Assistant Professor of Neurology at George Washington University Medical School.

2. Consultant District of Columbia Children's Hospital.

3. Bethesda, Maryland
4. Same as NINDB-83 (c)

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Maternal Condition During Pregnancy and the Course of Birth in Relation to Neurological Abnormalities in the Infants and Pathologic Lesions in Products of Abortion.

Principal Investigator: Anatole S. Dekaban, M.D.

Other Investigators: Dr. T.E. Cone and Dr. H.H. Hill, National Naval Medical Center; Dr. L.J. Geppert and Dr. H.L. Riva, Walter Reed Army Hospital

Cooperating Units: National Naval Medical Center and Walter Reed Army Hospital.

Man Years (calender year 1959):
Total: 0.3
Professional: 0.1
Other: 0.2

Project Description:

Objectives: Analysis of various abnormal factors occurring in pregnant mothers or complications of birth which may cause or contribute to neurological abnormalities in infants.

Methods Employed:

1. Prenatal care of mothers under research and their individual final assessment.
2. Recording and evaluating of the course of birth and pertinent abnormalities.
3. Examination of the newborn infants during initial hospital stay.
4. Follow-up examination of infants.
5. Gross and microscopic examination of the products of abortion.

Patients Material:

1. All pregnant women who were receiving prenatal care at the National Naval Medical Center and Walter Reed Army Hospital and subsequently were delivered in these hospitals between March 1, 1956 and March 1, 1957.

Clinical Project

Major Findings: The total of 4,156 pregnancies and products of these gestations were studied in great detail regarding premature care, confinement, infant's state at birth and his subsequent course up to one year of age. At the present time the analysis of the demographic data on this material is completed and ready for publication. The following two tables give some insight into the demographic aspects of this study.

TABLE I

Classification of the outcome of gestation in 4,156 pregnancies.

Abortions	312
Previables	9
Stillbirths	22
Neonatal Deaths	59
Infantile Deaths	10
Surviving full term infants with complicated gestation, delivery or abnormal clinical state at birth	921
Surviving full term infants with normal gestation, delivery and normal clinical state at birth	2,567
Surviving premature infants with complications of gestations, delivery or abnormal clinical state at birth	103
Surviving premature infants with normal gestation, delivery and normal clinical state at birth	153
Total	4,156

delive y of abnormal [illegible] Group B [illegible]
normal controls, Group C - [illegible].

MATERNAL AGE	GROUP A	GROUP B	GROUP
Under 18 years	54	26	1[illegible]
Between 18 & 28 years	820	516	15[illegible]
Between 29 & 38 years	368	175	12[illegible]
Over 38 years	35	12	2[illegible]

The evaluation of the remainder of the material will follow and it is likely to throw some light on the etiology, pathology and pathogenesis of those conditions which are included in the group of mental deficiency, epilepsy and cerebral palsy.

Significance to Neurological Research: It is postulated that various environmental factors acting during prenatal, intranatal and early postnatal life may be responsible for brain damage and the associated clinical sequelae in infants. This study may reveal the relative importance of certain factors and also their incidence. Since careful and uniform examinations are being conducted during all stages of prenatal and postnatal life, final analysis of the findings should be significant.

Proposed Course of the Project: Completion of this [illegible] and preparation of the material for publication will require considerable time.

Part B included yes

Serial No. NINDB-66 (c)

PHS-NIH
Individual Project Report
Calendar Year 1969

Part B: Honors, Awards and Publications

Dekaban, Anatole, et. al.: Correlation of the medical condition of the offspring and of the outcome of 4,156 pregnancies with maternal state during gestation, type of delivery and condition of the infants during first 4 days of life. Part I: Demographic characteristics and summarized results of the investigation. In press.

Honors and Awards Relating to this Project: None

Serial No. NINDB-67 (c)
1. Surgical Neurology
2. Developmental Neurology
3. Bethesda, Maryland
4. Same as NINDB-84 (c)

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Pathological Lesions in the Central Nervous System Occurring During Prenatal, Intranatal and Early Postnatal Life.

Principal Investigator: Anatole S. Dekaban, M.D.

Other Investigators: Martha Boring

Cooperating Units: None

Man Years (calendar year 1959):
Total: 0.6
Professional: 0.2
Other: 0.4

Project Description:

Objectives: The causation and pathology of the majority of patients with mental deficiency and cerebral palsy are largely unknown. Detailed examination of the brains of children who suffered from such disorders and correlation of these findings with the clinical data is expected to provide valuable information for elucidation of etiology of such conditions and eventually they may suggest possible preventive measures.

Methods Employed: Detailed examination of brains and spinal cords from patients who suffered from cerebral palsy or allied conditions by means of:

1. Gross examination and dissections.

2. Microscopical study of sections which were treated with chromatic silver, myelin and fat stains as well as by various histochemical procedures.

Material: Twenty-three brains from children who suffered acute birth injury or who were in chronic phase of cerebral palsy were studied.

Serial No. NINDB-67 (c)

Major Findings: Eight brains from patients suffering from cerebral palsy and allied conditions were studied in detail and part of the information obtained was published. Currently, careful evaluation of pathology and pathogenesis of acute birth injury is made on material consisting of 15 brains with such lesions. 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.

Significance to Neurological Research: Such studies are of great importance as the number of brains examined in detail in the instances of cerebral palsy and allied conditions is rather small. Studies relating to this project resulted in 2 publications during 1959.

Proposed Course of the Project: Further accumulation of data is needed.

Serial No. NIMDB-67 (c)

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B. Honors, Awards and Publications

Rice, E.C. and Dekaban, Anatole: Congenital hemiplegia resulting from cerebral malformation. AMA Arch. Pathology 68: 348-351, 1959.

Dekaban, Anatole: Arhinencephaly in an infant born to a diabetic mother. J. Neuropath. and Exper. Neurol. 18: 620-626, 1959.

Honors and Awards Relating to this Project: None

Serial No. NINDB-68 (c)
1. Surgical Neurology
2. Developmental Neurology
3. Bethesda, Maryland
4. Same as NINDB-85 (c)

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: The Incidence and the Type of the Central Nervous System Abnormalities Encountered in Offspring Born to Diabetic Mothers.

Principal Investigators: Anatole S. Dekaban, M.D.
Robert L. Baird, M.D.

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1959):
Total: 0.6
Professional: 0.2
Other: 0.4

Project Description:

Objectives: Our clinical and neuropathological studies indicated that severe abnormalities must occur not infrequently in infants born to diabetic mothers. (Dekaban, A. and Magee, K.: Occurrence of neurological abnormalities in infants born from diabetic mothers. Neurology 8: 193-200, 1958). It became important to evaluate statistically the incidence of these abnormalities in larger series of offspring born to diabetic mothers and to analyze the findings in light of findings in series of normal controls.

Methods Employed:

1. Critical assessment of maternal diabetes and her total pregnancies.

2. Examination of all her offspring.

Material: The outcome of 234 pregnancies in 48 diabetic women and in 249 pregnancies in 48 normal controls were analyzed. The mothers were personally interviewed and the offspring examined.

Clinical project

Major Findings: The outcome of 235 pregnancies in diabetic and prediabetic women were investigated and the results obtained were compared with the corresponding data in the 249 pregnancies of matched normal controls. The percentage of abortions and previable deaths in the diabetic sample was 29.9, in the prediabetic sample 21.5 and in the normal controls 12.4. Stillbirths occurred with the following frequency: 11.5 per cent in the sample of diabetic mothers, 5.1 per cent in the prediabetics and 1.2 per cent in the normal controls. The neonatal deaths amounted to 8.3 per cent of all pregnancies in the diabetic women, 1.3 per cent in the prediabetics and 3.6 per cent in the sample of normal controls. These values demonstrate clearly that the fetal wastage in the diabetic women is significantly greater than in the normal controls. The fetal loss during the prediabetic stage falls about midway between that in diabetic and normal control mothers.

The main purpose of our study was to test the hypothesis that the morbidity dating since birth in the offspring of diabetic mothers is also increased as compared with the sample of normal controls. There were six abnormal surviving offspring in the sample of 157 diabetic pregnancies which amounts to a total of 3.8 per cent (or 7.6 per cent of the surviving children). In the normal control sample of 249 pregnancies there was only one abnormal surviving offspring which constitutes the incidence of 0.4 per cent. The difference in percentages in these two groups was significant at P less than 0.01 indicating that our hypothesis is very likely correct. The abnormalities in these children include mental deficiency, congenital malformations, birth injury and epilepsy.

Three of the six abnormal surviving children born to diabetic mothers had severe congenital malformations, two had mental deficiency and one sequelae of birth injury and epilepsy. Of the forty-one offspring of diabetic women who were stillborn or died during the neonatal stage sixteen had comprehensive postmortem examination and the major findings are as follows: pulmonary hyaline membrane disease was found in eight patients, congenital malfomations in three, cerebral birth injury in two and no gross, demonstrable lesion in the remaining three. Factors, possibly pertaining to the increased fetal loss and to the higher rate of morbidity among the offspring of diabetic mothers are discussed in publications listed below.

Significance to Neurological Research: The [illegible] observations which are the result of our studies established for the first time that maternal diabetes should also be included to the known causes of mental deficiency and cerebral palsy in a proportion of offspring.

Proposed Course of the Project: The question of extension of this project to include experimental approach to the metabolic derangement during diabetic acidosis in pregnant females is currently under discussion.

Part B included Yes [x] No [ ]

Serial No. NINDB-68 (c)

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards and Publications

Dekaban, Anatole and Baird, Robert: The outcome of pregnancy in diabetic women. Part I. Fetal wastage, mortality and morbidity in the offspring of diabetic and normal control mothers. J. Pediat. 55: 563-576, 1959.

Dekaban, Anatole: The outcome of pregnancy in diabetic women. Part II. Analysis of clinical abnormalities and pathological lesions in offspring of diabetic mothers. J. Pediat. In press.

Honors and Awards Relating to this Project: None

Serial No. NINDB-69 (c)
1. Surgical Neurology
2. Developmental Neurology
3. Bethesda, Maryland
4. Same as NINDB-86 (c)

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Measurements of External and Internal Orbital Distance in Males and Females from Birth to Adulthood.

Principal Investigator: Anatole S. Debban, M.D.

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1959):
Total: 0.3
Professional: 0.1
Other: 0.2

Project Description:

Objectives: It has been found that the measurement of the interpupillary distance in humans for the purpose of estimation of hypertelorism and abnormality of the sphenoid bone is unsatisfactory. It is thought that either external or internal orbital distance or index thereof should take place of the measurements of the interpupillary distance.

Methods Employed:

1. Measurements of the above-named distances in human males and females at progressive ages beginning from zero to 20 years of age.

2. Correlation of physical measurements of a small group of children with measurements made on cephalometric x-rays.

3. Statistical analysis in various age horizons.

Material: Measurements of all horizons have been taken. This amounts to the total of 600 head measurements.

Clinical Project

Major Findings: Part of the data derived from this study was analyzed and published in the book "Neurology of Infancy", pages 35-37. Entirely new are data pertaining to the norm in various ages of the interorbital distance and also to the volume of the skull as measured in live subjects.

Significance to Neurological Research: To make the estimation of conditions such as hypertelorism more scientific, the measurements of stable bony structures rather than movable organ as eyeball, should be performed. As an example a concommitant divergent strabismus can be given; in this instance measurement of the interpupillary distance for the estimation of the abnormality of the sphenoid bone would obviously give false results.

Proposed Course of the Project: The remainder of the material will be evaluated and when suitable patients with specific clinical entities will become available reports for publications will be prepared.

Serial No. NINDB-69 (c)

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards and Publications

Dekaban, Anatole: Pages 35-37 in Book: Neurology of Infancy. 1959, Williams & Wilkins, Baltimore.

Honors and Awards Relating to this Project: None

Individual Progress Report
Calendar year 1959

Part A.

Project Title: Preparation of the Normons of the Normal Development of the CNS in Mice and Experimental Production of Congenital Malformations of the CNS.

Principal Investigator: Anatole S. Bekoham, M.D.

Other Investigators: Marie J. Kendall, B.A.

Cooperating Units: None

Man Years (calendar year 1959):
Total: 0.6
Professional: 0.2
Other: 0.4

Project Description:

Objectives: The purpose of this project is the production and analysis of congenital abnormalities of the CNS and the provision of norms for the development of the CNS in mice.

Methods Employed:

1. Dissection of the CNS of mice of 16 pregnancy developmental stages.

2. Preparation and staining of serial sections.

3. Identification and outlining of main structures on the low power microphotographs.

4. X-ray radiation of pregnant mice from a strain which does not show any significant incidence of spontaneously occurring abnormality of the CNS.

5. X-ray radiation of pregnant Black C 57 mice in several stages of pregnancy with similar parameters to these for mice in Swiss Albino; the

strain used here show an abnormally high incidence of spontaneous malformations of the CNS.

6. Gross, skeletal, and microscopical examination of the obtained specimen.

Material: Mice strain: NIH stock "general purpose Swiss Albino" and Black C 57.

Major Findings: An atlas of normal mouse brain has been prepared and bound. It is in current use in our laboratory.

Careful dissection of brain and brainstem of fetus and young mice in eleven age horizons were performed. The specimens are sectioned serially and stained. It needs to be stressed that to obtain one perfect set of serial sections for one horizon it is usually necessary to process and section six to twelve brains. Only those sets which are in ideal conditions can be utilized for description.

Production of malformations by means of x-radiation. We are considerably limited in space for maintenance of mice. Since only certain age mice can be used, we have to harbour them until they attain it. Then, only about 20 percent of those kept become pregnant as a result of restricted duration of mating time. In strain "general purpose Swiss Albino" 98 litters were obtained from irradiated mothers. Approximately 10 percent of these had major abnormalities, about 25 percent minor abnormalities and the remaining are free of detectable pathology. Similar parameters of irradiation and technique were applied to strain Black C 57 and so far 45 litters were obtained.

Significance to Neurological Research: An experimental approach to congenital malformations of the CNS is necessary to help us understand certain obscure malformations occurring in humans. The provision of norms of the central nervous system has to precede the experimental production of congenital malformations, as there does not exist any proper guide in the form of an atlas or of a satisfactory reference during consecutive stages of the development of the mouse. Majority of the stages in this strain are not yet completed and final analysis of data and comparison with the findings learned from irradiation will be done during the coming year.

Proposed Course of the Project: For technical reasons we had to stop further irradiation of mice for the time being. It is planned to resume this experimental work beginning next year.

2. [illegible] Neurology Branch
2. Section on Clinical Psychology
3. Bethesda, Maryland
4. Same as NINDB - 88 (c)

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Effect of "fear-provoking" stimuli on visual discrimination in primates.

Principal Investigator: H. Lansdell

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1959):
Total: 0.0
Professional: 0.0
Other: 0.0

Patient Days (calendar year 1959):
None

Project Description:

Objective: To investigate the disruptive effects of certain stimuli on performance in a visual discrimination task in an attempt to quantify and systematize the nature of such "fear-provoking" stimuli, and to use such data to evaluate changes that may be specific to temporal lobe removal.

Major Findings: This project was discontinued as no laboratory space was made available.

Part B included Yes ☐ No ☒

Serial No. NINDB - [illegible]
1. Surgical Neurology Branch
2. Section on Clinical Psychology
3. Bethesda, Maryland
4. Same as NINDB - 89 (c)

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Psychological Evaluation of Temporal Lobe Disease.

Principal Investigator: H. Lansdell

Other Investigators: M. Baldwin, A. Mirsky, J. Caldwell and M. Mannarino

Cooperating Units: NIMH Section on Animal Behavior

Man Years (calendar year 1959):		Patient Days (calendar year 1959):
Total:	3.0	
Professional:	1.0	None
Other:	2.0	

Project Description:

Objective: To study patients with temporal lobe disorders with emphasis in the areas of intellectual ability, visual and auditory perception, linguistic functions and other more general "personality" features.

Methods Employed: Intelligence and personality tests; aphasia audiometric, and other specialized verbal tests; tests of visual perception. Tachistoscopic recognition. Continuous performance test. Auditory testing during neurosurgery on conscious patients.

Major findings: A few of the new tests used in the past year appear to hold promise for measuring the effects of temporal lobe surgery:

1) The MMPI, a personality questionnaire originally designed for "psychopathological" patients, has shown a consistent picture of improvement after surgery in five temporal lobe cases. Four "exploratory" cases without surgery did not. Three nontemporal and five pituitary operations did not show similar effects in the retest scores.

2) Gorham's Proverbs Test has shown considerable consistency in improved scores upon retest in 12 cases of neurosurgery (including 5 right temporal) but not in 2 cases of left temporal lobectomy. Six follow-up cases who had some time ago undergone left temporal surgery did not score as well as any of six follow-up cases with surgery on the right. This test would seem to be likely to distinguish the laterality of the surgery better than any other test.

3) Bass' Famous Sayings has a scale termed "Social Acquiescence" which appears to drop after temporal lobe surgery (4 out of 6) but not usually after nontemporal (1 out of 5).

4) An unpublished "Estimation Questionnaire" by Bruner and Pettigrew purports to measure "breadth of category" in thinking by requiring multiple-choice estimates of the dimensions of various things or events. Four right temporal cases reduced their estimates; six of seven other neurosurgical cases increased their estimates.

5) Schlosberg has published a set of 48 pictures of an actress posing in various emotional "states." Using his unpublished norms, it was found that in the second half of the test three right temporal cases made more errors of judgment postoperatively and five other neurosurgical cases did better.

There is some danger that the above results could be misleading: 1) The number of cases in some of the comparisons is rather small and the results may be chance variations. 2) The control groups are not perfectly appropriate at present, e.g., high IQ's in the case of pituitary operations, tumor pathology and smaller removals in the cases of nontemporal surgery. The present data suggest there is a rather complex verbal facility maintained by the integrity of the dominant temporal lobe, and a socially-important kind of interpretive function seems to suffer in some respects after the operations; but they respond (in the post-operative period at least) with a happier outlook to a questionnaire.

Significance of the program to the Institute: Several of the new tests appear to offer good possibilities for distinguishing the psychological effects of temporal lobe surgery from other forms of surgery, and even the side of the temporal lobe surgery. If the present indications prove to be reliable in future cases, they will provide important implications about the contribution of the temporal lobes to normal intellectual or emotional activity.

Proposed course of the project: The next year's cases should provide adequate numbers and better control data to check the above implications. And sufficient data on a variety of additional questionnaires, perceptual and intellectual tasks more recently adopted, will help us describe better some of the important functions of the temporal lobe in man.

Part B included Yes ☐ No ☒

Serial No. NINDB 73(c)
1. Surgical Neurology Branch
2. Section on Clinical Psychology
3. Bethesda, Maryland
4. Same as NINDB - 90 (c)

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Body Temperature in Chimpanzees with Bilateral Temporal Lobe Damage.

Principal Investigator: Mildred L. Blevins

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1959):
Total: 0.0
Professional: 0.0
Other: 0.0

Patient Days (calendar year 1959):
None

Project Description:

Objective: To record the rectal temperature of normal chimpanzees and chimpanzees with damage to both temporal lobes.

Major Findings: This project was discontinued as the principal investigator has left the Institute.

Part B included Yes ☐ No ☒

Serial No. NINDB-74(c)
1. Surgical Neurology Branch
2. Section on Pain
3. Bethesda, Maryland
4. NINDB-91(c), 1958

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Fluothane Studies.

Principal Investigator: Kenneth D. Hall, M. D.

Other Investigators: Philip Geisler, M. D., Forbes H. Norris, Jr., M.D., Wm. L. Pritchard, M.D.

Cooperating Units: None

Man Years (calendar year 1959):
Total: 0.0
Professional: 0.0
Other: 0.0

Patient days (Calendar Year 1959)
0

Project Description:

Objectives: Same as NINDB-91(c), 1958.

Methods Employed: Same as NINDB-91(c), 1958

Major Findings: Same as NINDB-91(c), 1958.

Proposed Course of the Project: This project was discontinued upon resignation of the principal investigator.

Serial No. NINDB-75(c)
1. Surgical Neurology Branch
2. Section on Pain
3. Bethesda, Maryland
4. NINDB-92(c), 1958

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Hypothermia in Neuroanesthesiology

Principal Investigator: William Lee Pritchard, M. D.

Other Investigators: Charles A. Bucknam, M. D.
Shelley Chou, M. D.

Cooperating Units: None

Man Years (calendar year 1959):
Total: 1.3
Professional: 0.8
Other: 0.5

Patient days
(Calendar Year 1959)
0

Project Description:

Objectives: Hypothermia has been well accepted as a useful adjunct to certain neurosurgical procedures. Further evaluation of established techniques on patients and development of more effective techniques in the animal laboratory are the objectives of this project.

Methods Employed: Hypothermia by immersion in ice water bath is being employed on all patients with space-occupying lesions and vascular lesions. Observations on "brain fall-away", retractability, and amount of bleeding are made at operation. Various physiological parameters are monitored during the procedure. Post-operatively, the patient is watched carefully for signs of cerebral edema or hemorrhage.

In the animal laboratory a technique has been developed by which the brain may be selectively cooled by the shunting of inflow blood to the brain through a cooling apparatus. The outflow blood from the brain is then shunted through a warming apparatus. A sigmamotor pump is used to pump the blood from each heat exchanger to the brain and back to the heart, respectively.

Another technique is currently being studied in which profound hypothermia of the total body is produced to levels where cardiac standstill results. This is effected by the employment of an extracorporeal pump-oxygenator with partial cardio-pulmonary bypass, circulating the venous blood from the animal through an oxygenator, a heat exchanger, and then back into the animal's arterial system.

Major Findings: Further experience with ice water immersion hypothermia to levels of 30°C. in patients has substantiated the previous impression that this technique is beneficial in neurosurgical procedures. Bleeding has been less of a problem, brain retraction is facilitated by an increased extracerebral space, and post-operative edema and hemorrhage have been less frequently seen. Cardiac arrhythmias have not been observed at these temperature levels and there have not been any other serious complications of hypothermia.

The laboratory investigation on dogs has been rewarding in that it was possible to work out the technique of selective brain cooling so that the brain could be lowered to a temperature of 12-15°C. while the esophageal temperature was maintained at 25-30°C. The majority of the animals survived, but a few of them succumbed to ventricular fibrillation. However, it would seem that the multiple cannulations and vascular surgery in the neck might be somewhat cumbersome for clinical application.

With this in mind, total body hypothermia by blood stream cooling is now being evaluated. Insufficient work has as yet been done to allow conclusions.

Significance to Neurological Research: The clinical applications of hypothermia would indicate that low temperatures, even in the moderate range which has been used, are of value in neurosurgical procedures.

Experimentally, it has been shown that much lower brain temperatures may be reached by selective blood stream cooling. This procedure may be too cumbersome to allow clinical application. However, if the total body cooling procedure can be sufficiently refined, very deep levels of hypothermia could be attained and unusual advantages would be afforded the neurosurgeon in having a practically bloodless field and considerably more space to work in.

Proposed Course of the Project: It is intended to continue the use of moderate levels of hypothermia on patients, and to perfect the laboratory procedures utilizing deep hypothermia in the hope that clinical application may be feasible in the near future.

Part B included: Yes ☐ No ☒

Serial No. NINDB-76(c)
1. Surgical Neurology Branch
2. Section on Pain
3. Bethesda, Maryland
4. NINDB-93(c), 1958

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Succinyl Choline in Awake Craniotomy

Principal Investigator: William Lee Pritchard, M. D.

Other Investigators: Maitland Baldwin, M. D.
Charles A. Bucknam, M. D.

Cooperating Units: None

Man Years (calendar year 1959):
Total: 1.4
Professional: 0.9
Other: 0.5

Patient days (Calendar Year 1959)
0

Project Description:

Objectives: To produce a state of anesthesia in which the subject, patient or animal, is awake, able to cooperate, and suffering no discomfort. Such a state is particularly valuable when electroencephalographic and cortical stimulation studies are required, such as in the surgery of epilepsy and in studying the electrical activity of the chimpanzee brain.

Methods Employed: The subject is induced by conventional anesthetic technique and is then paralyzed by intravenous administration of succinyl choline. He is then allowed to awaken and is mechanically respirated, while in a paralyzed state. The drip of succinyl choline is then carefully titrated to the point where the subject can communicate by sign language and motor activity resulting from cortical stimulation may be observed. Thus sensory phenomena and motor responces may be noted by observers.

Major Findings: This technique has been further refined to the point at present so that it is being well tolerated by seizure patients and the desired results are being obtained. Improvements have resulted largely from trials with chimpanzees.

Significance to Neurological Research: This method has developed to become a valuable addition to the anesthetic management of patients undergoing epilepsy surgery.

Proposed Course of the Project: Attempts to further refine this technique will be made by increased use on seizure patients.

Part B included: Yes ☐ No ☒

Serial No. NINDB-77(c)
1. Surgical Neurology Branch
2. Section on Pain
3. Bethesda, Maryland
4. NINDB-94(c), 1958

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: The Effect of Hypertonic Urea Solution on Intracranial Pressure.

Principal Investigator: William Lee Pritchard, M. D.

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1959):
Total: 0.3
Professional: 0.3
Other: 0.0

Patient days (Calendar Year 1959)
0

Project Description:

Objectives: Evaluation of the use of hypertonic urea solution for the reduction of cerebral edema.

Methods Employed: Patients with intracranial mass lesions undergoing surgery are observed for relief of edema or reduction of brain volume following the administration of intravenous urea solution. Patients on the ward having clinical evidence of increased intracranial pressure are given urea, following which clinical signs are carefully observed. Blood studies and urine volumes are followed.

Major Findings: The desired effect of reduction of cerebral edema and intracranial pressure has been uniformly observed, both in the operating room and on the wards. No serious side effects have been noted, with the exception of two cases in which there were transient ischemic syndromes in the extremities in which the urea was administered by vein.

Significance to Neurological Research: These findings support those from other institutions regarding the outstanding value of this new clinical tool.

Proposed Course of the Project: The project will continue in the manner outlined above.

Part B included: Yes ☐ No ☒

ANNUAL REPORT
OPHTHALMOLOGY BRANCH
NATIONAL INSTITUTE OF NEUROLOGICAL
DISEASES AND BLINDNESS

Calendar Year 1959

The activity of the Branch in 1959 benefited greatly from the appointment of three investigators on the Visiting Scientist program. They were Dr. K. Tansley, Dr. P. Lele and Dr. K. Tasaki. In my opinion their contributions, which are not as yet published, will be of considerable value in the various fields. Where new techniques are developed and taught, their studies serve as a platform for further investigations by permanent members of the staff. The appointment of these scientists expired in August. All who had the opportunity to work with them are grateful for what they could learn, and feel the loss of these most competent workers.

The arrival of the eminent Dr. W. Rushton, working with Dr. Fuortes as a Visiting Scientist since July of this year, appears to be of the greatest impact on the research efforts of this Branch since its inception. The members of this Branch, and may I say of the Institute, will consider themselves fortunate to witness the investigative approaches of such a brilliant man.

Dr. O'Rourke, who assisted most effectively in administrative matters and as a teacher in the past year until July, was forced to terminate his assignment as a consultant because of his obligations to Georgetown University. His talent of organization and professional competence are painfully missed.

The two-year stay of all four Clinical Associates and one Research Associate ended or is ending this year, and new young ophthalmologists will be joining the clinical staff. Such rotation of the young investigators unavoidably causes difficult situations in view of its adverse effect on the continuation of projects both of clinical and laboratory nature. But the fact remains gratefully acknowledged that these talented young men have substantially contributed to various project areas during their two years and that they have been stimulated to further work in the line of their interest in new positions.

In view of the number of individual projects, the results can only be briefly summarized. Again, in the limelight of basic research interests are the studies on elementary

processes of vision conducted by Dr. Fuortes and co-workers. The experiments on the Limulus eye showed that physiologic light stimuli decrease the resistance of the nerve cells, but to only a moderate degree and specifically, that resistance of the cell membrane is not greatly changed during impulse activity. There are various explanations for this phenomenon. Dr. Fuortes feels that the experimental results on inhibition of the Limulus eye support the theory of non-involvement of cell somata by impulse activity and that impulses originate in the axon. Properties of transmission from photoreceptor structures to the nerve cell of the Limulus have been studied by analysis of subliminal responses to dim light. Dr. Fuortes proposes the concept that discrete liberation of quantities of a transmitter substance is responsible for the transient depolarizing impulses which he had reported to occur under steady dim illumination. Dr. Fuortes and Dr. Rushton plan to study the fascinating possibility that each quantity of the transmitter substance is liberated by a single quantum of light.

Dr. K. Tasaki in Dr. Fuortes' laboratory worked on hyperpolarizing potentials elicited by light in the fish retina and thought that these potentials originated either in large cells or in extracellular compartments.

Results of Dr. Tansley's co-worker's ERG studies on pure-cone mammalian retinae (squirrels) are not as yet available, but evidence was obtained that certain squirrels have only cones as demonstrated by the absence of a shift in the peak luminosity of the spectral sensitivity curve from the light- to the dark-adapted state. The investigators hope that these studies on tree and ground squirrels will contribute to our knowledge of the fundamental mechanisms of color vision.

In the clinical counterpart of this work, Drs. Copenhaver, Gunkel, and Goodman examined the physiology of cone and rod vision of patients with various forms of retinal degeneration and color defects in which in addition to their elaborate methods of psychophysical testing, spectral sensitivity curves were obtained. The usefulness of the methods used in this laboratory for clinical purposes were shown in the results obtained on patients with various types of juvenile amaurotic family idiocy, as they allowed for distinction among the infantile, late infantile and juvenile form.

Dr. Gunkel played an important role in these studies, as a factor of effecting continuity in the ever-changing list of investigators in this laboratory (Bornschein, Iker, Goodman, Dodt, Tansley, Copenhaver and Jones). Adaptometry and retinal

profile studies are his responsibility alone, and his unusual talent for modifying or constructing new instruments, optical devices and most complex apparatus is appreciated by everyone.

Among the studies on aqueous dynamics and related problems of physiology and pathology of the intraocular pressure, the investigations of Lele and Grimes on the neural mechanism involved in regulating these functions deserve emphasis. The elaborate technique of Dr. Lele in securing intact and functioning eye nerve preparations allowed clarification of obscure conditions which could not be explained in previous experimental work, as for instance, the poor reproducibility of pressure-induced afferent impulses in vivo. The investigators attacked the problem in a comprehensive and systematic way by studying afferent nerve discharges of long ciliary nerves in response to intraocular pressure increases and obtained in almost all instances impulses in contrast to the many failures with the technique used previously in this laboratory. Again, in contrast to previous findings, no spontaneous activity signaling resting pressures was observed in such preparations or in vivo. The search for the site of origin of pressure-induced afferent activities did not render conclusive results, I believe, but merely suggested that such nerve structures are contained in the outer coats of the eye. The manyfold experiments of stimulating long and short ciliary nerves or the ciliary ganglion under various conditions are of great interest not only because of the effects on intraocular pressure and outflow but also because of the applicability of Dr. Lele's technique for related studies in this field.

For a systematic approach to studies of afferent and efferent impulse activity in the posterior ciliary nerves, a thorough knowledge of the complex anatomy of these nerves is necessary. Simple dissection does not permit the demonstration of fine branches, but they can be made visible under the dissecting microscope by the use of a modified silver technique (Grimes). With this method, differences in a given species and between species (cat, monkey, human) can be demonstrated.

Dr. Macri's important work on the intraocular pressure problem centered around the correlation of the venous pressure and the eye pressure, and attempts to demonstrate with his ingenious technique the effect of Diamox on both pressures. Dr. Macri's new concept implies that the carbonic anhydrase inhibitor lowers the venous pressure of the intraocular vasculature selectively without interfering with the general blood pressure. His distinction between a monophasic and biphasic outflow pattern, as reported last year, was the subject of further experimentation. The change from one pattern to another

by pharmacological agents (epinephrine) or by sectioning preganglionic sympathetic fibers poses intriguing problems which need further clarification. In view of his interest in vascular effects connected with the intraocular pressure, Dr. Macri elaborated and studied for the first time, using a cast technique, the cat and monkey venous ocular circulatory system which is closely connected with the aqueous outflow system. As a species variation of the cat anastomosing vessels connect the anterior ciliary vein and the vortex veins. The cast technique also showed beautifully the filling of intraocular aqueous veins in the cat and the as yet unexplained connection between anterior chamber and the suprachoroidal space in the monkey.

A similar area, intraocular venography, was further perfected by Dr. Cohen. Using an intricate radiological technique, he succeeded in demonstrating venous channels of very small caliber. He also showed that introductions of Hypaque in the anterior chamber is well tolerated and can be used clinically to establish the presence of subclinical forms of choroidal detachment.

The clinical program dealing with intraocular pressure pathology (glaucoma) is developing more and more into a well-rounded and planned study, due to the stimulating interest and experience of Dr. Okun and the increase of personnel. His specific plans for study are well taken and promising, as the diurnal changes of outflow patterns, the effect of various sympathomimetic and anticholinesterase drugs on the aqueous outflow and measurements to estimate the rate of aqueous formation in health in patients with borderline glaucoma and those with frank glaucoma of various types.

The outstanding contribution to the cataract project is related to the demonstration of the ultrafine structures of the epithelium and the fibers in the normal and cataractous lens by Dr. Wanko and Miss Gavin. As far as comparisons with light microscopic studies are concerned, a fair parallel of findings obtained with the two techniques was demonstrated in some respects, but our knowledge of fine changes in the cytoplasm and nucleoplasm was vastly enriched by electron microscopy. This new information was particularly exciting in the visualization of X-ray induced changes in cytoplasmic components at an early stage after irradiation, whereas in the past, such changes were considered as relatively late effects. Thecooperation of the electron microscopists and the biochemist (Dr. Resnik) proved fruitful, since it was possible to show that the fine filamentous elements described by Dr. Wanko as possibly characteristic for the lens are products of all interactions of soluble proteins and the fixative. Dr. Wanko and Miss Gavin are studying

at present the fine morphology of lens material from patients with senile cataract which should allow interesting comparisons with the results reported by these investigators on various forms of experimental cataract.

Dr. Resnik has made great progress in his physiochemical studies of lens proteins. He has demonstrated the great complexity of the soluble lens proteins of which 8 fractions have been separated. Transformation of these fractions induced by freezing or by the use of solvents indicate the difficulties in arriving at an understanding of this protein system. Dr. Resnik collaborated in various enzyme studies with Dr. Kuhlman which will be taken up later as these studies do not deal to any great measure with the lens.

The cooperative study of the histological laboratory of this Branch with the laboratory of Nutrition and Endocrinology of NIAMD resulted in a piece of work which might have practical interest. Dr. M. Reid of NIAMD had carried out nutritional studies on the guinea pig for many years and has observed the frequent occurrence of cataracts when only tryptophane was deficient in the diet. The growth curves of these animals approached the normal, but cataract developed regularly and early. It was shown that animals on a moderately supplemented diet strived at a normal scale and that lens changes developed in other apparently healthy animals. The histologic features of this cataract differed from other types of experimental lenticular opacities as they did not involve the equatorial area where new fibers form. The clinical similarity of tryptophane deficiency cataract and that of various forms of perinuclear cataract in the human was striking.

The greatest number of patients admitted to the ward are those with uveitis. Although they are studied thoroughly with all available means little has been accomplished in this as in other ophthalmology departments in establishing the etiology, pathogenesis and rationale of therapy of this disease. An approach based on the understanding of immunological mechanisms might improve the precarious situation of our ignorance. It was for this reason that Dr. van Alphen's interest in the immunological relations of ocular tissues is so timely. It provides hope that the responses of eye tissues to disease will be better comprehended. For the time being Dr. van Alphen plans to study the immune responses of the cornea and lens in certain dermatoses. He might expand his work to the uveal problem in the future.

Records of a large group of patients with uveitis and positive toxoplasma dye tests admitted in the past 5 years to the Institute, have been studied by Dr. Kaufman with emphasis

on certain diagnostic and therapeutic aspects. Some of the conclusions are of interest. The readily available toxoplasmine skin test seemed to be reliable as in the great majority of patients with positive reaction was in agreement with the positive result of the toxoplasma dye test. A false positive skin test was extremely rare. Patients with a positive serological skin test and uveitis reacted satisfactorily to antitoxoplasma chemotherapy in about 50%. The addition of corticosteroids to the therapy with Daraprim and sulfa in patients who did not respond to the chemotherapy alone, improved the condition but infrequently. Early onset of the uveitis under the age of 20 years and an acute or subacute course can be considered favorable for a therapeutic success, whereas in a chronic course the prognosis is less good.

Dr. O'Rourke has tabulated the results of his thyroid hormone turnover studies in uveitis (30 patients with uveitis and 10 controls). He states that hypometabolism co-exists with many chronic diseases including uveitis and that no definite conclusions can be drawn from the data available at the present time.

The following investigations cannot be grouped under one of the main projects. They are partly clinical and partly laboratory studies.

Dr. van Alphen has completed his fundamental study on basic factors in refraction anomalies. The manuscript is not available as yet but the statistical analysis it contains would not lend itself to a short abstract. Dr. van Alphen arrives at the conclusion that the often-defended and often-questioned theory of Steiger on the origin of refraction anomalies is untenable. Pertinent to Dr. van Alphen's theory which he considers tentative is the behavior of the choroid as a sheet muscle. His examinations on correlated pressure changes in the subscleral space and the anterior chamber examined with a variety of techniques and strain gauge measurements on the exposed choroid strongly suggest that the assumption of this function of the choroid is correct.

In the clinical study on detection of ocular tumors by isotope tracer methods, Dr. O'Rourke collected the results from 8 patients with malignant melanomas in the choroid which were studied with respect to the radiophosphorous uptake in the diseased tissue. Counts of beta-emissions obtained by the routine anterior counting procedure were unreliable and sometimes misleading. Posterior counting provided information for a correct localization of the tumor although the highest radioactivity did not always lie directly over the center of the tumor. This discrepancy was demonstrated by comparing the count sheets with the pathologic sections.

A new project on a cytological level originated from a comparative study suggested by the Academy of Ophthalmology and Otolaryngology in which the use of alpha-chymotrypsin in cataract surgery and the side effects of using this enzyme for zonulolysis were investigated. For demonstrating possible injurious effects on the corneal endothelium it was necessary to develop a technique in which the entire cell population of this tissue could be examined. With this method it was shown that irrigation of the anterior chamber with 0.9 solution of NaCl and with the enzyme produced damage to the endothelium, but the lesions were wider spread when alpha-chymotrypsin was used. One of the significant results of this study is the demonstration of karyokinetic cell division in the normal endothelium of young adult animals. It was generally assumed in the past that the corneal endothelium multiplies by amitosis. It is planned to extend studies on the endothelium as this cell layer plays an important role in maintaining the normal transparency of the cornea and its healthy condition influencing the means of corneal transplantation with penetrating grafts. Little is known about the physiology and pathology of this tissue, suggesting that the studies should be extended to tissue culture and electron microscopic techniques.

Dr. Wanko and Miss Gavin have collected more material from muscle biopsies for electron microscopic examinations. They described in normal muscle fibers heterogenous accumulation of packed granules which, on the basis of histochemical reactions, might be considered to be a lipofuscin.

A clinical and histological study carried out by Dr. Paton and me on a tri-racial isolate population in Halifax county which was shown to have a familial disorder involving the eyes (Dr. Graham of Duke University and Dr. Witkop of the NDI) revealed that the eye lesions consisted of plaques of hyperplastic epithelium with signs of dyskeratosis and acanthosis located in the perilimbal zone. The middle layer of epithelium was the site of dyskeratotic changes, whereas the superficial epithelium underwent parakeratotic changes. Later it was discovered that the evaluated members of this population had also buccal mucosa lesions which were interpreted by the dentist as "white sponge nevus." It appears then that a new type of familial dyskeratosis involves outstandingly the conjunctiva bulbi and is accompanied by similar changes in the buccal mucosa.

The following studies by Drs. Paton and Copenhaver are essentially case reports, but represent new and interesting observations. Dr. Paton described the occurrence of a primary malignant melanoma of the choroid in a patient who had a similar lesion in the lids and the adjacent skin of the same site. Such co-existence of malignant melanomas at these two sites have not been

reported in the past. Based on a previous report from this Branch, the fundus picture of angioid streaks was described in detail by Dr. Paton in three patients with sickle cell anemia. It appeared unlikely that these two diseases co-existed by chance. The patients did not exhibit signs of pseudoxanthoma elasticum and Paget's disease, the usual systemic diseases connected with angioid streaks. The new observations might necessitate a change in the concept of the pathogenesis of angioid streaks.

Dr. Copenhaver gave a dramatic account of the detection of the self-inflicted nature of a lesion which has been labeled with a whole battery of diagnostic possibilities. The patient had lost the right eye by self-mutilation and presented a large ulcer of the bulbar conjunctiva and the adjacent tissues of the other eye when she was admitted to the hospital as a diagnostic problem. She was observed to induce injury to her only eye by placing tablets of aspirin into the conjunctival sac. After discharge a psychiatrist obtained a confession from the patient as to her self-mutilating tendency.

Not grouped with other reports are the findings of two new studies by Dr. Kuhlman who applied Lurie's technique of microchemical enzyme determinations to the primary and secondary aqueous humor of rabbits. Malic and lactic dehydrogenase present in small amounts in the normal aqueous humor appeared increased three- to four-fold in samples of the secondary aqueous. The enzyme contained in old animals was lower than in young rabbits.--Following the investigations on the enzyme systems in the cornea as reported last year, Dr. Kuhlman subjected another collagenous tissue--the collagen of the developing epiphyseal plate--to microchemical procedures similar to those employed previously in examinations of the normal and irradiated corneal epithelium. The following enzyme activities were assayed: lactic, malic, and glucose-6-phosphate dehydrogenase; phosphoglucoisomerase; aldolase; alkaline phosphatase. Primary spongiosa and cartilage cells contained more total phosphorous solids and acid-soluble material than unorganized cartilage cells. The activity of both lactic dehydrogenase and phosphoglucoisomerase was higher in the organized cartilage area and lower in the unorganized cartilage area. Malic dehydrogenase activity paralleled calcification, but was more uniformly distributed. Aldolase activity was not detectable by the assay method used. 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 general, an overall increase in enzyme activity with the maturation of animals. The interpretation of the accumulated data must await further studies.

In concluding this summary it might be justified to point to the participation of the members of this staff at various meetings and to the considerable number of scientific papers completed in the report period as listed in the two enclosures. It speaks for the unlimited energy and devotion of the investigators to their work, for the outstanding technical assistance, for the dedication of the nursing staff and the secretarial staff, that the program could be continued and expanded. All members of this Branch are grateful for advice and help they received by the Directors whenever such help was sought.

Ludwig von Sallmann, M.D.
Chief, Ophthalmology Branch
National Institute of Neurological
Diseases and Blindness

PUBLICATIONS *
Ophthalmology Branch
1959

1. Cohan, B.E.: Experimental intraocular venography, A.M.A. Arch. Ophth. (in press).

2. Copenhaver, R.M. and Gunkel, R.D.: The spectral sensitivity of color-defective subjects determined by electroretinography, A.M.A. Arch. Ophth. 62:55-68, 1959.

3. Copenhaver, R.M. and Goodman, G.: The electroretinogram in infantile, late infantile and juvenile amaurotic family idiocy, A.M.A. Arch. Ophth. (in press).

4. Copenhaver, R.M.: A report of an unusual self-inflicted eye injury, A.M.A. Arch. Ophth. (in press).

5. Fuortes, M.G.F.: Discontinuous potentials evoked by sustained illumination in the eye of Limulus, Arch. ital. Biol. 97:243-250, 1959.

6. Fuortes, M.G.F.: Integrative mechanisms in the nervous system, Amer. Nat. 93:213-224, 1959.

7. Frank, K., Fuortes, M.G.F., and Nelson, P.G.: Voltage-clamp of motoneurone soma, Science, 130:38-39, 1959.

8. Gavin, M.A. and Lloyd, B.J., Jr.: Knives of high silica content glass for thin-sectioning, J. Biophys. Biochem. Cytol. 5:507, 1959.

9. Kaufman, H.E. and Caldwell, L.A.: Pharmacological studies of pyrimethamine (Daraprim) in man, A.M.A. Arch. Ophth. 61:885-890, 1959.

10. Kaufman, H.E., Remington, J., Melton, M.L., and Jacobs, L.: Relative resistance of slow-growing strains of toxoplasma gondii to pyrimethamine (Daraprim), A.M.A. Arch. Ophth. 62:611-615, 1959.

11. Kaufman, H.E. and Thomas, L.B.: The vitreous opacities diagnostic of familial primary amyloidosis, New Eng. J. Med. (in press).

12. Kaufman, H.E.: Primary familial amyloidosis, Annals of Intern. Med. (in press).

13. Remington, J.S., Jacobs, L. and Kaufman, H.E.: Adult toxoplasmosis, New Eng. J. Med. (in press).

* Publications listed as "in press" in the report of 1958 are not included here.

14. Kuhlman, R.E. and Resnik, R.A.: Enzymic studies of X-irradiated cornea and lens with special reference to glutathione reductase, Biochem. J. 72:261-265, 1959.

15. Kuhlman, R.E.: Species variation in the enzyme content of the corneal epithelium. J. Cell. and Comp. Physiol. 53:314-326, 1959.

16. Kuhlman, R.E.: A microchemical study of the developing epiphyseal plate, J. of Bone and Joint Surgery, (in press).

17. Kuhlman, R.E. and Kaufman, H.E.: A microchemical study of the aqueous humor enzyme-protein interrelations, A.M.A. Arch. Ophth. (in press).

18. Kuhlman, R.E. and Resnik, R.A.: The oxidation of C^{14}-labeled glucose and lactate by the rabbit cornea, Arch. Biochem. Biophys. (in press).

19. Lele, P.P. and Grimes, P.A.: The role of neural mechanisms in the regulation of intraocular pressure in the cat, Exper. Neuro. (in press).

20. Paton, D. and Thomas, L.B.: Simultaneous occurrence of the primary malignant melanomas of the eye and the skin, A.M.A. Arch. Ophth. 62:645-652, 1959.

21. Paton, D.: Angioid streaks and sickle cell anemia, (A report of two cases), A.M.A. Arch. Ophth. (in press).

22. Resnik, R.A. and Kenton, E.B.: Lens Proteins: II. The effect of pH on alpha crystallin, Am. J. Ophth. 48:52-55, 1959.

23. Resnik, R.A., Wanko, T. and Gavin, M.A.: Observations on the lens proteins alpha and beta crystallin, Am. J. Ophth. (in press).

24. Resnik, R.A., Wanko, T. and Gavin, M.A.: Observations on a cytoplasmic component in lens fibers, J. Biophys. Biochem. Cytol. (in press).

25. Tasaki, K.: Some observations on the retinal potentials of the fish, Arch. ital. Biol. (in press).

26. von Sallmann, L., Collins, E.M. and Grimes, P.A.: Mimosine cataract, Am. J. Ophth. 47:107-117, 1959.

27. Reid, M.E. and von Sallmann, L.: Nutritional studies with the guinea pig. VI. Tryptophan (with ample dietary niacin). J. of Nutrition, (in press).

28. von Sallmann, L., Reid, M.E., Grimes, P.A. and Collins, E.M.: Tryptophan-deficiency cataract in guinea pigs, A.M.A. Arch. Ophth. 62:662-672, 1959.

29. von Sallmann, L.: Experimental studies of some ocular effects of alpha chymotrypsin, Trans. Am. Acad. Ophth. Otol, (in press).

30. von Sallmann, L.: Familial dyskeratosis of the perilimbal conjunctiva, Trans. Am. Ophth. Soc., (in press).

31. von Sallmann, L. and Paton, D.: Hereditary dyskeratosis of the bulbar conjunctiva and oral mucosa. I. Ocular Manifestations, A.M.A. Arch. Ophth. (in press).

32. Wanko, T. and Gavin, M.A.: Electron microscope study of lens fibers, J. Biophys. Biochem. Cytol. 6:97-102, 1959.

33. Wanko, T., von Sallmann, L. and Gavin, M.A.: Early changes in the lens epithelium after Roentgen irradiation, A.M.A. Arch. Ophth. (in press).

1959 Presentations
Ophthalmology Branch

Fourth Conference on Ophthalmic Biochemistry, Cambridge, Massachusetts, February 21-22, 1959:

Kuhlman, R.E.: The Oxidation of C^{14}-labeled Glucose and Lactate by the Rabbit Cornea.

60th Annual Meeting of the American Roentgen Ray Society, Cincinnati, Ohio, September 22-25, 1959:

Cohan, B.E.: Experimental Intraocular Venography (exhibit)

Midwinter National Meeting of the Association for Research in Ophthalmology, Edgewater Park, Mississippi, February 19-21, 1959:

Resnik, K.A.: Observations on the Lens Protein, Alpha and Beta Crystallin.

Wanko, T.: Structural Changes in the Lens Epithelium after Roentgen Irradiation. An Electron Microscope Study.

American Medical Association Meeting, Atlantic City, New Jersey, June 8-12, 1959:

Wanko, T.: The Crystallin Lens (exhibit)

American Society for Pharmacology and Experimental Therapeutics, Coral Gables, Florida, August 31-September 3, 1959:

Macri, F.J.: Acetazolemide and the Venous Pressure of the Cat Eye.

American Society of Parasitologists, Penn State College, Pennsylvania, August 30-September 2, 1959:

Kaufman, H.E.: The Relationship of Toxoplasma Dye Test and Neutralization Antibodies.

Conference on Inhibition in the Nervous System and Y-Aminobutyric Acid, Duarte, California, May 22-24, 1959:

Fuortes, M.G.F.: Inhibition in Limulus Eye.

95th Annual Meeting of the American Ophthalmology Society, Hot Springs, Virginia, May 28-30, 1959:

von Sallmann, L.: Familial Dyskeratosis of the Perilimbal Conjunctiva

American Academy of Ophthalmology and Otolaryngology, Chicago, Illinois, October 11-16, 1959:

von Sallmann, L.: Some Ocular Effects of Alpha Chymotrypsin.

Symposium "Long Term Effects of Chronic Radiation Exposure," Bethesda, Maryland, October 29-30, 1959:

von Sallmann, L.: Delayed Effects of Chronic Radiation. (Discussion)

Combined Clinical Staff Meeting, National Institutes of Health, Bethesda, Maryland, March 26, 1959:

von Sallmann, L.: Primary Amyloidosis (moderator)

ADDENDUM

1958 Presentations
Ophthalmology Branch

Symposium "Integrative Mechanisms in Biology" Meeting of the American Society of Naturalists, Washington, D.C., December 29, 1958:

Fuortes, M.G.F.: Integrated Mechanisms in the Nervous System.

Washington Society for Electron Microscopy, Fifth Meeting, Bethesda, Maryland, December 12, 1958:

Wanko, T.: The Fine Structure of Lens Epithelium.

Addendum to Serial No. NINDB 44 (c) 1958
1. Ophthalmology Branch
2. Microbiology Section
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
Calendar Year 1959

Part B. Honors, Awards and Publications

Publications other than abstracts from this project:

Kaufman, H.E. and Thomas, L.B.: The vitreous opacities diagnostic of familial primary amyloidosis, New Eng. J. Med. (in press).

Honors and Awards relating to this project: None

Addendum to Serial No. NINDB-59 (c) 1958
1. Ophthalmology Branch
2. Physiology Section
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
Calendar Year 1959

Part B. Honors, Awards and Publications

Publications other than abstracts from this project:

Copenhaver, R. M., and Gunkel, R. D.: The spectral sensitivity of color-defective subjects determined by electroretinography, A.M.A. Arch. Ophth., 62:55-68, 1959.

Honors and awards relating to this project: None

Addendum to Serial No. NIMDB-85 (c)
1. Ophthalmology Branch
2. Chemistry Section
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
Calendar Year 1959

Part B. Honors, Awards and Publications

Publications other than abstracts from this project:

Kuhlman, R.E. and Resnik, R.A.: Enzyme studies of X-irradiated cornea and lens with special reference to glutathione reductase. Biochem. J. 72:621-265, 1959.

Kuhlman, R.E. and Resnik, R.A.: The oxidation of C^{14}-labeled glucose and lactate by the rabbit cornea, Arch. Biochem. Biophys. (in press).

Honors and Awards relating to this project: None

Serial No. NINDB-78 (c)
1. Ophthalmology Branch
2.
3. Bethesda, Maryland
4. Same as NINDB-4[illegible] (c)

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Basic Factors in Refraction Anomalies

Principal Investigator: Gerard van Alphen, M.D.

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1959): Patient Days: None
Total: 0.5
Professional: 0.5
Other: 0

Project Description:

Objectives: Same as last year and essentially aimed at completing a theory on the origin of refraction anomalies.

Methods:

1) Statistical: factor analysis with rotation of the correlation matrix of the five optical elements based on the data of stenatrom.

2) Physiological: the degree that the tension in the choroid counteracts the intraocular pressure is determined by measuring the (a) pressure in the suprachoroidal space as described last year. (b) relative tension in the choroid by direct strain gauge measurement at various sites, before and after sympathetic and parasympathetic stimulation. (c) the effect of (b) on the relationship of intraocular pressure versus scleral elasticity.

Major Findings: Three oblique factors may explain the correlations between the five optical elements in the human eye. The load of these factors indicates existing links between the various optical elements and these links can be tentatively explained. A major assumption in this concept is that the choroid essentially behaves as a sheet muscle and therefore counteracts part of the intraocular pressure. For this assumption additional evidence is obtained from strain gauge measurements on both in situ experiments and experiments on isolated eyes, in monkeys and cats.

Significance to Program of Institute: As Steiger's theory on the origin of refraction anomalies, although widely adhered to, is obviously untenable, no adequate explanation exists for the mechanism of emmetropia or amétrópia. The loads of the 3 basic factors involved reveal links between the various optical elements and a tentative interpretation of these links may form a basis for a theory on refraction anomalies.

Proposed Course of Project: To pursue the effect of the tension in the choroid on the elasticity of the sclera by strain gauge measurements on eyes in situ and isolated eyes. To complete a concept as to the possible mechanism of emmetropia or its aberrations.

Part B included: Yes ☐ No ☒

Serial No. NINDB-79 (c)
1. Ophthalmology Branch
2.
3. Bethesda, Maryland
4. Same as NINDB-48 (c)

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Immunological Relations in Ocular Tissues

Principal Investigator: Gerard W. van Alphen, M. D.

Other Investigator: Sarah Robinette, B. S.

Cooperating Units: None

Man Years (calendar Year 1959): Patient Days: None
Total: 1.5
Professional: 0.5
Other: 1.0

Project Description:

Objectives: To examine possible immunological relations between skin, conjunctiva, cornea and lens; to determine whether last year's findings of immunological interrelationships between various eye tissues is confined to the species or whether organ specificity can be demonstrated; to test the possibility of autosensitization against various eye tissues; to investigate the reaction of the lens after passive cellular transfer of hypersensitivity to the anterior chamber of the eye; to find immunological clues for ocular involvement in patients with various allergic dermatoses.

Methods Employed: After enucleation of one eye, guinea pigs are immunized to their own lens, own corneal epithelium and own vitreous. The tissues are homogenized with Freund's adjuvant to enhance antibody production. Their blood is tested for antibodies by the Ouchterlony method. Anti-calf eye tissue sera are obtained by immunizing guinea pigs with calf lens, vitreous, corneal epithelium, corneal stroma, conjunctiva, and

skin. The sera are tested for mutual cross reactions and for cross reactions with various eye tissues of some mammals and some other major representatives of the vertebrates by means of agar diffusion methods. Rabbits are immunized with repeated injections on homologous lens in the neck and the foot pads. Lymphocytes are collected from the regional lymph glands and transferred to the anterior chamber of the eye.

Major Findings: Skin, conjunctiva, corneal epithelium, and corneal stroma are related immunologically, as well as lens, corneal epithelium and vitreous; however, skin and lens are not. Of the various eye tissues tested, organ specificity is almost exclusively confined to the lens; and anti-calf lens serum not only cross reacts with the lens, but also with the vitreous of the different vertebrates. Corneal epithelium appears to be a relatively strong antigen as compared to corneal stroma. Antibodies against own eye tissues could not be demonstrated by present methods. The results of passive cellular transfer of hypersensitivity to the anterior chamber of the eye and its effect on cornea and lens is as yet conjectural.

Significance to Program of Institute: A knowledge of immunological relations between various tissues is theoretically intriguing and necessary for an understanding of the response of the eye to disease. It supplies a theoretical basis for certain experimental and clinical observations which assume immunological relations between skin, conjunctiva and cornea, but it obviously does not support alleged immunological mechanisms in dermatoses associated with cataracts. The relatively high antigenicity of corneal epithelium as compared to corneal stroma strongly supports recent suggestions that the removal of epithelium of a corneal transplant may decrease an immune response.

Proposed Course of Project: To repeat the experiments on autosensitization in inbred guinea pigs (strain 13) and to complete current experiments. To study patients with certain dermatoses showing ocular manifestations of cornea and lens.

Part B included: Yes ☐ No ☒

Serial No. NIMDB-80 (c)
1. Ophthalmology Branch
2.
3. Bethesda, Maryland
4. Same as NIMDB-43 (c)

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Clinical Glaucoma Study

Principal Investigators: Edward Okun, M.D.
Ludwig von Sallmann, M.D.

Other Investigators: Bruce Cohen, M.D.
Ottiwell Jones, III, M.D.
Richard Oglesby, M.D.

Cooperating Units: None

Man Years (calendar year 1959): Patient Days: 1,453
Total: 5.6
Professional: 4.1
Other: 1.5

Project Description:

Objectives:

1) The main objective is to evaluate suspected and known cases of glaucoma in an effort to find earlier means of diagnosis and better means of control. The principal tool of investigation is repeated tonography, with and without provocative testing.

a) The mechanism of diurnal changes in intraocular pressure are investigated by means of tonography.

b) The mechanism of action of various drugs and operative procedures is studied by tonography.

c) The effect of instantaneous blood pressure changes on the intraocular tension is to be investigated by tonographic means, in association with the Heart Institute.

2) To further investigate the disturbed intraocular fluid dynamics in glaucoma, the inflow mechanisms are studied. Just as tonography gives an indication of the ease with which fluid leaves the eye, the inflow studies give an indication of the ease with which fluid enters the eye.

Methods Employed: Each patient acceptable for the study is admitted to the hospital for a minimum of several days in order that an extensive glaucoma workup can be performed. In addition to the history and general physical examination, specific tests include tonometry with day curve, applanation tonometry, biomicroscopy, gonioscopy, repeated tonography with and without provocative tests, and studies of recovery from tonography as determined with the applanation tonometer. In addition, visual field studies are carefully performed using the Goldmann perimeter and Gunkel tangent screen. When the patient's intraocular fluid dynamics have been observed over a time interval, the response to indicated therapy is subsequently evaluated. Patients are followed at four to six month intervals after discharge, at which time they are re-evaluated.

Major Findings: At the present time, the number of patients in this study is too small, and their period of follow too short to draw many conclusions from this long-term project. On an individual basis, we feel that we have encountered some extremely provocative cases illustrative of a broad spectrum of glaucoma forms. In one or two cases of secondary glaucoma the diagnosis was suspected on the basis of tonography alone, and subsequently validated when episodes of increased tension occurred. In other cases, repeated provocative tests and tonography have failed to incriminate glaucoma during an initial admission and the diagnosis made in subsequent months. At the present time, the cases of borderline glaucoma have not been followed for a sufficient time to judge the value of the data obtained.

Proposed Course of Project: It is hoped that the size of this project will continue to increase. A reservoir of well-studied patients who reside in the Washington area is being built up and it is hoped that by continuing studies on this increasing population a means of earlier diagnosis can be established. The next logical step will be the evaluation of various means to delay the onset of manifest glaucoma.

Significance to Program of Institute: The described project is of timely importance to the Public Health Service due to the growing awareness of doctors and laymen alike concerning the significance of early glaucoma diagnosis. Through clinical experiments with glaucoma, laboratory investigations are further inspired. It is emphasized that this Institute offers a unique opportunity for such detailed observations of glaucoma patients, for routine laboratory studies can be repeatedly performed under a considerably longer period of hospitalization than is available to private institutions. Through the quality of prolonged study rather than through the quantity of patients examined, this project should offer information which cannot be readily gained elsewhere.

Part B included: Yes ☐ No ☒

Serial No. NINDB-81 (c)
1. Ophthalmology Branch
2.
3. Bethesda, Maryland
4. New Project

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Simultaneous Occurrence of Primary Malignant Melanoma of the Eye and Skin

Principal Investigators: David Paton, M. D.
Louis B. Thomas, M. D.

Other Investigators: None

Cooperating Units: Department of Pathologic Anatomy
National Cancer Institute

Man Years (calendar year 1959): Patient Days: 35
Total: 0.5
Professional: 0.5
Other:

Project Description:

Objectives: To contribute to the question, first, of coexistence of primary malignant melanomas of the eye and melanomas at other sites; second, of the familial occurrence of this uveal tumor.

Methods Employed: Routine clinical and histological examinations were performed.

Major Findings: The case reported had a pigmented skin lesion for six years covering the lids and adjacent tissue of the right eye, which was repeatedly treated with electrocautery during this span. Biopsies of a skin nodule indicated that the lesion was a malignant melanoma. The patient was admitted to the NIH. A pigmented area near the macula of the right eye was detected, and wide excision of the skin lesion and orbital

exenteration was performed in the NCI. The histological examination of the skin showed junctional nevus and a nodule with the characteristics of superficial malignant melanoma. In the conjunctiva, signs of pre-cancerous melanosis were observed and the choroid tumor was diagnosed as an early malignant melanoma with spindle cells of type A and B. Clinically, however, the appearance of the choroidal melanoma was compatible with the benign lesion. The choroidal tumor and the cutaneous melanoma were considered as independent primary neoplasms which occurred simultaneously in this patient. The patient's brother was subjected to enucleation of the right eye for malignant choroid melanoma nine years ago.

Significance to Program of Institute: This is a new observation of the simultaneous occurrence of primary malignant melanomas in the skin and the choroid. A familial occurrence of such eye tumors is extremely rare.

Proposed Course of Project: This project has been terminated.

Part B included: Yes [X] No [ ]

Serial No. NINDB-81 (c)

PHS - NIH
Individual Project Report
Calendar Year 1959

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Paton, D. and Thomas, L.B.: Simultaneous occurrence of primary malignant melanomas of the eye and the skin. A.M.A. Arch. Ophth. 62:645-652, 1959.

Honors and Awards relating to this project: None

Serial No. NINDB-82 (c)
1. Ophthalmology Branch
2.
3. Bethesda, Maryland
4. Same as NINDB-50 (c)

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Intraocular Angiography

Principal Investigator: Bruce E. Cohan, M. D.

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1959): Patient Days: None
Total: 1.0
Professional: 1.0
Other: 0

Project Description:

Objectives: Recent studies previously reported have demonstrated the initial pathways of aqueous humor outflow in the rabbit by means of radiographs of the eye taken after replacement of aqueous humor by water soluble radiopaque solutions. It is thought that by modifying these techniques and devising new ones, further information may be obtained concerning queous outflow channels and intraocular vessels in an essentially intact eye.

Methods Employed:

1) The aqueous humor is replaced by the same volume of sodium diatrizote (Hypaque) in a control rabbit eye and in an eye immediately following cyclodialysis. Radiography of these eyes is then carried out.

2) Through a small conjunctival incision the anterior ciliary vein of the cat's eye is isolated, divided between ligatures and both cut ends cannulated. Radiographs are taken during injection of sodium diatrizote (Hypaque) by hand or with a screw-type electrical infusion pump. Clinical and

histologic studies of the eyes are made at varying intervals after the x-rays are obtained. A number of radiographic techniques are employed to obtain the intraocular venous patterns in various projections.

Major Findings: The techniques mentioned above have resulted in the successful demonstration of (1) the roentgen-anatomy at the acute cyclodialysis in the rabbit eye, and (2) the roentgen-anatomy of the intraocular venous systems in the essentially intact cat eye, including the intrascleral venous plexus, the veins of the ciliary body, the choroidal venous system and venous plexus of the optic nerve. Clinical and histologic studies of the cat eyes made at varying intervals after the procedure revealed essentially no permanent damage.

Significance to Program of Institute: It is hoped that studies mentioned above may lead to better understanding of:

1) The dynamic anatomy of the intrascleral venous plexus which is intimately related to the mechanism of aqueous outflow and, therefore, glaucoma.

2) The dynamic anatomic pattern of the veins of the ciliary body and choroid which are of importance in the physiology and pathology of intraocular pressure and in disease of the uveal tract, i.e., uveitis.

3) The possibilities for future possibile investigation of the intraocular veins in man by angiographic methods.

Proposed Course of Project: It is proposed to complete the initial sutdies utilizing these new techniques.

Part B included Yes [XX] No [ ]

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Cohan, Bruce E.: Experimental intraocular venography. A.M.A. Arch. Ophth. (In Press).

Honors and Awards relating to this project:

Scientific Exhibit "Experimental Intraocular Venography" was awarded the Bronze Medal of the American Roentgen Ray Society at its 60th Annual Meeting in Cincinnati, Ohio, September 22-25, 1959.

Serial No. NINDB-83 (c)
1. Ophthalmology Branch
2.
3. Bethesda, Maryland
4. Same as NINDB-46 (c)

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Thyroid Hormone Turnover in Uveitis

Principal Investigator: James F. O'Rourke, M. D.

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1959)
Total: 0.5
Professional: 0.5
Other: 0

Patient Days:

Outpatient Visits:

Project Description:

Objectives: Radiothyroxine turnover studies in uveitis: a measure of peripheral utilization of thyroid hormone in patients with uveal inflammation.

Methods Employed: Patients with uveitis are screened for evidence of thyroid dysfunction. Those showing hypometabolism but euthyroid status, are selected for the turnover study. Fifty microcurie injections of I^{131} thyroxine are made intravenously and the plasma radioactivity is measured daily for 15 days thereafter.

From the data provided, the half-time turnover of thyroxine is calculated linearly as well as derived from formula. Additional factors studied are the measure of the extrathyroidal thyroxine pool, percentage of iodine pool utilized daily per kilogram of body weight and per square meter of body surface.

Ten control patients have also been studied, ranging in age from 18 to 68 years.

Major Findings:

Thirty uveitis patients and 10 controls

	Range			Mean		
	Normal Value	Control Patients (10)	Uveitis Patients (30)	Norm. Value	Control Patients (10)	Uveitis Patients (30)
T 1/2	5.6-9.5	5.6-8.8	7.3-14.2	6.71	7.53	10.21
% of Iodine pool utilized daily	9.0-12.2	7.87-12.37	4.91-10.50	10.52	9.25	6.81
Iodine utilized daily (micro. gm/70 kg.)	48-69	45-63	23-72	52	54	47
Iodine utilized daily (micro. gm/1.73 Sq.M)	41-71	41-65	21-71	51	53	44

The results of the study so far do not tell anything about uveitis etiology or its responsiveness to treatment. A report on the measurements taken to date is being prepared for publication. It is likely that hypometabolism co-exists with many chronic diseases, including uveitis, but the significance of the association could only be shown by: (a) demonstrating that thyroid function or dysfunction can influence the immune response in patients with uveitis (Dr. Bergenstal has good ideas about how to study this) and, (b) demonstrating in hypometabolic patients that the kinetics of thyroxine turnover and the natural history of uveitis are each influenced by therapy with thyroid hormones. (Thyroxine or Triiodothyronine).

Significance to Program of Institute: Laboratory findings connected with the most threatening unsolved problem in clinical ophthalmology, that is, uveitis, are of paramount interest. However, at present no definite conclusions can be drawn from the available data of the present study.

Proposed Course of Project: Dr. O'Rourke has terminated his assignment as consultant to the Ophthalmology Branch as of July 1959. The project cannot be continued in the near future. It is hoped that it can be reactivated at a later date.

Part B included: Yes [ ] No [XX]

Serial No. NINDB-84 (c)
1. Ophthalmology Branch
2.
3. Bethesda, Maryland
4. Same as NINDB-47 (c)

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Detection of Ocular Tumor by Isotope Tracer Methods

Principal Investigator: James F. O'Rourke, M.D.

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1959): Patient Days: 332
Total: 0.5
Professional: 0.5
Other:

Project Description:

Objectives: Radiophosphorous uptake of malignant melanoma of the posterior choroid: comparison of trans-conjunctival and trans-scleral counting methods.

Methods Employed: Five hundred microcuries of sodium radiophosphate is given intravenously to patients showing solid detachment of the retina. Both anterior (trans-conjunctival) and posterior (trans-scleral) counting is done after 24 hours under general anesthesia in the operating room as part of the procedure of planned enucleation. In each case four quadrants are counted for one minute each, the first quadrant being the area shown to have highest radioactivity during a preliminary scan. Anterior counts are made at the equator with the globe rotated maximally, using a forceps. Posterior counts are made on bare sclera following surgical exposure and detachment of one rectus muscle.

The comparisons made between the two counting techniques are the following: (a) difference in total radioactivity (anterior versus posterior) as expressed by counts per minute recorded for four quadrants, (b) difference in radioactivity recorded over the highest single quadrant in each area, expressed as percent increase above the four quadrant mean, (c) accuracy of localization, that is, correspondence of the highest single quadrant with the known site of the tumor bed.

Major Findings:

1) Total radioactivity detected by the posterior counting technique exceeds the anterior value by 74% in six patients studied (range 53% to 120%).

2) (a) The highest anterior quadrant count exceeded the mean value by 25.3% in 8 cases counted (range 5.2% to 48.8%). (b) The highest posterior quadrant count exceeded the mean value by 74.3% (range 47% to 100.3% in 6 cases).

3) (a) The highest anterior quadrant count failed to correspond to the tumor bed location in six or eight cases studied. (b) The highest posterior quadrant count was localized correctly in the quadrant containing the tumor in six consecutive studies made.

4) The point of highest radioactivity during posterior counting (as marked on the sclera during operation) did not always lie directly over the center of the tumor mass as demonstrated on pathologic section.

Significance to Program of Institute: The study as conducted to date has failed to develop any really new knowledge; it merely demonstrates something that is already well known by workers in this field. A report on the above findings is being prepared. Further clinical studies are needed to extend the series but suitable cases for this purpose are rare. Basic studies are possible using the Cloudman S-91 melanoma; these might be used to examine the tumor uptake of gamma-emitting sources or to devise an autoradiographic method of ocular counting.

Proposed Course of Project: It is planned to continue the project when patients with uveal malignant melanomas or with other intraocular tumors can be admitted.

Part B included: Yes ☐ No ☒

Serial No. NINDB-85 (c)
1. Ophthalmology Branch
2. Pharmacology Section
3. Bethesda, Maryland
4. New Project

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Study on a Possible Second Aqueous Outflow Pathway in the Eye

Principal Investigator: Frank J. Macri, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1959): Patient Days: None
Total: 0.8
Professional: 0.4
Other: 0.4

Project Description:

Objectives: Casts made by injection into the anterior chamber showed filling of the ciliary body of three species: cats, rabbits and monkeys. In monkeys, the material was also found, having a sheet-like appearance, in the suprachoroidal space (twelve of twelve monkeys). This filling was found in addition to that of the aqueous veins, etc. Whether or not this represents a second outflow pathway or anatomic damage due to perfusion of cast material is not known. This observation together with the knowledge that outflow of the eye could be altered to a "biphasic" pattern in which the proportionality of outflow to pressure was lost, indicated the possibility of a second outflow system. Preliminary experiments on monkey eyes showed the presence of fluorescein in the suprachoroidal space after the material was injected into the anterior chamber, lending further support for this study.

Methods Employed: In these initial experiments, enucleated, arterially perfused eyes are used. The intraocular and venous pressures are measured so that true outflow pressure may be calculated. A small incision (2-3 mm.) is made in the sclera and the choroid and sclera separated circularly for a distance of about 5 mm. A piece of thin Saran Wrap is inserted between the two layers of tissue. A piece of #10 polyethylene tubing, bent to coincide with the curvature of the eye, is then placed in the suprachoroidal space, through the incision and above the Saran Wrap insert. The insert prevents the tearing of the choroid by the tubing during its placement. By means of a preplaced matress suture, the tubing is tied in place and the incision tightly closed. It is felt that if flow to the suprachoroidal space does exist, this procedure may allow for its quantitation. Total flow from the anterior chamber will also be determined so that, by difference, flow through the aqueous veins may be calculated.

Major Findings: There are no major findings as yet because the project has only recently been initiated.

Significance to Program of Institute: Should this study show that a second aqueous outflow mechanism functions under physiologic conditions, this finding would be new and of great interest for all studies on aqueous dynamics.

Proposed Course of Project: Continuation of this project with modified techniques is planned.

Part B included: Yes [ ] No [X]

Serial No. NINDB-86 (c)
1. Ophthalmology Branch
2. Pharmacology Section
3. Bethesda, Maryland
4. New Project

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Anatomy of the Venous Circulation of the Cat Eye and the Aqueous Outflow Channels in the Cat, Rabbit and Monkey.

Principal Investigator: Frank J. Macri, Ph.D.

Other Investigator: None

Cooperating Units: None

Man Years: (calendar year 1959)
Total: 0.4
Professional: 0.2
Other: 0.2

Project Description:

Objectives: The cat eye is being rather extensively used to elucidate mechanisms which affect or control the intraocular pressure. Before this study was undertaken there was no detailed anatomic description of either the venous circulatory system or of the aqueous outflow system in this species. This fact together with the observation that intraocular pressure and venous pressure are intimately related made it of great importance to determine the venous and aqueous outflow systems.

Methods Employed: Casts are made by injection of Tygon paint, hardening for 24 hours in 70% alcohol and then digestion with NaOH.

Major Findings: The major difference in the venous anatomy of the cat as compared to the human or monkey was the large anastomotic connection between the anterior ciliary

vein and the ampulla of the vortices. 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. 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 well seen and their interconnections noted.

Casts made by anterior chamber injection showed filling of the ciliary body as well as the expected filling of aqueous veins, etc. In the monkey the material went beyond the ciliary body and was found in the suprachoroidal space as a broad, thin sheet. In no case was the cast noted to communicate with the vasculature of the ciliary body area.

Significance to Program of Institute: The elucidation of the venous network allowed an explanation for the similarity of venous pressure recorded from the vortex and anterior ciliary vein in the cat. It was of fundamental interest that filling of the ciliary body was obtained; this being more extensive in the monkey in which cast material was seen in the suprachoroidal space. This is suggestive of a second outflow pathway which is at present being investigated.

Proposed Course of Project: This project will be terminated.

Part B included: Yes [ ] No [X]

Serial No. NINDB-87 (c)
1. Ophthalmology Branch
2. Pharmacology Section
3. Bethesda, Maryland
4. Same as NINDB-42 (c)

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Study on the Pharmacodynamics of Various Agents Affecting Intraocular Pressure

Principal Investigator: Frank J. Macri, Ph.D.

Other Investigator: Ludwig von Sallmann, M. D.

Cooperating Units: None

Man Years (calendar year 1959):
Total: 0.8
Professional: 0.4
Other: 0.4

Patient Days: None

Outpatient Visits: None

Project Description:

Objectives: To test various drugs for their ability to alter intraocular pressure and to determine their mechanisms of action.

Methods Employed: Various factors known to be of importance in the maintenance of IOP are measured and recorded. These are the "facility of aqueous outflow" (flow expressed as cmm/min/mm.Hg), venous pressure in different veins of the eye, elasticity and aqueous inflow. In addition to the measurements of IOP and the local venous pressures, the systemic arterial blood pressures are also recorded to determine the correlation between these functions. Cats and monkeys are used.

Major Findings: It was reported last year that two patterns of aqueous outflow could be discriminated by the methods employed. The first pattern which we called "monophasic" was one in which outflow (expressed as "C", cmm/min/mm.Hg) was proportional to outflow pressure throughout the range of pressures studied. The second pattern called "biphasic" was one in which this proportionality was upset and in which "C"

decreased progressively as the intraocular pressure was elevated. The values of "C" in the biphasic outflows were always greater than those of the monophasic at pressures close to the normal for the animal studied.

Diamox, an agent found effective in lowering intraocular pressure of glaucomatous patients, was studied rather intensively. The only activity ascribed to Diamox in the literature is its ability to inhibit carbonic anhydrase and through this action a decrease of aqueous inflow is postulated. At this time, we take no exception that this is, in part at least, a mechanism whereby the intraocular pressure is lowered.

It was reported last year that Diamox was capable of reducing the venous pressure of the cat and monkey eye and also converted a monophasic outflow to one which was biphasic. Further work done during the current year has confirmed these findings. Two questions then arise:
1. Does the fall of venous pressure evoke the fall of intraocular pressure induced by Diamox, or vice versa?
2. What is the mechanism of the biphasic outflow?

1. Diamox lowering of venous pressure.

A. It was first ascertained that Diamox acted directly on the eye to lower the venous pressure of this organ. This was done by perfusing enucleated cat eye through the ophthalmic artery with Eagle's Basal Medium containing 10% plasma. Perfusion with fluid containing Diamox, in concentrations similar to those used "in vivo", produced falls of both the intraocular pressure and venous pressure.

B. The intraocular pressures of 15 cat eyes (in vivo) were maintained constant at different pressure levels by connecting the anterior chambers of these eyes with pressure bottles. In 12 of these eyes the venous pressure was found to fall after the administration of Diamox. No significant change of systemic blood pressure was noted. In another series of animals, the blood pressure was raised and lowered by various means, so that an influence on the venous pressure of the eye would be exerted. In every case, the intraocular pressure was found to change in the same direction and to the same degree as the venous pressure. Raising or lowering the intraocular pressure from normal levels only produced falls of venous pressure. It may then be said that the intraocular pressure is directly dependant on venous pressure and that Diamox lowers intraocular pressure, in part at least, through this mechanism.

2. Mechanism of biphasic outflow.

A biphasic outflow could be induced by section of the preganglionic fibers of the superior cervical ganglion. Stimulation of this nerve produced a rise of both intraocular pressure and venous pressure, as reported last year, as well as reversing the outflow back to the monophasic pattern. Reversal of the biphasic pattern could also be effected by topical application of a 10% emulsion of phenylephrine or anterior chamber injection of epinephrine or arterenol.

Often times, after the stimulation of the ciliary ganglion (effectiveness as noted by miosis and a synchronous fall of both intraocular pressure and venous pressure) the monophasic outflow pattern would convert to the biphasic form. Whether this represents suppression of the sympathetic nervous pathways is not clear. It appears then that the conversion of the monophasic outflow to the biphasic one can be regulated by the autonomic nervous system.

In these studies it was found that reducing vitreous volume by removing aliquots of vitreous could convert a monophasic outflow to the biphasic; or if the biphasic form existed previously this would be further exaggerated by the procedure. The reverse phenomenon was observed by increasing vitreous volume. As a working hypothesis, it is concluded that the two outflow patterns cited are dependant on the volume in the vitreous compartment and that this volume (probably through the vasculature) is under the control of the autonomic nervous system.

Significance to Program of Institute: Studies on aqueous dynamics might contribute to the understanding of the pathology of intraocular pressure in relation to the glaucoma problem.

Proposed Course of Project: All studies on this project will be continued.

Part B included Yes [X] No [ ]

Serial No. NINDB-88 (c)
1. Ophthalmology Branch
2. Chemistry Section
3. Bethesda, Maryland
4. Same as NINDB-54 (c)

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: A Study of the Proteins of the Lens

Principal Investigator: Robert A. Resnik, Ph.D.

Other Investigators: John Papaconstantinou, Ph.D. and Theodor Wanko, M.D.

Cooperating Units: Department of Embryology
Carnegie Institute of Washington

Man Years (calendar year 1959): Patient Days: None
Total: 2.0
Professional: 1.0
Other:

Project Description:

Objectives: To obtain physical and chemical data on the lens protein.

Methods Employed: Methods and techniques commonly used in the physical-chemical characterization of polymers and polyelectrolytes, are used. In addition, techniques used for the studies of proteins are employed.

Major Findings: The fractionation of the soluble lens protein from the lens cortex by column chromatography has indicated the complexity of this system. A total of eight fractions, A-H, has been obtained. Of these only G and H are homogeneous when subjected to boundary electrophoresis. Fractions A, B, C, D, E, and F each contain two to three components. Some of the proteins in these fractions are capable

of undergoing various transformations. For example, the amount of protein in fraction F increases at the expense of that in fraction G. This transformation may be induced by freezing and thawing a mixture of the lens proteins. In addition, similar treatment of fraction G alone results in the appearance of fraction F. The transformation is due to the effect of solvent, pH 7, 0.005 M phosphate buffer. Fractions A-E have been referred to in the past as beta-crystallin while F-H have been denoted as alpha-crystallin. This work has been done with Dr. John Papaconstantinou.

In collaboration with Dr. T. Wanko, electron microscopic observation of electrophoretically isolated samples of three fractions of the lens proteins has been carried out. It has been observed that the fraction denoted as alpha-crystallin contains compound, elongated structures. The samples obtained from the remainder of the soluble proteins contain spheres of different sizes.

The electron microscopy of the lens protein has been discontinued at this time because of the complexity of the lens protein system.

Significance to Program of Institute: The identification of those proteins in the lens which are particularly susceptible to alteration or interaction brings us one step closer to a basis for correlating chemical changes in the lens with its transparency.

It is now recognized that the low density elements observed by the electron microscopist in sections of osmium-fixed lens are the lens proteins.

Proposed Course of Project: The chemical basis for the transformation of some of the lens proteins will be studied. It is possible that this may be correlated with the gelation of alpha-crystallin below its isoelectric point.

Part B included: Yes [X] No [ ]

Serial No. NINDB-88 (c)

PHS - NIH
Individual Project Report
Calendar Year 1959

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Resnik, R.A. and Kenton, E.B.: Lens protein: II. The effect of pH on alpha-crystallin. Am. J. Ophth. 48:52-55, 1959.

Resnik, R.A., Wanko, T. and Gavin, M.A.: Observations on the lens proteins alpha- and beta-crystallin. Am. J. Ophth. (In Press)

Honors and Awards relating to this project: None

Serial No. NINDB-89 (c)
1. Ophthalmology Branch
2. Chemistry Section
3. Bethesda, Maryland
4. New Project

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: An Investigation of the Enzyme Systems Present in Cornea and Other Collagenous Tissues

Principal Investigator: Robert E. Kuhlman

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1959): Patient Days: None
Total: 1.0
Professional: 1.0
Other: 0

Project Description:

Objectives: To study the calcification of the collagen of the developing epiphyseal plate.

Methods Employed: Total solids, lipids, phosphorous, and acid soluble material were determined. In addition, the following enzyme activities were assayed: lactic, malic, and glucose-6-phosphate dehydrogenase; phosphoglucoisomerase; aldolase; alkaline phosphatase.

Major Findings: The total phosphorous, solids, and acid soluble material were all found to be higher in the primary spongiosa and cartilage cells as opposed to the unorganized cartilage cells

The activity of both lactic dehydrogenase and phosphoglucoisomerase is higher in the organized cartilage area and lower in the unorganized cartilage area.

Malic dehydrogenase activity parallels calcification, but is more uniformly distributed.

Aldolase activity was not detectable by the assay method used.

Glucose-6-phosphate dehydrogenase doubles in activity as the primary spongiosa develops.

Alkaline phosphatase activity increases with each advancing stage of calcification. There is, in general, an overall increase in enzyme activity with the maturation of animals.

Significance to Program of Institute: Past studies in this laboratory have indicated that the activity of the enzyme of the cornea vary both with age and the specific layer of cornea studied. It was therefore decided to study the development of other collagenous tissue.

Proposed Course of Project: This project is terminated.

Part B included: Yes [X] No [ ]

Serial No. NINDB-89 (c)

PHS - NIH
Individual Project Report
Calendar Year 1959

Part B. Honors, Awards and Publications

Publications other than abstracts from this project:

Kuhlman, R.E.: A microchemical study of the developing epiphyseal plate, J. of Bone and Joint Surgery, (In Press).

Honors and Awards relating to this project: None

Serial No. NINDB-90 (c)
1. Ophthalmology Branch
2. Chemistry Section
3. Bethesda, Maryland
4. New Project

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: An Investigation of the Enzymatic Systems Present in the Aqueous Humor

Principal Investigator: Robert E. Kuhlman, M.D.

Other Investigators: Herbert E. Kaufman, M.D.

Cooperating Units: None

Man Years (calendar year 1959): Patient Days: None
Total: 0.5
Professional: 0.5
Other: 0

Project Description:

Objectives: Microchemical procedures for the assay of enzymes and metabolites in micro samples of aqueous humor are developed and analyses performed.

Methods Employed: Lactic, malic and glucose-6-phosphate dehydrogenase, together with protein concentration, were determined in the aqueous humor and blood of rabbits.

Major Findings: Glucose-6-phosphate dehydrogenase was not detectable in the aqueous of rabbits. Malic and lactic dehydrogenase were present in the aqueous of normal rabbits in relatively small amounts. The activity of these enzymes increased three to four fold in samples of aqueous humor subsequently removed. The protein concentration in these samples of aqueous was about 40 times greater than the first samples drawn. Because of the complications of increased protein concentration, it is difficult to interpret the results obtained [illegible] secondary [illegible]

The enzyme levels in aqueous obtained from old animals was lower than that found in young animals.

Significance to Program of Institute: The results implicates age as a factor related to the activity of enzymes in the aqueous humor. The traumatic effects of removing small amounts of aqueous emphasized that due consideration be given to this when interpreting such data. It is possible that acute uveitis caused by allergy or endotoxin may cause a relatively low enzyme to protein ratio in the aqueous similar to that observed in secondary aqueous.

Proposed Course of Project: This project is terminated with these results.

Pa[illegible] Involved: Yes [X] No [ ]

Serial No. NINDB-90 (c)

PHS - NIH
Individual Project Report
Calendar Year 1959

Part B. Honors, Awards and Publications

Publications other than abstracts from this project:

Kuhlman, R.E. and Kaufman, H.E.: A microchemical study of the aqueous humor enzyme-protein interrelations, A.M.A. Arch. Ophth. (In Press)

Honors and awards relating to this project: None

Serial No. NINDB-91 (c
1 Ophthalmology Branch
2. Physiology Section
3. Bethesda, Maryland
4. Same as NINDB-39 (c

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Studies on Central Nervous System Control of Intraocular Pressure. (Anatomy of Posterior Ciliary Nerves)

Principal Investigators: Ludwig von Sallmann, M. D.
Patricia Grimes, B. A.

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1959): Patient Days: None
Total: 1.0
Professional: 0.5
Other: 0.5

Project Description:

Objectives: Studies on the anatomy of the posterior ciliary nerves, as outlined in last year's report, have been extended first to determine the variations of this nerve supply to the eye in one species (cat) and, second, to establish interspecial differences. Human material was subjected to using the same technical procedures as used in the cat and monkey preparations. This technique and dissection has been elaborated by Miss Grimes. An improved photographic technique allowed clear demonstration of pertinent observations.

Methods Employed: The orbital contents are removed completely including the nerve supply as far back as the fifth nerve ganglion. The extraocular muscles are carefully dissected away and the [illegible] ciliary nerves. For examination [illegible]

washed in water for at least one hour, and is then placed in an 0.5% solution of silver nitrate for two hours. At the end of this time, the eye is transferred to 10% formalin for 10 minutes, and, finally, to a 5% solution of sodium thiosulfate for approximately 30 minutes. Treatment with silver nitrate and the subsequent steps are carried out in darkness. In the resulting preparation nerves are stained a dark brown while ganglionic tissue remains white. Other tissues are unstained. Dissection is accomplished under water using the Zeiss stereomicroscope, and photographs are taken as warranted.

In some instances fresh material is dissected, and the relationship of certain branches to the ciliary and posterior ganglion is determined. These branches are then removed, fixed in 1% osmium tetroxide, embedded, and cross-sectioned. Data on fiber-size distribution in the various nerves thus may be obtained.

Major Findings: The examinations of the human orbital nerves showed a distribution of posterior ciliary branches similar to those observed in the monkey. Occasionally a V nerve branch was seen to travel along the optic nerve to the posterior pole of the globe without undergoing anastomosis in the orbit. Such branches not fusing with postganglionic branches of the ciliary ganglion have been demonstrated in the cat but not in the monkey. On the other hand, the human ciliary ganglion received a heavy branch from the V nerve corresponding to the fine branches to the ganglion in the monkey. Such connections between the V nerve and the ganglion could not be demonstrated in the cat.

Significance to Program of Institute: The understanding of the course, ramifications and fusion of posterior ciliary nerves makes it possible to select the proper pathways for studies of afferent and efferent impulses and to stimulate nerves which could be classified. The advantage of the knowledge of the topography of these nerves and their distribution is shown in the study by Lele and Grimes.

Proposed Course of Project: The investigation has been completed. It is planned to present the results at the meeting of the Association for Research in Ophthalmology in 1960.

Publ. Included: Yes [ ] No [X]

Serial No. NINDB-92 (c)
1. Ophthalmology Branch
2. Physiology Section
3. Bethesda, Maryland
4. New Project

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: An Unusual Self-Inflicted Eye Injury

Principal Investigator: Richard M. Copenhaver, M.D.

Other Investigator:

Cooperating Units: None

Man Years (Calendar Year 1959): Patient Days: 121
Total: 0.2
Professional: 0.2
Other: 0

Project Description:

Objectives: To provide a basis for the differential diagnosis of ulcerations in the conjunctiva or other parts of the diseased eye and self-inflicted lesions.

Methods Employed: Clinical observations; histological examinations of biopsies from the conjunctiva.

Major Findings: A thirty-four-year-old white female had lost the right eye because of a relapsing ulcerative process of the cornea and conjunctiva of unknown etiology. The eye was enucleated because of pain at the request of the patient. The bulbar conjunctiva of the left eye developed a large ulcer, the nature of which could not be diagnosed. Lethal midline granuloma, actinomycosis, tuberculosis, syphilis, collagen disease, lymphogranuloma venereum, granuloma inguinale, histoplasmosis, sporotrichosis

and cryptococcosis were mentioned as diagnostic possibilities. The location of the extensive conjunctival lesion suggested self-mutilation, and the biopsy, which showed necrotic tissues surrounded by a wall of healthy epithelium, supported this suspicion. Maderosis, broken lashes and small corneal abrasions provided evidence that the patient had inflicted injury to her only eye. Later it was discovered that she introduced parts of aspirin tabloid into the conjunctival sac. The patient's arms were placed in casts. Following this, the conjunctival ulcerations and other signs of irritation improved rapidly. The psychiatric consultant suggested that the manipulations on her eye might be a compulsive symptom of this inhibited, isolated woman. Psychiatric examination carried out after the patient was discharged accomplished a confession of the patient as to the self-mutilation of the eye which had led to the loss of one eye and extensive damage to the other.

Significance to Program of Institute: See Objectives.

Proposed Course of Project: The study has been terminated.

Part B included: Yes ☒ No ☐

Serial No. NINDB-92 (c)

PHS - NIH
Individual Project Report
Calendar Year 1959

Part B. Honors, Awards and Publications

Publications other than abstracts from this project:

Copenhaver, R.M.: A report of an unusual self-inflicted eye injury. A.M.A. Arch. Ophth. (in press).

Honors and Awards relating to this project: None

Serial No. NINDB-93 (c)
1. Ophthalmology Branch
2. Physiology Section
3. Bethesda, Maryland
4. New Project

PHS - NIH
Individual Project Report
Calender Year 1959

Part A.

Project Title: Angioid Streaks and Sickle Cell Anemia

Principal Investigator: David Paton, M.D.

Other Investigators: Ludwig von Sallmann, M.D.

Cooperating Units: None

Man Years (calendar year 1959): Patient Days: 93
Total: 0.5
Professional: 0.5
Other:

Project Description:

Objectives: "Angioid streaks" on the fundus have been correlated with pseudoxanthoma elasticum or Paget's disease. The observation of this is rare, but characteristic fundus pictures in two patients with sickle cell disease suggests that pathogenic factors, other than those considered now, might be responsible for the development of the lesion.

Methods Employed: Funduscopy, biomicroscopy, fundus photography, and histological examination of skin biopsy, in addition to the usual hematological, radiological and physical examinations.

Major Findings: The fundus picture observed in two young Negro males with sickle cell anemia were typical for angioid streaks; the maculae were not involved and the vision not impaired. Vascular calcifications seen frequently in patients with angioid streaks could not be demonstrated. Skin biopsies taken from the neck and axilla showed no evidence of

pseudoxanthoma elasticum. The observation of a third young Negro with sickle cell disease was made available for study from the Wilmer Institute. Again, the fundus showed typical angioid streaks. Histological examination of eyes of patients with sickle cell disease and angioid streaks are not available as yet. The absence of signs of pseudoxanthoma elasticum and Paget's disease or classification of vascular calcifications indicate that either the anemia, retinal hemorrhages or the occlusive vascular disease plays a role in the pathogenesis of the fundus changes.

Significance to Program of Institute: The concept that angioid streaks are exclusively connected with pseudoxanthoma elasticum and Paget's disease has to be corrected and other pathogenic factors such as occlusive vascular disease have to be considered as possibly correlated with the typical fundus picture.

Proposed Course of Project: Studies on this rare eye condition will be continued when patients are available.

Part B included: Yes [X] No [ ]

Serial No. NINDB-93 (c)

PHS - NIH
Individual Project Report
Calendar Year 1959

Part B. Honors, Awards and Publications

Publications other than abstracts from this project:

Paton, D.: Angioid streaks and sickle cell anemia. (A report of two cases) A.M.A. Arch. Ophth. (In Press)

Honors and Awards relating to this project: None

Serial No. NINDB-94 (c)
1. Ophthalmology Branch
2. Physiology Section
3. Bethesda, Maryland
4. Same as NINDB-40 (c)

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: The Study of Neural Mechanisms in the Regulation of Intraocular Pressure in the Cat

Principal Investigator: P.P. Lele, M.D.

Other Investigators: Patricia Grimes, B.A.

Cooperating Units: None

Man Years (calendar year 1959): Patient Days: None
Total: 2.0
Professional: 1.0
Other: 1.0

Project Description:

Objectives: In an effort to evaluate the role of the central nervous system in the regulation of intraocular pressure, experiments in this laboratory have been directed toward definition of the afferent and efferent limbs of a possible reflex mechanism. Previous studies using the isolated cat eye confirmed the observations of von Sallmann *et al* (1958) who described afferent impulses in the long ciliary nerves evoked by increased intraocular pressure. Although use of the isolated preparation simplified the experiments and allowed for accurate determination of anatomic structures, study of the pattern of afferent activity indicated probably fatigue of the nerve endings during prolonged increases in pressure. It was, therefore, planned to compare the results of experiments using the isolated eye with those *in situ*.

There have been numerous statements in the literature concerning pressure changes following stimulation of various efferent nerves supplying the eye. von Sallmann and Lowenstein (1955), and Gloster and Greaves (1957) described IOP changes in cats produced by stimulation of certain points in the dorsal hypothalmus. In addition to these observations, Perkins (1954) reported that antidromic mechanical stimulation of the fifth nerve in rabbits produced a marked increase of the IOP. Investigations have been carried out, therefore, to determine the effect on the IOP of electrical stimulation of the third and fifth cranial nerve using both in vitro and in situ preparations.

Methods Employed: Isolated Eye. The animal under light pentobarbital anesthesia is killed by air embolism. The eye with intact nerve supply is dissected out as rapidly as possible and placed in a bath of warm oxygenated Krebs-Ringer-Glucose solution. The extraocular muscles are carefully removed. In an effort to maintain the eye in the best possible condition the ophthalmic artery is cannulated and perfused with warm oxygenated KRG solution at such pressures as to maintain the IOP at approximately 20 mm. Hg. The third and fifth nerves are then dissected and prepared either for recording afferent activity or for stimulation. Two 22 gauge needles are inserted into the anterior chamber for recording IOP and for increasing the pressure by infusion.

In situ preparations. The animal is anesthetized with pentobarbital and the femoral artery and vein are cannulated for blood pressure recording and injection respectively. The skull is then opened and the frontal lobe of one cerebral hemisphere is removed to expose the roof of the orbit and the ophthalmic division of the fifth nerve in its intra-cranial course. The orbit is unroofed and the superior rectus muscle is removed to expose the nasociliary nerve and the proximal portions of the long ciliary nerves. The long ciliary nerves are then freed and prepared for recording of activity or stimulation at either the central or peripheral ends.

The ciliary ganglion, also, may be exposed by retraction of the lateral recti.

Major Findings: An afferent nerve discharge is evoked in the long ciliary nerves of both the isolated and in situ preparations when the IOP is raised by infusion into the anterior chamber. In the isolated eye the response consists of a transient phase, characterized by rapid firing which lasts as long as the pressure is rising, and a sustained phase of slow firing, the frequency of which declines slowly for 2-3 minutes. The total response includes the activity of not more

than 6 units. Experiments in situ yielded responses which were similar to those of the isolated eye except that the transient phase was less pronounced, probably due to adaptation of the globe to the higher testing pressures, and the sustained response continued for the length of the stimulus. The relationship between the amount of evoked activity and the pressure level was non-linear, and there was no evidence of different units coming into activity at different levels of IOP. No spontaneous activity was seen in 90% of 50 nerves tested.

Experiments designed to find the site of origin of the pressure-induced activity indicate strongly that it arises from nerves lying in the outer coats of the eye which respond to stretch.

Continuous "spontaneous" efferent activity was recorded from the central ends of the cut short ciliary nerves. Raising the pressure in the ipsilateral or contralateral eye failed to produce any detectable changes in the level of recorded activity. Efferent activity in the long ciliary nerves was also found to be unaffected by changes in the ipsilateral or contralateral eye pressure.

Stimulation of the retrobulbar nerves, however, did produce changes in the eye pressure. 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 ciliary ganglion (or its motor root) in the isolated perfused eye produced a symmetrical constriction of the pupil with a fall in intraocular pressure which is maintained for the length of the stimulus. The pressure change is reversed when the flow of fluid through the vessels is discontinued. Under these conditions there is a rise in pressure during stimulation. These same effects are observed in situ with and without circulation. Stimulation of the long ciliary nerves produced pupillary dilitation in all cases but there was no pressure change without circulation of fluid in the vessels. In the isolated perfused eye a fall of IOP occurred during stimulation. The magnitude of this fall was approximately 1/3 of that resulting from parasympathetic stimulation. No pressure changes were observed when the eyes of animals previously subjected to cervical sympathetic neurectomy were used (antidromic stimulation of afferent fifth nerve fibers).

Despite the observed pressure effects due to stimulation of the sympathetic and parasympathetic supply, retrobulbar nerve block did not affect the resting intraocular pressure in the anesthetized cat, nor did it affect the rate

of recovery of the IOP from small imposed changes. This is an interesting observation in view of the fact that IOP in man falls following nerve block (de Roetth).

In view of the relatively small amount of afferent activity elicited by IOP changes and the absence of "spontaneous" activity signalling resting pressures, it is doubtful that this sensory path carries enough information to participate normally in the regulation of IOP. However, the techniques employed do not permit recording the activity of very small fibers which may be involved in such regulation. Although there are pressure effects resulting from third and fifth nerve stimulation these do not appear to effect long-term control of the IOP.

This evidence indicates that in the cat the pathways investigated do not contribute to the normal maintenance of pressure in the eye.

Significance to Program of Institute: The results of these experiments have implications for any experimental studies of the intraocular pressure. The isolated eye preparation of functionally viable neuromuscular apparatus proved to be very suited for the problems under study and supplement essentially the results obtained in the living eye. This technique is also adaptable for studies on the ciliary body and pressures in various parts of the eye as indicated in the project of Dr. van Alphen, listed under basic factors in refraction anomalies.

Proposed Course of Project: This project will be carried on by Dr. von Sallmann due to the departure of Dr. Lele. Since there are so many reported differences between the rabbit eye and the cat eye with regard to neural effects in the IOP, it is suspected that species differences may be of considerable importance in the investigation of this problem. It is planned, therefore, to continue this work using the monkey eye, which is closer in structure to the human eye than is the rabbit or cat and should yield data more applicable to the situation in man.

Part B included: Yes [X] No [ ]

Serial No. NINDB-94 (c)

PHS - NIH
Individual Project Report
Calendar Year 1959

Part B. Honors, Awards and Publications

Publications other than abstracts from this project:

Lele, P.P. and Grimes, P.A.: The role of neural mechanisms in the regulation of intraocular pressure in the cat, Exp. Neurology, (In Press).

Serial No. NINDB-95 (c)
1. Ophthalmology Branch
2. Physiology Section
3. Bethesda, Maryland
4. Same as NINDB-60 (c)

FHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Design and Construction of Ophthalmic Instruments

Principal Investigator: Ralph D. Gunkel, O.D.

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1959): Patient Days: None
Total: 0.5
Professional: 0.5
Other: 0

Project Description:

Objectives: To make improvements of instruments used in clinical and laboratory ophthalmological work. Specifically, to design and/or construct such devices as are suggested or required by current projects.

Methods Employed: The trend of this project has been to involve the investigator more and more deeply in work with visiting scientists. Since the clinical associates usually are here for two years only, it has been helpful to have Dr. Gunkel provide a measure of continuity in the electroretinography and psychophysical testing programs. Techniques and instruments must usually be modified for each new study, and continued collaboration appears to be the most productive method.

Work with the modified adaptometer continues, in that perimetric light sense studies are routinely made on clinical patients having certain types of retinal degeneration, but the potential usefulness of this type of measurement appears to be much greater than time has permitted us to realize.

Numerous optical devices were constructed or adapted for the work with Dr. Tansley. These included sector discs and shutters for providing photic stimuli of different duration, flicker rates, or on/off ratios. Also required was an intense bleaching light which could be changed in color and introduced into the pupil without interfering with entry of the stimulating beam.

Frequent measurements of the spectral emission of our high-pressure Xenon lamp were required and occasional measurements of the absolute brightness of the stimulus beam.

Contact lenses and other devices were constructed as needs arose in the use and handling of laboratory animals.

Major Findings: The light sense studies have demonstrated characteristic types of photopic profiles in four different kinds of color blindness deficiency. They also have shown macular impairment in numerous cases where it was suspected but not demonstrated by visual field testing.

The obvious result of the development of the various laboratory aids and devices has been to facilitate the work of the project involved. These findings are discussed more specifically under the projects of Dr. Tansley and Dr. Copenhaver.

Significance to Program of Institute: It is expected that this work will be useful for the purpose of distinguishing photopic and scotopic mechanisms (work with Dr. Tansley) and that continuation of the psychophysical testing of patients with color sense disturbances and various forms of retinal degeneration will provide important information for the diagnosis of such diseases.

Proposed Course of Project: Dr. Rushton's study of retinal pigments and their relation to vision and color perception (both normal and abnormal) will require the construction and/or modification of a number of instruments. Part of Dr. Rushton's work can add validity (or discredit) to the study of retinal profiles, and the two phases of investigation are certainly very intimately related. For this reason, collaboration with Dr. Rushton appears to be the most promising possibility for the coming year.

Part B included: Yes ☐ No ☒

Serial No. NINDB-96 (c)
1. Ophthalmology Branch
2. Physiology Section
3. Bethesda, Maryland
4. Same as NINDB-58 (c)

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Functional Studies in Retinal Anomalies and Diseases (Electroretinography, Adaptometry, and Perimetric Light Sense Studies).

Principal Investigators: Richard M. Copenhaver, M.D.
Ralph D. Gunkel, O.D.
George Goodman, M.D.
Eberhard Dodt, M.D.

Other Investigators: None

Cooperating Units: None

Man Years (Calendar year 1959):
Total: 1.3
Professional: 1.3
Other: 0

Patient Days: 100

Outpatient Visits: 23

Project Description:

Objectives: This study which is a continuation of a previous project is concerned with the investigation of visual function in patients with retinal abnormalities utilizing new adaptometric, perimetric and electroretinographic tests, in addition to clinical examination. Special emphasis is placed upon those conditions where there is a selective affection of the scotopic or photopic retinal processes. When possible, other affected members of the family are studied.

The objectives of this study are as follows:

1) To aid in the differential diagnosis, prognosis and genetic counselling of patients with retinal abnormalities.

2) To investigate the clinical usefulness of adaptometry, perimetric light sense studies and electroretinography.

3) To study the physiology of rod and cone vision in these patients by means of perimetric and electroretinographic techniques which permit a separate evaluation of the photopic and scotopic response.

Methods Employed: After clinical ophthalmologic examination the following special tests were performed:

1) Adaptometry: The course of dark adaptation is determined for a paramacular retinal area on the Goldmann adaptometer.

2) Perimetric light sense testing: The absolute light threshold is determined for red and blue stimuli from 0 to 40 degrees over one or more meridians in the visual field. The thresholds for blue light afford a "rod profile" of the retina, and the thresholds for red light represent the modified "cone profile" of the retina. Special attachments have been added to the Goldmann adaptometer to make these studies possible.

3) Electroretinography: The ERG's are obtained by means of contact lens electrodes and recorded on an EEG machine. The use of an intense light source supplied by a xenon lamp in conjunction with double interference and neutral density filters makes possible not only the separation of scotopic and photopic function but allows the study of various photopic mechanisms associated with color vision.

Major Findings: In addition to the major findings described in last year's report the following can be added:

A cone monochromat was discovered who exhibited normal visual function except for a complete absence of color discrimination. This anomaly is extremely rare and was thought to be of interest to color theory. Scotopic function as tested by 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 that part of the color loss is due to a retinal defect but did not contribute to the question of the site of the other abnormalities which must have been present to give so severe a color deficiency.

The spectral sensitivity curves (Dodt, Copenhaver and Gunkel) determined by electroretinography were also studied in albinos, Caucasians and Negroes where the degree of retinal pigmentation is quite different. It was found that where the retinal pigmentation 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 dependent on the absorption spectrum of blood itself. Thus, in future clinical studies utilizing the method of spectral electroretinography this factor must be taken into consideration.

Two children each with infantile amaurotic family idiocy and the late infantile and juvenile stages of amaurotic family idiocy had complete clinical evaluations including electroretinography, (Copenhaver and Goodman). The electroretinographic potentials were found to be novel in the type of disease occurring earliest and nearly absent in late infantile and juvenile amaurotic family idiocy. These findings correlate closely with what is known of the retinal histopathology in these diseases and our concepts of the site of origin of the electroretinogram. This study was not only of theoretical interest, but indicated the electroretinogram might be a useful tool in the early diagnosis of juvenile amaurotic family idiocy and a great help in clarifying the diagnosis between such conditions as Tay-Sach's disease and metachromatic leukoencephalopathy. This is a valuable technique because it supplies the clinician with an objective test in individuals who are infantile and demented.

Significance to Program of Institute: The functional study of retinal diseases may lead to a further clarification of basic problems in retinal physiology which will result in a better understanding of numerous clinical diagnostic problems.

Proposed Course of Project: Dr. Ottiwell Jones has taken the place of Dr. Copenhaver. With the advice of Dr. Rushton and the help of Dr. Gunkel he is going to continue and to extend studies on the outlined perimeters. Patients are continuously referred from various medical centers for psychophysical testing and, thus, subjects for such studies are available.

Part B included Yes [X] No [ ]

PHS - NIH
Individual Project Report
Calendar Year 1959

Part B. Honors, Awards and Publications

Publications other than abstracts from this project:

Copenhaver, R.M. and Goodman, G.: The electroretinogram in early infantile, late infantile and juvenile amaurotic family idiocy. A.M.A. Arch. Ophth. (in press).

Honors and awards relating to this project: None

Serial No. NINDB-97 (c)
1. Ophthalmology Branch
2. Physiology Section
3. Bethesda, Maryland
4. Same as NINDB-57 (c)

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: ERG Reactions of Pure-Cone Mammalian Retinae

Principal Investigators: Katharine Tansley, D.Sc.
Richard M. Copenhaver, M.D.

Other Investigator: Ralph D. Gunkel, O.D.

Cooperating Units: None

Man Years (Calendar Year 1959):
Total: 1.0 Patient Days: None
Professional: 1.0
Other: 0

Project Description:

Objectives: To study the spectral sensitivity, dark adaptation, and flicker fusion frequencies of various members of the squirrel family, these having the only pure-cone retinae known amongst mammals.

Methods Employed: The apparatus, consisting of a Xenon high pressure lamp and a set of double interference filters to produce nearly monochromatic light stimuli, already in use here, will be employed to measure the spectral sensitivity curves of various squirrel species. Both flickering and single flash stimuli will be used to study flicker responses and dark adaptation curves.

Major Findings: Adequate data have been obtained for reliable sensitivity curves on several different species

of squirrel. This will be analyzed and presented for publication early in 1960.

Some new observations regarding the flicker ERG and the depressed off-effect at high stimulus intensity will be presented. Evidence that certain species of squirrel have only cones and not rods was accumulated by demonstrating the absence of a shift in the peak luminosity of the spectral sensitivity curve from the light to dark adapted states, these high critical fusion frequencies and the absence of the type of spectral sensitivity curve usually found in the presence of rhodopsin. Two "humps" were found in the sensitivity curves of these squirrels and were thought to be representative of at least two photosensitive cone pigments. The maximum of these peaks were about 490 mμ and 535 mμ. It appears probable that the green sensitive pigment identified by other workers in vivo bleaching experiments is responsible for the green "hump" found in the electro-spectral sensitivity curves. The blue "hump" was less predictable and thought to represent a highly labile blue sensitive photopigment.

Acquisition of a Zeiss photocoagulator has facilitated a study of the effect on the ERG of deliberate damage to the retinal circulation. A discussion of these findings will probably comprise a third publication. It can be said, however, that the destruction of the retinal vessels produced a retinal degeneration in some of the animals limited to the entire inner retinal layers down to and including part of the bipolar cell layer. The marked reduction of the electroretinogram following this degeneration was taken to confirm the hypothesis that the positive potentials originate on the bipolar cell layer while the negative retinal potentials arise on the deeper retina.

Significance to Program of Institute: One of the great difficulties encountered in studying the human ERG is the separation of the photopic (cone) response from the scotopic (rod) response. Most members of the squirrel family possess pure-cone retinae so that a good knowledge of the reactions which can be obtained from such animals should be most helpful in providing information on the responses of a purely photopic mechanism uncontaminated by those of the scotopic mechanism. Investigations already carried out in Europe have shown that the

reactions of the retina of two species--a tree squirrel and a ground squirrel--are in some ways quite unlike those of the more usual mixed rod and cone retina of which the human is an example. The tree squirrel spectral sensitivity curve is much narrower and appears to reflect the activity of one only of the three postulated mechanisms for color vision--the "green" mechanism. The ground squirrel apparently has two, the "blue" and the "green". It is hoped therefore that a more complete study on several species of both tree and ground squirrels will provide information about the fundamental mechanisms for color vision. Dark adaptation has, so far, only been studied on a tree squirrel which gives a curve similar to that found by psychophysical methods for the human fovea.

It is believed 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 yet been systematically studied. We intend to repair this omission and hope to discover whether in this respect also the reactions of the squirrel retina resemble those of the human photopic mechanism.

Proposed Course of Project: The experimental part of the project has been completed, and data are being analyzed for presentation in three publications. Completion of these reports will terminate this project.

Part B included Yes ☐ No ☒

Serial No. NINDB-98 (c)
1. Ophthalmology Branch
2. Physiology Section
3. Bethesda, Maryland
4. Same as NINDB-56 (c)

PHS - NIH
Individual Project Reports
Calendar Year 1959

Part A.

Project Title: Electrophysiology of the Eye

Principal Investigator: M.G.F. Fuortes, M.D.

Other Investigators: Kyoji Tasaki, M.D.
W.A.H. Rushton, F.R.S.

Cooperating Units: Laboratory of Neurophysiology, NINDB
Marine Biological Laboratory, Woods Hole, Massachusetts

Man Years (Calendar Year 1959)
Total: 4.0
Professional: 2.0
Other: 2.0

Patient Days: None

Project Description:

Objectives: Study of the changes evoked by light in the eyes of invertebrates (Limulus) and vertebrates (fish) has been continued. Part of the experiments on the Limulus eye have been performed in Woods Hole, Massachusetts, where fresh animals could be obtained daily. Taking advantage of Dr. K. Frank's kind hospitality, additional experiments on nerve cells of vertebrates have been performed in the Spinal Cord Section of the Laboratory of Neurophysiology (NINDB) in order to determine whether certain properties found in Limulus are also common to other structures.

Methods Employed: Glass micropipettes filled with 3 molar KCl were introduced in the cells to be studied and were used both for recording potentials and for passing

currents. In Dr. Frank's laboratory two pipettes were introduced into the same cell and various responses were studied with voltage-clamp method.

Major Findings: In accordance with the plans and problems outlined in the preceding report, three major questions have been investigated:

1) Site of impulse initiation;

2) Subliminal responses to light.

1) It was found during the past year that resistance of nerve cells in the Limulus eye decreases moderately during illumination. Spikes recorded during illumination are smaller than those recorded (in comparable conditions) in darkness. This can be explained assuming that the resistance of the soma membrane does not decrease very drastically during impulse activity. Direct measurement of membrane resistance during an impulse could not be performed on Limulus but the results obtained on spinal motoneurones with voltage-clamp support the assumption.

In order to explain this result it may be thought: a) that the mechanism of impulse generation in somata is different from that of axons (where membrane resistance decreases by a factor of 500 or so): b) that impulse activity does not involve cell somata or that it only involves part of cell somata.

All experimental findings obtained so far are in agreement with the second hypothesis, which in particular is strikingly supported in Limulus by experiments on inhibition. Whereas following excitatory illuminations frequency of firing bears a strict relation with the membrane potential of the soma, inhibitory illumination decreases frequency of firing without perceptibly changing the recorded membrane potential. This is what one would expect if both the place of origin of the rhythmical impulses and the site of inhibitory action were at a distance from the soma. It is found, in fact, that inhibitory synapses are on the axons, at 100 μ - 200 μ from the soma.

The conclusion of this work is that both in the eye of Limulus and in the motoneurones of cats impulses originate in the axon.

2) The study of the properties of transmission from photoreceptive structures to nerve cells in Limulus has been continued by means of an analysis of subliminal responses to dim lights. It has been found that (following dark adaptation) steady dim illumination produces an irregular series of transient depolarizing pulses. Since previous work from this laboratory had led to the conclusion that a chemical substance liberated by the photoreceptor is responsible for evoking nerve cell depolarization, it is reasonable to think that the transient depolarizations recorded during dark adaptation are 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. Dr. William Rushton, Visiting Scientist from Cambridge, England, will now be in charge of this problem.

Dr. Kyoji Tasaki has completed his work on fish retina as planned and left the Section in September. He has confirmed Svaetichin's finding that hyperpolarizing potentials are elicited by light in the fish retina. He found that 1) hyperpolarizing potentials may be obtained in the absence of a negative DC shift; 2) electric currents through the microelectrode do not affect size and properties of the hyperpolarizing potential change. 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.

Significance to Program of Institute: It is hoped that the work described may be useful for a better understanding 1) of the nature of such elementary processes of vision as: a) mode of excitation of photoreceptors and b) transmission of excitation to nerve cells; 2) mechanism of generation of impulses in nerve cells; 3) elementary interaction between nerve cells in the eye.

Proposed Course of Project: Next year's work will concentrate on the properties of subliminal responses with particular reference to an analysis of the number of light quanta required to elicit a response in different conditions. The changes induced by light adaptation will also be studied. It is hoped that some fundamental properties of the process of vision will be revealed by the planned experiments.

Part B included Yes ☒ No ☐

Serial No. NINDB-98 (c)

PHS - NIH
Individual Project Report
Calendar Year 1959

Part B. Honors, Awards and Publications

Fuortes, M.G.F.: Integrative mechanisms in the nervous system, Amer. Nat., 93:213-224, 1959.

Frank, K., Fuortes, M.G.F., and Nelson, P.G.: Voltage-clamp of motoneurone soma, Science, 130:38-39, 1959.

Fuortes, M.G.F.: Discontinuous potentials evoked by sustained illumination in the eye of Limulus, Arch. ital. Biol., 97:243-250, 1959.

Tasaki, Kyoji: Some observations on the retinal potentials of the fish, Arch. ital. Biol. (in press).

Honors and Awards relating to this project: None

Serial No. NINDB-99 (c)
1. Ophthalmology Branch
2. Cytology and Histopathology Section
3. Bethesda, Maryland
4. New Project

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Studies on the Corneal Endothelium

Principal Investigator: Ludwig von Sallmann, M.D.
Leo Caravaggio, M.S.

Other Investigators: Eleanor Collins

Cooperating Units: None

Man Years (calendar year 1959): Patient Days: None
Total: 4.0
Professional: 2.5
Other: 1.5

Project Description:

Objectives: Physiology and pathology of the corneal endothelium is not well understood although its importance in certain disease entities, particularly Fuchs' dystrophy, has been appreciated following advances of biomicroscopic techniques. The prominent role of the condition and preservation of the endothelium for successful penetrating corneal grafts has been repeatedly emphasized in recent work. The limitations of our knowledge of this cell layer in health and disease rests in the difficulties to evaluate morphological changes on routine sections. Attempts to use flat mount techniques in the past have been made on portions of the endothelium, but examination on whole flat mounts of this cell layer have not been reported or carried out. The present study deals with two aspects of

the problem; first, to determine the regeneration of the endothelium under physiological conditions and second, to examine the process of healing of chemically or mechanically induced wounds.

Methods Employed: The flat mount technique devised for the lens epithelium which have been subjected to the Feulgen reaction, was successfully modified for the corneal endothelium. Mr. Caravaggio succeeded in separating the endothelium attached to the Descemet's membrane from the remaining layers of the cornea and in mounting such preparation of young adult rabbits in their entirety. The number of cells in the whole endothelium was calculated and the mitotic index determined. Injury to the endothelium was induced by irrigation of the anterior chamber with 0.9% solution of sodium chloride, by similar treatment with chymotrypsin and by removing circumscribed strips of the endothelium with a blunt instrument. Other eyes were subjected to routine histological examination.

Major Findings: In contrast to the generally held opinion that karyokinetic cell division does not occur in the cornea of the adult animal under physiologic condition, it was established that mitosis takes place and that 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. It seems superfluous, therefore, to resort to the assumption that endothelial cells multiply by amitosis in efforts to replace dead cells.

The infusion of 0.5% sodium chloride solution into the anterior chamber leads to endothelial damage indicated by gaps in the regular cell mosaic, by cell degeneration and a remarkable increase of cell division in the area of the lesion. This injury is more extensive when the enzyme alpha-chymotrypsin, 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 the ingrowth of cell elements from the margin of the wound. Here again a great number of dividing cells are observed in the surrounding zone.

Significance to Program of Institute: Knowledge of the behavior of the undisturbed endothelium and of the endothelium subjected to various injuries is of importance for the understanding of endothelial disease of primary nature as well as that accompanying other diseases of the eye. It might open ways for attempts to influence such disease states therapeutically or to prevent injuries of the cell layer by routine surgical procedures, by storage and keratoplastic techniques.

Proposed Course of Project: It is planned to study age-induced morphologic changes of the endothelium and at a later date to approach more basic aspects by tissue culture techniques and electron microscopy.

Part B included: Yes [X] No [ ]

Serial No. NINDB-99 (c)

PHS - NIH
Individual Project Report
Calendar Year 1959

Part B. Honors, Awards and Publications

Publications other than abstracts from this project:

von Sallmann, L.: Experimental studies of some ocular effects of alpha chymotrypsin, Trans. Am. Acad. Ophth. Otol., (In Press).

Honors and Awards relating to this project: None

Serial No. NINDB-100 (c)
1. Ophthalmology Branch
2. Cytology and Histopathology Section
3. Bethesda, Maryland
4. New Project

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Eye Changes in a Familial Type of Dyskeratosis of the Conjunctiva and the Oral Mucosa.

Principal Investigator: Ludwig von Sallmann, M. D.
David Paton, M. D.

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1959): Patient Days: 34
Total: 0.2
Professional: 0.1
Other: 0.1

Project Description:

Objectives: To study clinically and histologically the eye lesions in a familial disease which involves predominantly the perilimbal conjunctiva and the oral mucosa. It occurs in a large tri-racial isolated population in Halifax County, North Carolina.

Methods Employed: 128 of 390 persons in the eye disease lineage were repeatedly examined biomicroscopically. Scrapings from the conjunctiva were obtained from 21 patients and biopsies from the perilimbal area in 7. These 7 patients and 3 uninvolved members of the family were admitted to the Ophthalmology Branch for detailed study. The biopsy material was subjected to routine histologic examination with a battery of staining procedures and phase contrast microscopy.

Major Findings: Other investigators (Dr. Graham and C. Witkop, Jr., D.D.) have studied the socio-economic and genetic aspects of the disease. It appears that the latter is inherited as a simple Mendelian dominant. The eye changes occupy the perilimbal conjunctiva and consist of firm granular semi-translucent proliferations raised above the surface of the surrounding tissue. The shape of the lesion varied but most frequently it assumed a triangular form of horse-shoe-like configuration. The surrounding portions of the conjunctiva show a rather characteristic vascularization which explains the term "the red eye" used by the patients. Serious corneal complications were rare but sometimes dense membranes of proliferated tissue covered the cornea for periods of time. The histologic examination showed that the epithelium in the involved part of the conjunctiva was thickened to various degrees and that the epithelial hypoplasia was accompanied by degenerative changes of the dyskeratotic type and the signs of acanthosis. The epithelial cells of the middle layers were often swollen and rounded and the cytoplasm was acidophilic. Degenerative changes of the cell nuclei were common. With the Giemsa stain irregular light blue structures could be demonstrated near the nucleus or its remnants. The nature of these blue-staining components is not clear. Spotty keratinization and cytonecrotic signs became widespread towards the epithelial surface. The tunica propria was usually free of major pathology, but was sometimes the site of extensive round cell infiltration.

During the physical examination it was noted that a change in the buccal mucosa coexisted with the eye lesions. It was diagnosed by Dr. Witkop as "white sponge nevus of the oral mucosa" and was described as histologically similar to the conjunctival change. In view of this new finding the original impression that the lesion could be considered as an atypical limbal vernal conjunctivitis had to be discarded and the diagnosis of familial conjunctival dyskeratosis of the conjunctiva and the oral mucosa accepted.

Significance to Program of Institute: Although this disease has not been reported in other isolated populations where intermarriage is frequent, it cannot be excluded that the entity exists but has not been discovered.

Proposed Course of Project: This project has been terminated.

Part B included: Yes [X] No [ ]

Serial No. NINDB-100 (c)

PHS - NIH
Individual Project Report
Calendar Year 1959

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

von Sallmann, Ludwig and Paton, David: Hereditary dyskeratosis of the bulbar conjunctiva and oral mucosa. I. Ocular manifestations. Amer. Med. Assoc. Archives of Ophthalmology (In Press).

Honors and Awards relating to this project: None

Serial No. NINDB-101 (c)
1. Ophthalmology Branch
2. Cytology and Histopathology Section
3. Bethesda, Maryland
4. Same as NINDB-37 (c)

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Nutritional Cataract. Tryptophane Deficiency Cataract in Guinea Pigs.

Principal Investigator: Ludwig von Sallmann, M.D.
Mary E. Reid, Ph.D.

Other Investigators: Patricia Grimes, B.A.
Eleanor M. Collins

Cooperating Units: Laboratory of Nutrition and Endocrinology, NIAMD

Man Years (calendar year 1959): Patient Days: None
Total: 0.2
Professional: 0.1
Other: 0.1

Project Description:

Objectives: In the past, experimental cataract induced by deficiency of essential amino acids have been studied predominantly in the rat. When lens opacities develop in this species, the animal appears to be in an extremely poor state of health. Secondary effects due to the near starvation condition of the rat cannot be distinguished readily from the primary effect of the amino acid deficiency. The use of guinea pigs as the experimental animal obviates this complicating factor. Therefore, experiments are conducted on the guinea pigs to determine the tryptophane requirements for maintaining clear lens where the diet contained ample dietary niacin and all essential amino acids but tryptophane.

Methods Employed: Male guinea pigs 3-5 years old were placed on the experimental diet which contained 0.1% tryptophane, and in separate groups of animals, supplements of the amino acid ranging from 0.02 to 0.1%. These guinea pigs and control animals were regularly checked for their weight gain, examined at weekly intervals with the slit lamp, and killed at various intervals. Both eyes of 135 guinea pigs were enucleated and subjected to histologic examination. To amplify previously reported observations on the rat lens epithelium, several litters of rats (52 animals) were fed tryptophane-deficient diets of various types. Nine weeks after feeding began the right eyes were prepared for cytologic examination of the lens epithelium in whole mounts and the left eyes were used for histologic studies to allow comparison of the results with those obtained in guinea pigs.

Major Findings: Guinea pigs on a diet containing 0.1% tryptophane developed incipient cataractous changes as early as 1-2 weeks after the feeding was started. The growth of these animals was only slightly retarded. Most of the animals showed transient alopecia and for a short time had swollen abdomens and excreted soft, unformed feces. Small supplements of the amino acid added to the basal diet delayed onset of lenticular changes and prevented extensive lens damage. Maximal growth was obtained by the addition of 0.03% L-tryptophane, a level which is not sufficient for complete lens protection. The D-isomer had from 1/4 to 1/8 the growth-promoting activity of the L form. The presence of the D form did not appear to suppress the protective action of the L-isomer on the lens. In fact, at the higher supplemental levels, it might exert slight additional effects.

The initial histologic lesions were characterized by fiber hydrops predominantly around the sutures. Later the deep cortex was the site of fiber destruction. From there, the lesions spread towards the surface. The lens epithelium and all components of the lens equator preserved their morphological integrity in contrast to any of the histologically studied forms of experimental cataract. Rats suffered loss of weight instead of gaining weight. Cataracts developed four weeks after the diet was started. The histopathology of the rat cataract was similar to that outlined for the guinea pig.

Significance to Program of Institute: It was shown for the first time that the requirement of the lens for one essential amino acid (L-tryptophane) was greater than that for maximal growth of the body as a whole. There is the distinct possibility that early or congenital cataracts in the human are connected with transient deficiency of essential amino acids.

Proposed Course of Project: This project is terminated for the present. In view of other projects on experimental cataract underway, it might be extended to other forms of nutritional deficiencies.

Part 3 included Yes [X] No [ ]

Serial No. NINDB-101 (c)

PHS - NIH
Individual Project Report
Calendar Year 1959

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

von Sallmann, Ludwig; Reid, Mary E.; Grimes, P.A.; and Collins, Eleanor M.: Tryptophane-Deficiency cataract in guinea pigs. A.M.A. Arch. Ophth., 62:662-672, 1959.

von Sallmann, Ludwig and Reid, Mary E.: Nutritional studies with the guinea pig VI. Tryptophane (with ample dietary niacin). (In Press)

Honors and Awards relating to this project: None

1. Ophthalmology Branch
2. Cytology and Histopathology Section
3. Bethesda, Maryland
4. Same as NINDB-45 (c)

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Study of Ocular Toxoplasmosis and Its Therapy

Principal Investigator: Herbert E. Kaufman, M.D.

Other Investigator: Richard G. O'Connor, M.D.
David Paton, M.D.
Richard Copenhaver, M.D.
Bruce Cohan, M.D.
James O'Rourke, M.D.
Ludwig von Sallmann, M.D.

Cooperating Units: Laboratory of Tropical Diseases, NIAID (Toxoplasma Dye tests were carried out in this lab.)

Man Years (Calendar Year 1959) Patient Days: 3180
Total: 0.5
Professional: 0.5
Other: 0

Project Description:

Objectives: Evaluation of clinical observations made on patients with uveitis of possible toxoplasmic nature.

Methods Employed: The etiologic workup on patients with uveitis consisted of a routine hemogram, urinalysis,

sedimentation rate, X-ray of the chest and skull and often the hands, antistreptolysin O titer, brucella and tularemia agglutination test, serological tests for syphilis, measurements of the total protein and albumin and globulin fractions, blood sugar, blood urea nitrogen and serum calcium. Skin tests with toxoplasmin and toxoplasmin control, histoplasmin, coccidiodin, heterologous staphylococcal antigen, Dick test streptococcal antigen, and lymphogranuloma venerum, as well as the first and second strength purified protein derivative of tuberculin were also done. On many patients, samples of aqueous and serum were tested for precipitating antibodies.

Major Findings: Of the great number of patients admitted to the Ophthalmology Branch with uveitis, 106 were found to have a positive toxoplasma dye test titer in the absence of other obvious etiology, and were treated with Daraprim and sulfonamides. As a rule, the course lasts six weeks. Only patients with active lesions were suitable for evaluation of therapy. Ninety-nine of the 106 patients with positive dye tests of different levels had also a positive toxoplasmin skin test. A survey of an additional 100 patients with negative toxoplasma dye tests revealed only one person with a positive skin test. This person with "false positive" skin test was the only one in more than 200 subjects on whom the toxoplasmin control was positive. The results indicate that the skin test is a very reliable procedure. Forty-three of the patients with marked activity at the onset of treatment were free of signs of inflammation following the course of chemotherapy, and more than half of this group underwent complete remission. Corticosteroids were added to the treatment with toxoplasmacidal drugs when cases did not respond satisfactorily to the chemotherapy. In only 7 of 35 patients of this group did the signs of inflammation disappear.

The therapeutic response to Daraprim and sulfonamides seemed to be more favorable when the onset of the disease dated back to an age under 20 years. The chronicity of the disease appeared prognostically unfavorable. 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.

Significance to Program of Institute: Evaluation of the skin test as compared to the toxoplasma dye test is of practical importance because of the unavailability of dye tests in many states. The results with the use of toxoplasmacidal drugs are encouraging when they can be administered

in a relatively acute stage of the disease which had its onset at an early age. Thus, the results are a valuable contribution to the meager knowledge of the diagnosis and therapy of one type of uveitis.

Proposed Course of Project: Results will be reported by Dr. Kaufman in the near future and studies on the disease continued.

Part B included: Yes ☒ No ☐

Serial No. NINDB-102 (c)

PHS - NIH
Individual Project Report
Calendar Year 1959

Part B. Honors, Awards and Publications

Publications other than abstracts from this project:

Kaufman, H.E., and Caldwell, Lee A.: Pharmacological studies of pyrimethamine (Daraprim) in man, A.M.A. Arch. Ophth., 61:885-890, 1959.

Kaufman, H.E., Remington, J., Melton, M.L., and Jacobs, L.: Relative resistance of slow-growing strains of toxoplasma gondii to pyrimethamine (Daraprim), A.M.A. Arch. Ophth. 62:611-615, 1959.

Remington, J.S., Jacobs, L., and Kaufman, H.E.: Adult toxoplasmosis, New Eng. J. Med. (in press).

Honors and Awards relating to this project: None

Serial No. 103 (c)
1. Ophthalmology Branch
2. Cytology and Histopathology Section
3. Bethesda, Maryland
4. Same as NINDB 52 (c)

PH3 - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Electron Microscope Studies on Biopsies of Human Muscle Diseases

Principal Investigators: Theodor Wanko, M. D.
G. Milton Shy, M. D

Other Investigators: Mary Ann Lavin, M. S.

Cooperating Units: None

Man Years (calendar year 1959):
Total: 1.0
Professional: 0.5
Other 0.5

Patient Days: 1400

Project Description:

Objectives: To determine by means of the electron microscope detectable change in human muscle affected with various dystrophic diseases as compared with normal tissue.

Methods Employed: Material obtained from muscle biopsies on human subjects with muscle dystrophies of various kinds are immersed immediately after excision in 1% osmium tetroxide for fixation. This is followed by dehydration and, in some cases, by additional impregnation with 1% phosphotungstic acid, embedding in methacrylate and araldite and sectioning on a Servall ultramicrotome.

An RCA electron microscope, Model EMU-3C, is used in these studies. Control and comparative examinations are carried out with the aid of phase contrast and light microscopy.

Major Findings: During the last year more specimens of various diseases were collected. The main attention, however, was directed toward the morphology of a cytoplasmic inclusion, which occurs normally in the muscle fibers, vascular endothelia and pericytes. It is an agglomerate which consists of globules of various densities together with opaque granules, and shows no discernible boundary from the surrounding cytoplasm. This complex usually is found in the vicinity of nuclei and sometimes in close association with mitochondria. Examination of autopsy specimens from aged human heart muscle revealed the presence of a large number of conglomerates of similar composition. Concomitant light microscopic observations on frozen sections indicate the presence of lipid stained structures. In one instance in the literature such an agglomerate is shown in an electron micrograph and is termed "lipofuscin." A similar structure has also been shown to exist in the interstitial cells of the testis.

In order to obtain more information on the composition of this structure, sections of skeletal muscle have been subjected to chemical extraction with chloroform-methanol. Time and temperature of this extraction were systematically varied. As a result, the individual components of the agglomerate usually displayed loss of their over-all opacity and frequently there was a dissolution of the granular portions. However, this finding was not sufficiently consistent, and at the same time, other cellular components sometimes also showed a reduced opacity. The embedding medium was dissolved to varying degrees by the solvents as well. Therefore, these experiments did not seem to provide conclusive results and the following procedure has been initiated. Human heart muscle obtained at autopsies of aged individuals was chosen as the test material because of its abundance of the described agglomerates. Very small tissue blocks are subjected to extraction with lipid solvents such as chloroform-methanol, acetone, ether and pyridine prior to and after fixation in the usual manner. They are then embedded, sectioned and examined in the electron microscope and compared to untreated control specimens of the same series. No results on these experiments are available at present.

Significance to Program of Institute: It is possible that significant morphological differences between normal and dystrophic muscles might yield some insight into the cytopathology involved in these diseases.

The investigation on the heterogeneous agglomerate might yield information as to its chemical composition and, at the same time, might lead to a method of characterizing lipid structures of other cellular constituents by means of the electron microscope.

Proposed Course of Project: Studies on normal muscle will be continued with special attention being given to structural details at the Z disc level; to the two morphologically distinct types of myofilaments and to identification of the dense, heterogeneous agglomerate in muscle fibers and blood vessel walls.

Observations on myotonic dystrophy will be continued. Further studies of Werdnig-Hoffmann's disease, of familial periodic paralysis, of progressive muscular dystrophy and other neuro-muscular diseases will be pursued.

Part B included: Yes [ ] No [X]

Serial No. NINDB-104 (c)
1. Ophthalmology Branch
2. Cytology and Histopathology Section
3. Bethesda, Maryland
4. Same as NINDB-51 (c)

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Electron Microscopic Studies on Tissues of the Eye, such as Epithelium, Fibers and Capsule of the Lens, Epithelium of the Ciliary Body, the Optic Nerve and the Conjunctiva.

Principal Investigator: Theodor Wanko, M.D.

Other Investigators: Mary Ann Gavin, M.S. and Ludwig von Sallmann, M.D.

Cooperating Units: None

Man Years (calendar year 1959):
Total: 1.0
Professional: 0.5
Other: 0.5

Project Description:

Objectives: 1) To investigate the normal characteristics of lens tissue elements as seen in the electron microscope, 2) to investigate the ultrastructure of the lens epithelium and the lens fibers after cataractogenic agents had been administered, 3) to examine human cataractous lenses, obtained immediately after operation, in order to gain insight into the submicroscopic cellular pathology, 4) to study the morphology of the ciliary epithelium with the electron microscope. Information on the ultrastructure of the epithelium may lead to an understanding of its function, and particularly, its role in the formation of aqueous humor. 5) To study the normal characteristics of human conjunctival ultrastructure which should serve as a control in examination of pathological tissue.

Methods Employed: An RCA electron microscope, Model EMU-3C, is used in these studies. Control and comparative examinations are carried out with the aid of phase contrast and light microscopy. The tissues are obtained either from the anesthetized animal and immediately immersed in the fixation medium, or fixation is started in situ. Biopsy specimens of conjunctiva are immersed in the fixative immediately following excision. All tissues are then dehydrated in alcohol, dissected and embedded in Methacrylate. For the past several months araldite, an epoxy resin, has been used as an embedding medium parallel to Methacrylate. A satisfactory procedure was developed only after a long period of experimentation. Preliminary results indicate better preservation of the tissues as compared with Methacrylate. Sections are cut on a Servall ultramicrotome and transferred to collodion filmed grids covered with a fine carbon layer.

Major Findings:

1. Normal Lens

The electron microscope study on the general cytoarchitecture and on the pattern of distribution of fine structural elements in lens epithelium and cortical lens fibers of the normal mature experimental animal (rat, rabbit, guinea pig, calf, Rhesus monkey) has been concluded. These results are contained in last year's report.

A joint investigation with Dr. R. A. Resnik (Section on Biochemistry) dealt with the fractionation of the cellular components of the lens and the isolation of cytoplasmic structures, which from their appearance under the electron beam have been termed "low density elements." These structures were contained exclusively in a supernatant obtained after centrifugation for 16 hours at 105, 000xg in 0.88M sucrose. According to Dr. Resnik's results, they represent the product of interaction between the soluble lens proteins (alpha-, beta- and gamma-crystallin) and the fixative osmium tetroxide. They appear as spherical and filamentous structures of 100 to 120 A diameter. They have been observed both in sections of intact lens tissue as well as in sections of a pellet obtained after fixing and sedimenting the supernatant. A further separation and morphological investigation of the individual crystallins was attempted. The results at the present time remain inconclusive, since alpha-, beta- and gamma-crystallin are in reality generic terms for components which comprise several not yet separated and characterized proteins. Therefore, this study on the soluble lens proteins has been discontinued for the time being.

At present, a study of the lens sutures is being carried out. They represent an intricate system of intercellular relationship, and serial sections may provide information on their three dimensional arrangement. No major results are yet available.

2. Experimental Cataract

X-ray cataract: In addition to the groups of lenses examined 4, 10 and 14 days after exposure of the eye to 1500 R, as described in last year's report, a series of lens epithelia was studied 24 hours after the same amount of irradiation. This experiment indicates that the first morphological changes include displacement of nucleoli, conical tapering and elongation of mitochondria and partial disintegration of nuclei. The present observations were integrated with light microscopic studies. Gradual changes in fine structures in the lens involve the following structures in the following sequence: nucleoli, mitochondria, nuclei, endoplasmic reticulum, low density elements, cell membranes. The Golgi complex alone remained unchanged during the time intervals studied. This project on early changes of lens epithelium after Roentgen irradiation has been discontinued.

Myleran cataracts (2-4-6 weeks): Further studies of the initial phases of myleran-induced cataracts added no new data to those obtained last year. The changes observed are somewhat similar to those seen in later phases after x-irradiation. At the earliest phase, however, morphological changes are too advanced to provide information as to which structures in the epithelium are first affected.

Mimosine cataracts (5-7-14 days): In addition to last year's data it can be reported that initial changes in the fine structure in these lens epithelia involve the endoplasmic reticulum and nucleoli, both structures of a high ribonucleic acid content. Later, mitochondria and nuclei also undergo severe changes.

3. Human Cataracts

At present 7 human lenses with senile cataracts have been obtained. A few specimens from two of these lenses have been examined. It is noted that human lens epithelial cells contain the usual cytoplasmic constituents in an arrangement similar to that observed in the mature experimental animal. In human lenses one striking difference is the presence of an opaque conglomerate, usually located near the nuclei, wherein at least two different components can be differentiated. One is represented as a round, dense homogenous particle of ca. 200 μ in diameter. The other is a dense granular

formation of smaller size. Several of the larger particles are closely surrounded by a quantity of the granules. No distinct boundary between this conglomerate and the surrounding cytoplasm can be discerned. This complex bears a resemblance to cytoplasmic inclusions previously observed in adult human skeletal muscle fibers, capillary endothelium and heart muscle of aged humans where it is most abundant. The structure, at present, is tentatively identified as a lipofuscin-type of pigment; a more detailed explanation of it is contained in the report on the muscle biopsy project.

The parts of the project on the lens concerning myleran, mimosine and human cataracts will be continued.

4. Ciliary Body and Optic Nerve

Both projects have not been continued during the last year.

5. Conjunctiva

One normal and five pathological (Sjogren's syndrome) biopsy specimens have been collected so far. Their processing has been started. No major results can be reported as yet.

Significance to Program of Institute: Based on the previous study of normal fine structure of lens, a considerable amount of information on experimental cataract and some information on human cataracts has been collected. The investigations revealed that the reactions in the tissue seem to be specific to the type of cataractogenic agent employed. After further collection of data comparisons with changes in human cataracts will be possible.

The study of normal human conjunctiva should serve as a basis for investigations on pathological processes in that part of the eye.

Proposed Course of Project: Observations on human and experimentally induced cataracts will be pursued. The study on human conjunctival tissue has been initiated. More material on both projects will be collected.

Part B included: Yes [X] No [ ]

Serial No. NINDB-104 (c)

PHS - NIH
Individual Project Report
Calendar Year 1959

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Wanko, T. and Gavin, M.A.: Electron microscope study of lens fibers. J. Biophys. Biochem. Cytol., 16:97-102, 1959.

Wanko, T., von Sallmann, L., and Gavin, M.A.: Early changes in the lens epithelium after Roentgen irradiation. A correlated light and electron microscopic study. A.M.A. Arch. Ophth. (In Press)

Resnik, R.A., Wanko, T. and Gavin, M.A.: Observations on a cytoplasmic component in lens fibers. J. Biophys. Biochem. Cytol. (In Press)

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Honors and Awards

Honorable Mention - by A.M.A. for exhibit on "The Crystallin Lens," June 1959, Atlantic City, New Jersey.

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Electroencephalography Branch:

Enomoto, T. F.: Unilateral activation of the non-specific thalamic system and bilateral cortical responses. EEG Clin. Neurophysiol. 11: 219, 1959.

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AWARDS, RECOGNITIONS, AND ACTIVITIES

Staff Appointments to Universities:

Dr. Cosimo Ajmone Marsan, Instructor in Clinical Neurology, George Washington University

Dr. Maitland Baldwin, Associate Clinical Professor of Neurosurgery, Georgetown University

Dr. Anatole S. Dekaban, Assistant Professor of Neurology, George Washington University

Dr. G. Milton Shy, Associate Clinical Professor of Neurology, Georgetown University

Dr. Donald B. Tower, Associate Clinical Professor of Neurology and Associate Professor of Biochemistry, Georgetown University

Hospital Consultants:

Dr. Maitland Baldwin, U.S. Naval Medical Center, Bethesda, Md.

Dr. Anatole S. Dekaban, Children's Hospital, Washington, D. C.

Dr. G. Milton Shy, U.S. Naval Medical Center, Bethesda, Md. and D. C. General Hospital, Washington, D. C.

Awards and Honors:

Dr. Cosimo Ajmone Marsan, President, Eastern EEG Society, 1959.

Dr. Bruce E. Cohan, Bronze Medal for exhibit at American Roentgen Ray Society, September, 1959.

Dr. Ludwig von Sallmann, Lucien Howe Medal for 1959 "in recognition of his distinguished service to ophthalmology".

Dr. Donald B. Tower, Chairman, Section on Neurochemistry, American Academy of Neurology, 1957-1959; Editorial Board, Neurology, and Biochemical Pharmacology.

Mr. Jay B. Wells, Superior Accomplishment Award, NIH, 1959.

Annual Report* of the Basic Research Program, NIMH-NINDB

January 1 to December 31, 1959

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. Ideals contribute to the improvement of internal values. The internalization of an ideal is tremendously accelerated by social consensus. This may take the form of an institutional "tradition," or an ideal may become a social convention, an "institution" in its own right. Ideals may become "unwritten law." In this form they are more powerful than any statutory regulation. Most social behavior is governed by the force of social conventions, popular ideals, without need for legal intervention. Ideals can be too idly taken or, on the other hand, fall into disrepute. This leads to a deterioration of internal values which in turn is reflected by a deterioration in behavior. Ideals tend to "run downhill" and on this account need to be continuously striven for.

Ideals are not only important historic forces but they evolve historically. Hard-won human values are safeguarded and extended mainly through the evolution of improved ideals. To accomplish this, ideals need to evolve at a rate that will

* 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.

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 we are inclined to deprecate altruism, faith and mutual trust as being "unreal" or "impractical," we actually live by these ideals. Mankind could not have even 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. These vital mechanisms have earned for us our biological as well as social freedom. Coupled with awareness, such ideals can impel achievements that will still further enlarge human dignity and freedom. It seems necessary only to encourage a greater awareness of the opportunity before us. The rest would seem to follow. Improvement will be measured in "little pieces of the striving," in any single act intended toward the realization of an improved ideal.

The purpose of this essay is to discuss certain ideals relating to the pursuit of science, relating to the interface existing between science and society, and to the contributions which science should be making toward the encouragement 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 process which is essential to 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 desirability of scientists contributing in a professionally broad and responsible way to the determination of social purposes and means. Only through an effective and disinterested assumption of this responsibility can science escape from being a toy of technology, pitted against all manner of competitive special-interest seekers throughout society.

3. Science is revealing a new ethic which is based upon scientific rather than religious or philosophical grounds. Through these 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 would seem to be most urgently required in all this is first, a more successful interface between science and society, and second, a greater sense of professional responsibility and probity among scientists. This latter will call for the resumption of something akin to the idealistic spirit of craftsmanship, akin perhaps, to the professional notion of a guild.

All this will require individual internal actions as well as administrative changes relating to the conditions of scientific enterprise. Neither the individual internal actions nor the administrative changes will suffice alone. But they are achievable, together, as a natural outgrowth of the 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 the ideals and purposes of society and its institutions? Does the 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 the choice of both the ends and the means of 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 professional 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: i) that our own professional destiny is intimately bound up with the achievements of our immediate professional community, ii) that this group in turn has the capacity to contribute more effectively to the achievements of our society, and iii) 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. This ideal has been eroded insofar as individuals and institutions reject individual responsibility or fail to allow for it. Rejection is of course encouraged by those whose ambition favors their own limited interests. Failure to allow for it has two principal origins: one, a lack of faith in the willingness or capacity of individuals for bearing such responsibility; the other, an unwillingness to cope with the confusions that arise out of the widespread exercise of individual responsibility. The ideal has been given away insofar as those to whom it should belong have not conceived the need for or exercised such responsibility, or because they enjoy responsibility that is strictly limited 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 institution or society.

To the extent that individual responsibility and especially individual professional responsibility is precluded, or avoided, our institutions and society are made to depend upon undemocratic procedures. This is perhaps not disadvantageous in itself in the short view, and under a 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.

PROFESSIONAL EVALUATIONS INDISPENSABLE TO SCIENCE AND SOCIETY

It is often supposed that scientists deal rather exclusively with facts, and that they eliminate from their deliberations any considerations of value. It is supposed that facts are assembled by scientists and systematized in relation to other facts, whereupon out of the examination of such relations emerge general "laws of nature." Misunderstandings arising from this 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 supposition 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 both the scope and method of science.

1. 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, viz., 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 facts, 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 the truly great and lesser scientists.

The profession of science therefore demands an exercise of value discriminations and provides a continuing disciplined experience in making such evaluations. It can readily be conceded that only scientists are professionally qualified to make judgments concerning values that are intrinsic to science.

3. 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 non-conformity. 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 seems 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 non-logical 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, the 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 knowledgeable concerning the criteria underlying those discriminations which will foster genuine scientific excellence. They alone can prevent the freedom, which creativity requires, from being used as a shelter for inefficiency, superficiality or uncritical partisanship. Scientists are well aware that if their own profession does not provide these discriminative evaluations, they will nevertheless 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.

3. Evaluations of science in relation to society. Science consists of a collection of information, a body of theory and a methodology. All of the disciplines of science share in their 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 intellectual activities that is truly progressive. 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 scientists' internal discipline always to seek simpler and more general expressions to 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 part 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 sinecure on ways for the best utilization of such talent for improving, safeguarding and realizing a better 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 the heart of 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 a danger that science, whenever lumped together with technology, will be conceived as riding on the coattails of technology instead of 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 indispensible. It is mandatory that any institutional revisions be performed with conscious deliberation and wisdom instead of simply by 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 as are the decisions and interpretations of the Supreme Court in the field of law.

It is evident that scientists are needed not only for the evaluations of science, of scientists and of scientific performance, but also, as participants in 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 authority. 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 may 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 of 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 are foreign to a professional scientific code, 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 system of values 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 de-emphasizes 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 taken 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 of our opportunity, half of our capability, and half of 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 feat directly, because the popular conception embraces 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 the determination of the freedom as well as of the 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 systems of discrimination upon which plants depend are concerned with relatively elementary chemical and physical factors. 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 secure them for their own and their progeny's use. Animals are characteristically mobile, built for action. The evolving nervous system has been from the beginning 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 are in turn shaped by biological systems of value, whether these are consciously manifest or not. Built into such differentiating-action-generating systems are mechanisms for value discriminations affecting the preservation of the individual and the preservation of the species. Both of these kinds of discriminations and actions are vitally essential. Survival and evolution could not have gone very far in the creation of complex forms of life without having a biological foundation for cooperation and altruism as well as for combat. Cooperation and faith in some degree are absolutely essential for the reproduction and survival of most offspring, and are very far-reaching in yielding internal biological satisfactions. 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, and certainly not the 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.

Within the last few years, disciplines basic to neurology and psychiatry have contributed directly to a system of biological ethics that is based entirely on scientific grounds. The work of Dr. Paul MacLean is particularly significant in this regard because of his delineation of separate brain mechanisms which relate to preservation of the self and to preservation of the species. Work such as this promises to provide the first system of ethics to be developed without dogma. Scientific contributions relating to consciousness, appetite, emotion, learning, memory, motivation, value discrimination, decision making, and will are pertinent to any consideration of what we have to deal with in human nature. They

also show 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 limits and potentialities of all 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. The 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 science's chief social value. 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, the community, and the universe itself, is always in the 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 cultivated 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

1. 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 the 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; e.g., schoolboys, preachers,

artists, salesmen, teachers, thieves, sailors, playwrights, physicians, policemen, pilots, businessmen, factory workers, television sponsors, miners, research scientists, bankers, soldiers, etc. 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 a 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) relate 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 this reason it is quite understandable, although regrettable, that a research enterprise is likely to be evaluated according to standards of the marketplace.

> 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 it is equally unreasoned. As an example: pressures are exerted, directly and indirectly, by the predominant group of right-handed persons to disguise or eliminate left-handedness; at the very least, to require left-handed 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 competitive fields, 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 determines 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, well fitted 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.

2. Attracting the ablest scientists to an organization. There is no substitute 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 precious (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:

i) Recruitment and retention of top-level scientists is made easy.

ii) 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 knowledgable advocate for its scientific program and its professional support.

iii) Internal professional ideals to seek greater freedom, dignity and responsibility for the individual scientist, tend in a self-controlled, group-correcting way to elevate the standards of excellence of scientific performance entirely in the absence of administrative intervention.

iv) The stature and license of the administration as representative of this respected community of scholars becomes automatically enhanced.

v) Advocacy for the support of the organizations 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.

vi) 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 pre-logical and the logical steps of their scientific work. The issues at stake seem intangible, but the 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 non-scientific criteria.

It is clear beyond peradventure of doubt that these issues need to be settled by scientists according to standards of professional aspiration and professional 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 the 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 yet been bound down by ~~slowly~~ SLOVENLY traditions, or 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 de-personalized objectivity, aiming always toward the highest realizable levels of scientific achievement.

3. The extension of professional responsibility. Since World War II, the impact of science (as realized through technology) has outstripped most of the 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 the 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 the scientific and technical decisions he is forwarding, he becomes guilty of a 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 over-simplification 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 intra-cultural 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 any 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.

4. The ideal of professionalism. Our primary concern as scientists relates to the growth in intellectual and creative power of ourselves and our colleagues, to the improvement of 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 the 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 cue 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 the solution to the problem is evident. Not only is it necessary, in order to be creative, to be rid of interfering precommitted 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 suppression, 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 brings with it 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 guilds. By this is meant an increased sense of personal dedication, greater sense of individual responsibility to one's own work and to the work of one's colleagues, an enlarged sense of identification through individual and group accomplishment, a bigger 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 "organisational 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 a "creative temperament." These characteristics cannot be supplied from the outside. They can, and often are, however, eroded and given away. It is not the hazard of the 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 an 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 in formalities which is 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 and 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 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 ever 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 the prospects of tragic and debasing alternatives, this freedom can be greatly extended by the individual, the organization, the society and the species. This requires only a wider recognition and release of a 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

1. The Assembly of Scientists, NIH-NIMDS. 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 the establishment of the Institutes. Such an organization would serve as a general forum for improving communication among the scientists, as a means for the formulation and expression of opinion by the 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 for 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 the great universities.

Clearly, the 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-NIMDS, 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 NIMDS, 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 a 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.

3. 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 in each instance of a scientists working for a full year at the NIH, three disagreeable alternatives would be forced: i) either the Institutes would have to be expanded indefinitely, or ii) there would be inadequate space for essential research operations after only two or three years of such practice, or iii) 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 two to three years. Although this period might be extended for an additional year in exceptional cases, that would ordinarily be the final limit. The only possible exceptions would occur on the occasion of 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 opportunity. 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.

3. The Principle of "Sabbatical" Leave. Creative scientific endeavor demands a mastery of subject matter and the 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 recurrent opportunities to renew their mastery of the field, to learn new technical and conceptual skills and to obtain a new perspective on scientific values relating to their 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 their senior faculty members to be given extended periods of time away from regular duties, usually at seven-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, and 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 scientist belonging to the permanent staff, upon whom the Institutes stake their research mission and reputation, an opportunity at seven 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 proceeding 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.

4. 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 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 three years ago, has added to the preceptor-apprentice relationship 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.

5. 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 to label complex compounds by feeding plants with radioactive building blocks, by this means, 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 the 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 those 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 three 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 sixteen 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 twenty 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 twelve members of Boards of Scientific Councelors of the two Institutes, NIMH and NINDB, have continued to give encouragement, intellectual stimulation and to provide programmatic as well as scientific advice. Some thirty seven 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. He 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 NIH 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 extra-mural grant which will allow him to retain his post at the Enrico Fermi Institute in the University of Chicago and still 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 deliberate limitations on the length of time I felt it was reasonable to commit to such heavy administrative responsibilities. This year, shortly before the completion of three years in office, I asked the two Institute Directors' permission

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 is 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 three to five 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.

Robert B. Livingston

Robert B. Livingston, M.D.

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2. Livingston, R.B. Central control of afferent activity. Chapter 7 (pp. 177-186) and Discussion (pp. 205-206; 409-410; 668) In: Jasper, H.H., Proctor, L.D., Knighton, R. S., Noshay, W.C., and Costello, R.T. eds. Reticular formation of the brain. Boston, Little, Brown & Co., 1958, xiv + 766 pp.

3. Livingston, R.B. Central control of receptors and sensory transmission systems. Chapter 31 (pp. 741-760) In: Field, J., Magoun, H.W., and Hall, V.E., eds. Handbook of physiology, Section 1: Neurophysiology, vol. I. Washington, D.C., American Physiological Society, 1959, xiii + 779 pp.

4. Gernandt, B.E., Iranyi, M., and Livingston, R.B. Vestibular influences on spinal mechanisms. Exper. Neurol., 1959, 1: 248-273.

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SUMMARY OF ANNUAL REPORTS (1959)

William F. Windle

The Laboratory of Neuroanatomical Sciences is organized in five sections, one being a field station in Puerto Rico. The chief of each section 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 these separate accounts.

Section on Development and Regeneration

W. F. Windle, Chief

During 1959, Drs. Richard Sidman, Irene Miale and Ned Feder left the section for positions elsewhere, Drs. Sidman and Feder going to Harvard University, Dr. Miale to Cairo, Egypt. One research associate, Dr. Kenneth Wolf, completed his tour of duty and transferred to Massachusetts General Hospital for residency training. Dr. Earl Feringa joined the section.

The tissue culture unit has been transferred to the Section on Neurocytology and reorganized under Dr. S. L. Palay.

Research activities of the Section on Development and Regeneration fall into the 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 one 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 is the 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 data show that the cells of the primitive ependymal layer behave synchronously, and that the sites of DNA synthesis and mitosis are different. The views of Schaper and Sauer are confirmed: 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.

Using this same material, in additional experiments employing tritium-labeled thymidine in mice, it was found that the primordium of the cerebellum made its appearance at 11 days of gestation. At this time 3 zones comprising the primordium are recognizable. Cells of the ependymal region migrate into the intermediate zone to participate in formation of intracerebellar nuclei. Development of the various components of the cerebellum were traced on different days of gestation of mice. The cells destined to become Purkinje neurons migrate from the primitive ependymal region to their definitive positions in the future cerebellar cortex and rapidly differentiate there.

These basic studies on histogenesis of the nervous system by the tritium-labeled thymidine technique and radioautography have been discontinued reluctantly, those participating in them having left the laboratory. It is hoped that other studies will be undertaken by this method when personnel are recruited.

(b) Drs. L. Guth and C. J. Bailey have carried out studies to evaluate the relative roles of the sympathetic and parasympathetic pupillary nerve fibers and to determine whether autonomic neurons can maintain the function of autonomic effector organs other than those that they normally

innervate. They have observed that the atropinized pupil dilates significantly in darkness and that a significant portion of this dilation is 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. Furthermore, 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 retain their original specificity, inasmuch as the heterogenously reinnervated pupil dilated in response to decreased environmental temperatures. Although Dr. Bailey has left the section, Dr. Guth is continuing these studies.

Dr. Guth, collaborating with Dr. James Campbell of Columbia University and Dr. Lamar Soutter of Boston University, is trying to determine whether or not diaphragmatic function can be maintained by the recurrent laryngeal nerve in monkeys. The proximal recurrent laryngeal and distal phrenic nerve segments have been anastomosed in large Cynomolgus monkeys. Regeneration has been in progress for nearly one 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, for example, "bulbar" poliomyelitis, to try to enable patients to survive without the assistance of an artificial respirator.

The Chief of the Section and Dr. E. R. Feringa, collaborating with Drs. J. B. Campbell, A. Bassett and C. Thuline of the Department of Neurosurgery, 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

determining efficacy of regeneration in the central nervous system were laid down. These are: There must be proof that the spinal cord was severed, there must be clinically observable signs of restitution of motor and/or sensory functions; there must be proof of physiological conduction across the healed lesions, including electrical recordings from the spinal cord following stimulation above and/or below; transynaptic potentials must be demonstrated; finally, there must be unquestionable verification of re-establishment 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.

Dr. Feringa, collaborating with Drs. 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 asphyxiation 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, and with Dr. J. Cammermeyer as consultant.

Annual Reports (1959)

Asphyxia neonatorum was induced in monkeys near term by detaching the placenta at hysterotomy under local anesthesia, keeping the fetal membranes intact. Eleven to 16 minutes later the fetuses were delivered from their membranes and resuscitated by pulmonary insufflation with oxygen. The infant monkeys showed neurologic deficits during life. Five were killed by perfusion-fixation at 2 to 9 days of age. Brains of these and of 2 which were not asphyxiated were studied. A common pattern of structural alteration 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 seldom encountered.

Asphyxia neonatorum was induced near term by removing fetus in membranes and permitting its respiratory movements to stop before resuscitating with pulmonary O_2 insufflation. Six infants were killed 3 to 10 days, one 10 months later, by formalin perfusion. Six newborn and 4 juveniles were asphyxiated in N_2, resuscitated similarly (cardiac arrest in one), and killed 10 days later. Appropriate controls were provided. Brains of experimental and control animals were sectioned serially for Nissl and other stains. A consistent bilaterally symmetrical pattern of brain damage always followed asphyxia neonatorum, conforming to nuclear boundaries. No hemorrhages were seen. Neurons and neuroglia were destroyed focally in 3 or 4 days. Reactions of macrophages and astrocytes began before 9 days. The microglial reaction had cleared at 10 months, lesions being marked by asphyxia. Least damage occurred in an asphyxiated newborn requiring no resuscitation; most damage, after 11 minutes asphyxiation. Nuclei of auditory, vestibular, and somatic afferent pathways were prone to injury, with maximal effects in inferior colliculi. Cortical lesions occurred in two; a central cerebellar lesion in one. Nitrogen asphyxiation failed to produce this pattern of destruction; 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.

Annual Reports (1959)

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. There were indications that asphyxia even in small degree produced neurological deficits at least transiently. Asphyxiated animals and their control littermates were tested in an alternation maze during the first week of life or during the twelfth 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 these tasks, although relearning tests did not show that they forgot more rapidly than controls. The learning deficit seemed to become less marked as the animals grew older. There was no correlation of learning performance and degree of asphyxia. The histological examination of the brains of these asphyxiated pigs revealed damage similar to, though less severe than, that reported previously. Hemorrhages were seen only in acute stages. Chromatolytic changes in neurons, loss of neurons, and neuroglial reactions appeared, notably 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; the cerebral cortex and cerebellum appeared to be unaffected. 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

S. L. Palay, Chief

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. By improving the preservation of the central

nervous system and by careful study of the non-neural elements we have obtained a tentative picture of the two most numerous glial cells. These two cells have been variously described by other investigators with the result that a heated discussion has arisen over their distinguishing characteristics. The controversy is based upon the facts that neither side in the argument has achieved satisfactory fixation of the tissue and that neither side has made observations parallel to the other. As might be expected our observations indicate that both sides have some degree of error. The first cell, identified as the oligodendrogliocyte, occurs as a satellite of neurons and in rows in the fasciculi of the white matter. This cell has a rounded nucleus and a well-defined, irregular border, enclosing a cytoplasm densely packed with ribonucleoprotein granules. The endoplasmic reticulum is sparse, but, significantly, it has a tendency to swell under less than optimal conditions of fixation. Because of its tendency to swell, this cell has been only poorly preserved by other investigators and has been designated as the astrocyte by one group and the oligodendrogliocyte by the other group. The second cell type has been seen by only one of these groups of investigators. This cell, identified by us as the astrocyte in agreement with Luse, is more voluminous, has an indented or irregular nucleus, and a highly folded boundary. In sections it is surrounded by clusters of small, rounded or oval profiles, segments of the numerous slender processes protruding from its surface. The cytoplasm is less dense than that of the oligodendrogliocyte and the endoplasmic reticulum is prominent rather than the ribonucleoprotein granules. Also long, thin filaments occur in bundles in this cell and in the processes. This study is still in progress. Further work must be done to substantiate these definitions of the two cell types.

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 metabolism of GABA by the central nervous system is linked with cerebral pyridoxine metabolism and with oxidative systems by way of specific enzymes. It is postulated that glutamate and GABA are intermediates in the oxidation of α-ketoglutarate by brain and that these intermediates may be of major quantitative importance in cerebral oxidative metabolism. However, the rate of conversion from glutamate to GABA as measured in isotope experiments would lead to an extraordinarily high rate of oxidative metabolism, even if one assumed that all cerebral oxygen consumption was obligatorily linked to GABA metabolism. Thus the hypothesis must be tested further, by exploration of the size of the pool of active glutamic acid in the brain and by critical analysis of the methods for measuring the conversion rate to GABA.

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 the Linnean Society, London. Dr. Palay also was a member of the NIH Research Fellowship panel (Anatomy and Physiology) until July when he became a member of the NIH Study Section on Cell Biology. Dr. Brightman has been elected vice-president of the Washington Society for Electron Microscopy and will succeed to the presidency next year. Dr. Albers participated in a national conference on "Inhibition in the Central Nervous System and γ-aminobutyric acid."

Section of Experimental Neuropathology

J. Cammermeyer, Chief

Several series of studies have been completed as part of a long range plan to establish extra- and intra-spinal factors involved in the normal maintenance of spinal cord structure and function.

A recently completed comparative microscopical study of the vascular system revealed the widespread distribution of intervascular connective tissue fibers in 18 animal species. The presence of such fibers has rarely been correctly interpreted and the significance of establishing such a fibrillary system in normal animals is twofold, namely (a) to avoid that these fibrillary connections are diagnosed capillary fibrosis, and (b) not to overlook the physical properties of these connections which in cerebral edema may be stretched and result in distorted vascular lumens with abnormal flow of blood. Their large number in the spinal cord should be considered in evaluating the cause of myelopathies.

Another study was aimed at the oligodendrocytes along the vessels. In all animals these cells seemed to be arranged in clusters, rows or small groups near perivascularly arranged neurons or points of arborization of vessels. None was arranged in manner to support older concepts that these cells would be concerned with the function of neuronal perikarya or myelin sheaths. On the other hand the particular arrangement of these cells suggested that they are concerned

with intrinsic control of blood flow to neurons. This hypothesis, presented at the Conference on Microcirculation in Physiology and Pathology May 4, 1959 at the N.I.H has opened new avenues for anatomical, histochemical, physiological and pathological investigations. These observations have initiated further research to determine the exact distribution in a tridimensional model of central nervous tissue and to assess the reaction of these cells under abnormal conditions. The complex arrangements of oligodendrocytes and vessels was 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 outmost economical distribution of blood between activated neurons.

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, Oregon. 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) a striking diffusion of iron as indicative of disturbed iron metabolism in fat embolism; and (b) the aggregation of oligodendrocyte nuclei in small foci of necrosis with disappearance of neurons as indicative of relatively greater resistance of oligodendrocytes to ischemia than neurons.

An extensive review of histological material and of published studies by others formed the basis of a report at the Annual Meeting of American Association of Neuropathologists, June, 1959, and a review article will be published in Ergebnisse der Anatomie. Conclusive evidence of the artefactual nature of dark neurons is given. As a consequence, a revision must be made of contemporary views on the role of so-called dark neurons during normal cell activity and reaction to diseases. Only material free of dark neurons should be used for cytological and neuropathological studies. It is gratifying that it has been possible to establish the principle of fixation and autopsy for preventing normal neurons from artifactual changes.

With the aid of karyometric methods it has been possible to demonstrate that the size of neuroglial nuclei is greatly influenced by fixatives. The shape and staining of nuclei, in particular of astrocyte nuclei, varied greatly with the technique used. With the best procedure available

the astrocyte nuclei exhibited an extreme degree of pleomorphism in most regions and animal species except several of the small rodents. For investigations concerned with identification of neuroglial cell type only species with easily identifiable astrocyte and oligodendrocyte nuclei should be used.

As a consequence of these studies all histological material fixed by perfusion is prepared according to a scheme in which each step has been developed in this section: (a) Modification of Heidenhain's Susa solution as perfusate for fixation; (b) Autopsy delayed 4 hours after the perfusion followed by immediate transfer of blocks to alcohol and embedding in paraffin; and (c) microscopical sections stained by the periodic acid Schiff procedure combined with gallocyanin-chrom alum.

Two series of animals were studied by Dr. Helen 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. She reported for the first time in a systematic study the intricate manner in which fat is deposited; this permits the complex movement 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

Grant L. Rasmussen, Chief

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 has 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 has 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 the ventral cochlear nucleus of the same side. These efferents plus the one previously described (1958) together constitute a rich feed back 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.

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 organ of Corti than 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. 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).

Mr. Kent Morsat, a senior medical student of Yale rejoined the Section under the COSTEP program for two months and completed the study begun last year on fiber connections of the area postrema.

Section on Perinatal Physiology

C. M. Combs, Chief

During the past year the activity 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 has been asphyxia neonatorum.

Data on menstruation of rhesus monkeys under standard

conditions continue to be collected. Dr. Jacobson consistently has found the menstrual cycles of individual monkeys to be subject to wide variation which spreads throughout the year. There has been no systematic relation between regularity of cycles and fertility. Matings show that conception occurs throughout the year.

A comparison is now being made by Dr. Vollman between the reproductive performances of the monkey colonies in our Bethesda laboratories and in the San Juan laboratory. Dr. Vollman, who joined our staff in August is also beginning ovulatory tests on all of our monkeys using daily vaginal smears, crystallization of the cervical mucus, body temperature, and fluctuations in total and differential white cell count.

Data continue to be collected on maturation in infant rhesus monkeys and on the care required for rearing them. 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. The maturation of the ability to self-feed is assessed. Normal ranges for all of the above have been established. Special methods of feeding and regulation of body temperature have been developed. This knowledge has been applied to the care of infants damaged by either fetal or newborn asphyxiation.

Neurological deficits of experimentally induced asphyxia have been investigated by all members of the Section. 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 with pure oxygen through an endotracheal tube from a rubber bag. 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 accomplished 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 5-1/2 minutes after their last gasps, whereas the newborns could not be resuscitated if asphyxiated for 1-1/2 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.

Drs. Marisa I. Ramirez de Arellano, Max Ramirez de Arellano and D. L. McCroskey have continued to attempt to establish exact criteria for neurological examination in the infant monkey. It is hoped that their work, added to that previously done in this laboratory, will result in a complete publishable protocol which can be made available to other investigators.

Standardized neurological examinations have been performed periodically from the time of birth on. Motion picture records of the examinations are made at established periods. Electroencephalographic recordings are also periodically taken. 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 choreo-athetoid movements and a marked lag in the 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 five or six 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 3 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 12 mothers that were subjected to section survived. Several other newborn and young monkeys were made available for the study. A great deal of physiological and biochemical data were collected in this short period of time. This is being readied for publication, and plans are being made to continue the research in Puerto Rico in another 9 months. It is hoped that the same group of investigators will participate. The most substantial findings are summarized in the following paragraphs:

Observations have been made on 12 foetal monkeys in utero under pentobarbitone anaesthesia, ranging in gestation age from 115-158 days (term is about 160 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 foetal monkeys, an arm was also delivered through a separate incision and the brachial artery was catheterised so that samples could be withdrawn from tributaries of the ascending and the descending aorta simultaneously. In the 3 youngest monkeys (126 days gestation or less) a uterine incision was made over the neck of the foetus and a catheter was introduced into the left carotid artery.

The mean O_2 saturation of the femoral arterial blood was 58 per cent (range 51-63). Simultaneous samples taken from a brachial artery were, on the average, 9 per cent higher; values as high as 77 and 79 per cent 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 3 foetal monkeys, femoral arterial blood samples withdrawn at the peak of a uterine contraction (producing a rise of intra-uterine pressure of up to 35 mm Hg) contained less O_2 (1.5 - 9 per cent saturation) than did samples withdrawn during a quiescent period between contractions. On delivery of the foetus 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 intra-uterine 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. Jacobson has continued his studies on endometrial innervation in the monkey and has found a dense supply of fine terminal nerves in the endometrium. These nerves pass from the uterine muscularis into the endometrium where they approach the surface. In suitable sections the nerves were found arborizing around secretory glands and penetrating into the vicinity of the endometrial arterioles.

Dr. Combs, Dr. Dennery and Miss Saxon have been carrying out electroanatomical studies on connections

between the cerebellum, diencephalon and cerebral cortex. These experiments have all been carried out initially in cats, but similar 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 of 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 per cent 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 one to five. Mating activity commences late in July. Nearly all young are born during the period of 4 months commencing in February. Approximately 65 per cent of the mature females have infants born this year. At least since mid-1956, the monkey population has been divided into two 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 2 or 3 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, developmental 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. So far, blood cell counts and hemoglobin determinations have been done in 182 animals. New studies will be added shortly.

Other Activities, Laboratory of Neuroanatomical Sciences

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 NIH.

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 is a member of the Traineeship Review Board, NINDB. He serves on the Committee on Hearing and Bioacoustics, National Academy of Sciences-National Research Council.

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 Educational 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, which will be published in 1959.

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11. Windle, W. F. Discussion of: Experimental basis of reduced lipogenesis in white matter of central nervous system, Saul R. Korey. Trans. Am. Neur. Ass. 1959.

12. Wolf, M. K. and Samuel B. Aronson. Growth fluorescence and metachromasy of cells cultured in the presence of acridine orange. Anat. Rec., 1959, 133:441.

13. Wolf, M. K. Fluorescence and metachromasy of living tissue culture cells stained with acridine orange. Excerpta Medica.

Serial No [illegible]
1. Neuroanatomical [illegible]
2. Section on Develo[illegible]
and Regenerat[illegible]
3. Bethesda, Mary[illegible]

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: Aspects of experimental neuroembryology

Principal Investigator: L. Guth

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1959)
Total .5
Professional: .3
Other: .2

Project Description:

Objectives: To test and evaluate the neurogenetic concepts of neurotropism and of myotypic specification of neurons.

Methods employed: 1. Implantation of various embryonic tissues into the brain. 2. Cross-anastomosis of tibial and peroneal nerves in the rat and salamander.

Major findings: Cross-anastomosis of the tibial and peroneal nerves in the rat has been performed. No evidence of restoration of function was found. This result confirms those reported previously by Sperry.

Significance: 1. The experimental evidence opposing the doctrine of neurotropism has all been derived from experiments on regenerating nerves. These conclusions have been conceptually extended to ontogeny without additional experimentation. Such additional experimentation seems warranted in view of the great dissimilarities between ontogeny and nerve regeneration.

2. Weiss's theory of myotypic specification of neurons has long been puzzling. Further investigation of this

Part A Project Description (cont'd)

phenomenon will probably shed light both on the homologous response and on chemotropism.

Proposed course of project: To be continued.

Part B included: Yes

Serial No. NINDB-NA-DB-1

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Guth, L.: Translation: "Studies on Vertebrate Neurogenesis ("Etudes sur la Neurogenèse de Quelques Vertébrés") by S. Ramón y Cajal; Springfield, Illinois, Charles C Thomas (in press).

Honors and Awards relating to this project: None

Serial No. NINDB-NA-DR-1
1. Neuroanatomical Sciences
2. Section on Development and Regeneration
3. Bethesda, Maryland
4.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Histogenesis in the embryonic mammalian nervous system.

Principal Investigators: Irene Miale and Richard L. Sidman

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1959):
Total: 1.3
Professional: .9
Other: .4

Project Description:

Objectives: To analyze the behavior of cells in the immature mammalian nervous system in order to clarify mechanisms of normal and pathological development.

Methods employed: Autoradiography using tritium labeled thymidine (thymidine-H^3) was employed in pregnant mice. This labeled nucleotide is incorporated into deoxyribonucleic acid (DNA) of cells about to enter cell division and thereafter remains as a permanent marker in those cells. Labeled embryonic tissues were fixed in Bouin's fluid, embedded in wax, sectioned, and prepared for autoradiographic study by Leblond's dipped liquid emulsion technique.

Major findings: Pregnant mice were given tritium-labeled thymidine (5 μc/gm of mouse) intravenously at various stages of gestation. Some were killed at later stages of gestation while others were allowed to deliver their offspring, which were killed at regular intervals up to one month postnatally. A similar dose of thymidine-H^3 was given subcutaneously to young mice at selected postnatal stages, and they were killed at regular intervals thereafter. Whole embryos

or brains were sectioned serially at 5 or 10 μ and studied autoradiographically. Beginning on the eleventh day of gestation, it was possible to trace cells migrating from the actively proliferating primitive ependymal region through intermediate stages to their destination in the mature cerebellum. First the cells of intracerebellar nuclei and the Purkinje cells, then Golgi II neurons arose from this source. The external granular layer, which develops early and covers the surface of the cerebellum in the embryo, disappeared between 15 and 20 days postnatally. From this source certain elements of the cerebellar cortex, including granule cells and cells of the molecular layer, were derived. Proliferative activity in the external granular layer reached its peak in the first week after birth, at which time that in the primitive ependymal region had subsided. This dual source of cells for the cerebellum is unique in the development of the brain.

Significance: Autoradiography with thymidine-H^3 is a powerful new tool not heretofore used for the study of embryological processes. It allows labeling of cells at a well-defined period of their life cycle, and allows these cells to be followed through their subsequent migrations and differentiation. It should allow a detailed analysis of when and how the various parts of the brain form in embryonic life. This is of intrinsic value, and also will serve as a basis for analysis of developmental defects of the nervous system. A fair analogy can be drawn with congenital heart disease which has been clarified so well by relating the detailed embryonic development of the heart to the time during pregnancy when the mother was ill or injured. The use of this method has made possible a clearer picture of the sequence of development of the elements of the cerebellum than with the older nonexperimental techniques.

Proposed course of the project: Temporarily discontinued because both Dr. Miale and Dr. Sidman have left the laboratory. A manuscript is in process of being readied for publication. The title is: "A Dynamic approach to cerebellar histogenesis in the mouse using tritium labeled thymidine."

Part B included: Yes

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards, and Publications

Sidman, R. L., Irene L. Miale, and N. Feder, Cell proliferation and migration in the primitive ependymal zone; an autoradiographic study of histogenesis in the nervous system. Exp. Neurol., 1: 322-333, 1959.

Honors and Awards relating to this project: None

Serial No. NINDB-NA-DR-4
1. Neuroanatomical Scienc
2. Section on Development and Regeneration
3. Bethesda, Maryland
4.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Regeneration in the spinal cord.

Principal Investigators: J. B. Campbell (consultant), Earl R. Feringa and W. F. Windle

Other Investigators: A. Bassett (consultant), C. Thulin

Cooperating Units: Department of Neurosurgery, Columbia University, College of Physicians & Surgeons

Man Years (calendar year 1959):
Total: 1.4
Professional: 1.0
Other: .4

Project Description:

Objectives: To attempt to obtain anatomical, electrophysiological, and functional evidence of regeneration of the spinal cord in cats and monkeys.

Methods employed: In cats and monkeys the spinal cord was transected and repaired by the Millipore technique. Additional variables studied were: treatment with Piromen, filling the gap in the cord with multiple short segments of peripheral nerve, and fusion of the spine prior to cord transection. Standard electrophysiological tests were done to attempt to demonstrate electrical conduction across the transected area after 3 to 6 months.

Major findings: In the cats only one animal out of 15 showed evidence of coordinated walking movements after cord transection and repair by the Millipore technique. This

Part A Project Description (cont'd)

animal also received Piromen. Several other cats with simple transections of the cord showed electrical conduction from several centimeters above the gap to several centimeters below the gap and vice versa. Microscopical studies demonstrated axons crossing the gap in these animals.

In the cats with large spinal gaps filled with segments of peripheral nerves, we have seen axons growing into the graft area, and have electrophysiological evidence of conduction into, but not across, the graft area.

The new series of studies in the monkeys are incomplete.

Significance: Importance of studying the phenomenon of regeneration in the central nervous system is self evident. Results of this study may be applicable to almost any traumatic injury of the brain or spinal cord and may throw light on any one of a number of degenerative diseases.

Proposed course of project: Spinal fusions will be done in cats and monkeys. Experiments will be designed to explore further the value of the Millipore technique plus Piromen in fused animals as a means to obtain anatomical, electrophysiological, and "clinical" evidence of regeneration of the cord.

Efforts will continue to bridge gaps in the spinal cord with segments of fresh or banked peripheral nerves.

Part B included: Yes

Serial No NINDB-NA-DR-4

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Campbell, J. B. and W. F. Windle: Relation of Millipore to healing and regeneration in transected spinal cords of monkeys. Neurology (in press).

Honors and Awards relating to this project: None

Serial No. NIADB-NA-DR-6
1. Neuroanatomical Sciences
2. Section on Development and Regeneration
3. Bethesda, Maryland
4.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title. The functional role and specificity of the sympathetic nervous system in the regulation of pupillary function.

Principal Investigator: Lloyd Guth

Other Investigators: C. J. Bailey

Cooperating Units: None

Man Years (calendar year 1959):
Total: 1.3
Professional: 1.0
Other: .3

Project Description:

Objectives: To evaluate the relative roles of the sympathetic and parasympathetic pupillary fibers, and to determine whether autonomic nerve fibers can maintain the function of autonomic effector organs other than those which they normally innervate.

Methods employed: 1. Study of the size of the atropinized pupil before and after sympathectomy.

2. Transection of sympathetic rami T_{1-3} to allow collateral sprouts from rami T_{4-7} to innervate the pupillary ganglion cells of the superior cervical sympathetic ganglion.

3. Preganglionic vagosympathetic nerve anastomosis.

4. Stimulation of sympathetic rami T_{1-7} with physiological recording of the effects of this stimulation.

Part A Project Description (Cont'd)

Major findings: 1. The atropinized pupil dilates significantly in darkness, and a significant portion of this dilation is abolished by sympathectomy. It may be concluded that, contrary to current opinion, the sympathetic nervous system plays an active role (not merely a tonic one) in maintaining pupillary dilation in darkness.

2. Pupillary size was partially restored under the influence of the "foreign" nerve roots. However these roots did not mediate a "darkness reflex". These fibers apparently retained their original specificity inasmuch as the heterogeneously reinnervated pupil dilated in response to decreased environmental temperature.

3. None as yet.

4. None as yet.

Significance: 1. These results correct the erroneous opinion that the sympathetic nervous system is not reflexly activated by darkness.

2. We have found no evidence of central nervous system reorganization; however the experiment was contaminated apparently by regeneration from T_{1-3}, and so it is not possible to draw reliable conclusions from our observations.

Proposed course of project: 1. Completed.

2. To be continued during the coming year and extended by studying the effect of unilateral sympathectomy on the contralateral normal pupil.

3. To be initiated during the coming year.

4. To be initiated during the coming year.

Part B included: Yes

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Bailey, C. J. and L. Guth: Role of the sympathetic nervous system in the pupillary response to darkness. Exp. Neur. 1:166-170, 1959.

Honors and Awards relating to this project: None

Serial No. NINDB-NA-DR-7
1. Neuroanatomical Scienc
2. Section on Development and Regeneration
3. Bethesda, Maryland
4.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Heterogeneous reinnervation of the diaphragm

Principal Investigator: Lloyd Guth

Other Investigators: Karl Frank, Lamar Soutter, and James B. Campbell

Cooperating Units: Laboratory of Neurophysiology, NINDB, Massachusetts Memorial Hospital, Boston, College of Physicians & Surgeons, Columbia University

Man Years (calendar year 1959):
Total: .6
Professional: .4
Other: .2

Project Description:

Objectives: To determine whether diaphragmatic function can be maintained by the recurrent laryngeal nerve in rats and monkeys.

Methods employed: Anastomosis of proximal recurrent laryngeal and distal phrenic nerve segments.

Major findings: The recurrent laryngeal nerve can restore function to the denervated hemidiaphragm of the rat. The monkeys have not yet been subjected to careful physiological evaluation.

Significance: If the experiment succeeds in the monkey we may then consider application of this operative procedure to those human diseases characterized by pathology of the

Part A Project Description (cont'd)

phrenic nerve (e.g., "bulbar" poliomyelitis). It may enable such patients to survive without the assistance of an artificial respirator.

Proposed course of project: The experiment on the cat may be repeated on a larger group of subjects to make the observations more suitable for publication. The method will soon be tested here. That phase of the experiment will then be discontinued here, as it is an applied clinical investigation. It will probably be continued in Boston by clinical investigators.

Part B included: Yes

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Guth, L. and K. Frank: Restoration of diaphragmatic function following vagophrenic anastomosis in the rat. Exp. Neur. 1:1-12, 1959.

Honors and Awards relating to this project: None

Serial No. NINDB-NA-DR-2
1. Neuroanatomical Sciences
2. Section on Development and Regeneration
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: Histological studies of the nervous system of Macaca mulatta after asphyxia neonatorum

Principal Investigator: W. F. Windle

Other Investigators: J. Cammermeyer

Cooperative Units: University of Puerto Rico School of Medicine and USPHS Clinic, San Juan

Man Years (calendar year 1959):
Total: 1.3
Professional: .4
Other: .9

Project Description:

Objectives: To determine the pattern of structural changes in the monkey brain after asphyxiation at birth.

Methods employed: Fetuses of known gestational age were obtained from monkeys bred under controlled conditions by cesarean section close to term. Half the fetuses were asphyxiated by removal of the uterine contents without opening the chorionic sac. Other fetuses were delivered as controls. At varying times after delivery the infants were killed by the perfusion-fixation technique.

Serial sections of the experimental brains and brain stems and other sections of representative levels of the spinal cord were prepared. Every tenth slide was stained by the buffered thionin Nissl stain and alternate tenths by the Woelcke myelin stain. Selected sections were stained by Holtzer, PTAH and Bodian methods and by other techniques for iron and fat.

Experimental animals were compared with control animals of comparable age for gross and microscopical differences. Cinematographic and other records of experiments were used for reviewing functional observations. Studies have been carried out on six animals living 2 to 10 days and one living 10 months, one prematurely born, and three controls.

Part A Project Description (cont'd)

Major findings: Experimental animals, which showed extensive neurological deficits during life were paired with controls of similar age. The brain damage was almost perfectly symmetrical in the experimental animals and principally in the gray matter. There were changes in white matter and destruction of myelin (at birth the myelination is quite incomplete), but these were probably secondary to neuronal damage. There was a striking localization by cytoarchitecturally defined nuclei, with damage usually conforming closely to the anatomical boundaries, some nuclei being spared even though surrounded by damage. The pattern of changes was the same in all experiments, but the destruction varied in intensity with duration of asphyxiation.

The cerebral isocortex showed diffuse subtle changes of the neurons with less complete staining than in the control yet with no clearly abnormal neurons nor neuroglial changes at early ages. In a few folia of the vermis of the cerebellum there was loss of Purkinje cells and a slight neuroglial reaction. At 10 months, there was atrophy around the central sulcus of the cerebrum; a large central lesion occurred in the vermis of the cerebellum.

Other areas of the brain, brain stem, and spinal cord had 20 to 100 per cent of the neurons damaged, usually to the stage of ghost cells. There was a reaction of early macrophages and an astrocytic hyperplasia in most damaged areas. No hemorrhages, nor thrombosis were seen. At 10 months the microgiial reaction had cleared and lesions were marked by gliosis.

The inferior colliculus showed the most severe damage. Other areas of extensive damage were most of the thalamic nuclei, the subthalamic nucleus, interstitial nucleus of Cajal, globus pallidus, the whole reticular formation, the superior colliculus, oculomotor, trochlear, and abducens nuclei, most of the trigeminal nuclei, superior and medial vestibular nuclei, cochlear nuclei, superior olive, nucleus gracilis and cuneatus, most of the gray matter of the cord (except in thoracic segments) and the roof nuclei of the cerebellum.

Part A Project Description (cont'd)

Significance: This is the first experimental study of the effects of neonatal asphyxia in an infrahuman neonatal primate. Histopathology of the infant monkey brain is significantly different from that of adult man after asphyxiation and is different from that reported in human infants, where hemorrhages, cortical atrophy, hydrocephalus and vascular infarcts have been emphasized.

Proposed course of project: To continue as quickly as time and preparation of material permit.

Part B included: Yes

Serial No. NINDB-NA-DR-9

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Ranck, J. B., Jr. and W. F. Windle: Brain damage in the monkey, Macaca mulatta, by asphyxia neonatorum. Exp. Neur. 1:130-154 (1959).

Honors and Awards relating to this project: None

Serial No. NINDB-NA-DR-21
1. Neuroanatomical Sciences
2. Section on Development and Regeneration
3. Bethesda, Md. and San Juan, P. R.
4.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Psychological and histopathological deficits of asphyxia neonatorum in guinea pigs.

Principal Investigators: C. J. Bailey and W. F. Windle

Other Investigators: None

Cooperating Units: University of Puerto Rico School of Medicine and USPHS Clinic, San Juan

Man Years (calendar year 1959):
Total: .7
Professional: .5
Other: .2

Project Description:

Objectives: To determine the ability of guinea pigs to learn and remember simple maze problems at various ages after asphyxiation at birth. To extend earlier experiments of Becker and Windle to older ages and different test situations. To correlate physiological and psychological studies with histopathological observations on the brains.

Methods employed: As previously described: (a) asphyxiation by intrauterine ischemia at full term, (b) resuscitation by intratracheal insufflation with O_2, (c) study of neuromuscular and neurosensory deficits, (d) testing learning ability in maze and (e) histopathological correlations. Littermate controls were used.

Part A Project Description (cont'd)

Major findings: 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. There were indications that asphyxia even in small degree produced neurological deficits at least transiently.

Asphyxiated animals and their control littermates were tested in an alternation maze during the first week of life or during the twelfth 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 these tasks, although relearning tests did not show that they forgot more rapidly than controls. The learning deficit seemed to become less marked as the animals grew older. There was no correlation of learning performance and degree of asphyxia.

The histological examination of the brains of these asphyxiated pigs revealed damage similar to, though less severe than, that reported previously. Hemorrhages were seen only in acute stages. Chromatolytic changes in neurons, loss of neurons, and neuroglial reactions appeared, notably 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; the cerebral cortex and cerebellum appeared to be unaffected.

Significance: 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.

Proposed course of project: Experiments terminated

Part B included: Yes

Serial No. NINDB-NA-DR-21

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Bailey, C. J. and W. F. Windle: Neurological, psychological, and neurohistological defects following asphyxia neonatorum in the guinea pig. Experimental Neurology (in press).

Honors and Awards relating to this project: None

Serial No. NINDB-NA-DR-23
1. Neuroanatomical Sciences
S. Section on Development and Regeneration
3. Bethesda, Maryland

4. New

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Regeneration of peripheral nerves over long gaps.

Principal Investigator: J. B. Campbell (consultant) and Earl R. Feringa

Other Investigators: A. Bassett (consultant) and C. Thuline

Cooperating Units: Department of Neurosurgery, Columbia University, College of Physicians & Surgeons

Man Years (calendar year 1959):
Total: 1.4
Professional: .9
other: .5

Project Description:

Objectives: To explore methods of nerve grafting across large gaps in peripheral nerves.

Methods employed: Homologous nerve segments, banked by various methods (frozen, frozen irradiated, and frozen-dried), are implanted in gaps in the peripheral nerves of cats and goats. Subsequently, anatomical and functional regeneration are studied.

Major findings: In the cat good functional and anatomical regeneration was obtained across gaps one centimeter long which had been bridged with frozen nerve segments and wrapped in Millipore sheaths. The temporal history of the regeneration through the graft is being studied. Banked frozen irradiated nerve implants in cats have provided a superior method for

Part A Project Description (cont'd)

bridging large defects.

Studies in goats are in progress.

Significance: These experiments are designed to explore a method of bridging major defects in peripheral nerves and are expected to be directly applicable to human subjects. Other methods of bridging long nerve gaps in man have been disappointing.

Proposed course of the project: Experiments with frozen nerve grafts will be continued to study the time course of the process of regeneration.

Part B included: No

Serial No MINDB-NA-DS-24
1 Neuroanatomical Sciences
2 Section on Development
and Regeneration
3 Bethesda, Maryland
4. New

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Histological study of the nervous system of the Macaca mulatta after asphyxiation with nitrogen gas.

Principal Investigator: W. F. Windle

Other Investigators: Howard N. Jacobson

Cooperating Units: USPHS Clinic, San Juan, P.R.

Man Years (calendar year 1959):
Total: 1.2
Professional: .4
Other: .8

Project Description:

Objectives: To determine whether or not the pattern of structural changes in the monkey's brain after asphyxiation with nitrogen gas (anoxia) is similar to that after asphyxia neonatorum.

Methods employed: Monkeys delivered naturally by vaginal route or delivered by cesarean sections were placed in an atmosphere of nitrogen gas in an enclosed chamber until respiration had ceased. The animals were then resuscitated by insufflation of their lungs with oxygen. Experiments were performed between days 1 and 10 after birth. Four young adult monkeys were asphyxiated with nitrogen similarly. In one of these the asphyxiation was continued until cardiac arrest. The chest was quickly opened and massage of the heart restored circulation.

Serial sections of the brains of the monkeys were prepared and representative levels stained with buffered thionin Nissl stain; alternate sections stained by other methods.

Part A Project Description (cont'd)

Major findings: The pattern of structural change in the brain after asphyxia neonatorum of the monkey did not occur after asphyxiation with nitrogen gas and resuscitation. Even in the one animal with cardiac arrest and subsequent cardiac massage to restore circulation, a comparable pattern of structural change was not encountered.

Significance: These observations throw doubt on the assumption that the effects of asphyxia neonatorum are essentially due to anoxia.

Proposed course of project: The study is continuing and the results will be correlated with physiological observations and biochemical studies of changes occurring during birth.

Part B included: No

Serial No. NINDB-NA-NC-1
1. Neuroanatomical Sciences
2. Section on Neurocytology
3. Bethesda, Maryland
4.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Ultrastructure of nervous system.

Principal Investigator: Sanford L. Palay

Other Investigators: Spencer Gordon, Jack Rosenbluth, Milton W. Brightman

Cooperating Units: None

Man Years (calendar year 1959):
Total: 3.1
Professional: 2.5
Other: .6

Project Description:

Objectives: To study the organization of nervous tissue at the level of fine structure, particularly the relations between nerve cells and between the several cell types of supporting cells and the nerve cells.

Methods employed: The principal instrument employed is the electron microscope. Tissues are prepared for examination by fixation in osmium tetroxide, embedding in methacrylate or araldite, and sectioning. The sections are mounted on carbon-coated grids, stained with lead salts, and examined in the electron microscope. Thicker sections are mounted in glycerine and examined in the phase contrast microscope.

Major findings: Study of the glial cells in the central nervous system in collaboration with Dr. Gordon has revealed some distinguishing characteristics that will help to identify glia and will help to resolve the rather bitter disagreements which have marred the development of this subject. Two general types of glial cells have been identified. The first is a cell with a rounded nucleus and a well-defined irregular boundary enclosing cytoplasm that consists almost entirely of ribonucleo-protein granules and a sparse endoplasmic reticulum. The

Part A Project Description

reticulum is susceptible to swelling if the conditions of fixation are less than optimal. Because these cells are found as satellites around neurons and in rows in the white matter, they have been identified as the oligodendrogliocytes. The second cell is more voluminous, has an indented or irregularly elongated nucleus and a less easily defined boundary. Usually it is surrounded by clusters of small rounded or oval profiles which represent segments of the numerous slender processes protruding from its surface. The cytoplasm is less dense than that of the first cell and membrane-bound structures are prominent rather than the ribonucleoprotein granules. Frequently the cytoplasm of this cell type contains bundles of long thin filaments, about 80 Å in diameter, which also appear in the processes. This cell appears both in white and in gray matter and is often associated with blood vessels and the submeningeal and subependymal tissue. Its characteristics are consistent with the usual description of the astrocyte. In addition there are cells which do not fit exclusively either category and must be considered as intermediate types.

All cellular elements in the central nervous system fit closely together like the parts of a jigsaw puzzle. The slim crevices between them allow for about 100-200 Å of extracellular space in the one measurable dimension. It is probable that edema represents an increased intracellular fluid compartment, but our studies on this important point have not advanced sufficiently to render an opinion. As the descriptive analysis of the glia is a basic precursor of any satisfactory work on the extracellular space in the brain it will have to be completed first.

With all the interest expressed concerning the small extracellular space in the central nervous system, little attention has been paid to the fact that in almost all parts of the peripheral nervous system, neurons, their processes, and supporting elements are in the same close contact. With Dr. Rosenbluth we have been examining the interface between the capsular cells and the neuron in sensory ganglia. In the dorsal root ganglion, the plasmalemma of the capsular cell usually follows closely the contour of the neuron, separated by 100-200 Å. In certain areas, however, this distance increases to 0.1-0.3 μ. These bays are filled by ridges and microvilli projecting from the capsular wall. Rows of vesicles and tubules, 200-600 Å across, appear predominantly in the perineuronal zone of the capsular cytoplasm, apparently rising from invaginations of the plasmalemma. The surface of the

Part A Project Description (cont'd)

capsular cell facing the pericapillary interstitial space is coated by a basement membrane and exhibits broad, flat, overlapping cytoplasmic expansions. The cytoplasm of capsular cells contains granular endoplasmic reticulum, Golgi membranes, mitochondria, and assorted inclusions. Devious channels, 100-300 Å wide, bounded by membranes continuous with the plasmalemmae, cross the entire thickness. Single or multiple mesaxons also lead from either surface of the capsule cell to small axonal twigs enfolded into the cytoplasm.

The neuron does not display the surface irregularities of the capsular cell. Desmosomes or other specializations of adherent cell surfaces are absent. However, short, stubby neuronal processes frequently thrust into the inner zone of the capsule. In the neuron chains of vesicles, 200-600 Å in diameter, extend from the surface obliquely into the cytoplasm. Frequently, elongated flattened cisternae appear parallel to, and just beneath the surface, sometimes studded on their deep surface by ribonucleoprotein granules.

In the ganglia of the eighth nerve many of the neurons are surrounded by capsular cells that develop myelin. Some perikarya are coated by typical compact myelin, at least 0.1-0.5 μ thick, showing a periodicity of about 100 Å. The innermost lamella of the myelin sheath is separated from the neuronal plasmalemma by 100-200 Å. The outermost lamella is covered by a thin layer of capsular cell cytoplasm. The sheath completely encloses the perikaryon and is continuous with the myelin sheaths of the two processes issuing from its poles. Nodes of Ranvier do not appear in the parikaryal myelin sheath.

The capsules of unmyelinated eighth nerve ganglion cells resemble those of ordinary dorsal root ganglion cells. Intermediate forms exhibit incompletely compacted myelin consisting of multiple, closely apposed sheets of capsular cell cytoplasm 100-3,000 Å thick. This cytoplasm often contains endoplasmic reticulum, mitochondria, and rows of vesicles. In places the cytoplasm is absent and the two limiting surface membranes merge for short distances to form the major dense line of myelin.

The bipolar ganglion cells contain peripherally located Nissl bodies, prominent Golgi complexes, mitochondria, granular inclusions, and neurofilaments. In some of the heavily myelinated cells the ribonucleoprotein granules are sparse.

The observations raise questions concerning the role of the perikaryal myelin sheath in impulse propagation and in transfer of metabolites and nutrients across the neuronal surface.

part A Project Description

Significance: These studies provide the basis for a detailed understanding of the interrelations between neurons and their supporting structures. The close contact among all these elements suggests that mechanisms for free transport of nutrients and ions must be very efficient and must have intrinsic vectorial components. The myelin sheath around some perikarya suggests that certain generalizations for the mechanisms of impulse generation are either not applicable here or that a supposedly unrestricted extracellular space is not essential.

Proposed course of project: The results presented above are incomplete. The study must be extended, particularly the morphological work on glial cells. Some attempts at functional correlations will be made with the dorsal root ganglion cell in order to ascertain the significance of the bays and vesicles in the capsular cells. An additional study on the ependymal lining will be carried out in collaboration with Dr. Brightman. Further work will also be undertaken on synapses and dendrites in the cerebral cortex on the Mauthner cell in the teleost fishes.

Part B included: No

Serial No. NINDB-NA-NC-6
1. Neuroanatomical Science
2. Section on Neurocytolo
3. Bethesda, Maryland
4.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Relationship between neurosecretion and milk ejection.

Principal Investigator: Milton W. Brightman

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1959):
Total: .5
Professional: .3
Other: .2

Project Description:

Objectives: To determine whether a depletion of neurosecretory material occurs concomitantly with release of oxytocin from the hypothalamus and neurohypophysis in response to various stimuli.

Methods employed: About 175 female mice were divided into several groups: (a) virgin diestrous, (b) pregnant, (c) parturient, (d) females permitted to nurse their litters for periods ranging from 5 to 120 minutes, (e) nursing females presented with litters rendered incapable of suckling.

The brains and pituitary glands were stained with chrome-alum hematoxylin, coded and graded according to the amount of stainable material present.

Major findings: There was no demonstrable change in content of neurosecretory granules in the cells of the paraventricular and supraoptic nuclei, median eminence or pituitary stalk in any of the groups. The majority of the cells were only sparsely granular.

Part A Project Description:

The stimuli of suckling and of behavior associated with suckling (group e) resulted in a statistically significant depletion of neurosecretory meterial from the infundibular process.

Significance: In addition to osmotic stimuli, the act of suckling results in a release of hormone and of stainable material, from the infundibular process.

Proposed course of project: This project has been suspended

Part B included: No

Serial No. NINDB-NA-NC-7
1. Neuroanatomical Sciences
2. Section on Neurocytology
3. Bethesda, Maryland
4.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Species differences in cholinesterase activity of the central nervous system.

Principal Investigator: Milton W. Brightman

Other Investigators: R. W. Albers

Cooperating Units: None

Man Years (calendar year 1959):
Total: .8
Professional: .6
Other: .2

Project Description:

Objectives: To investigate the sites of "pseudocholinesterase" (PChE) activity in different species.

Methods employed: The Koelle histochemical method for cholinesterases.

Major findings: There is a remarkable species variation in the site of extraneuronal cholinesterase activity. Such activity occurs either in the endothelium, e.g., rat, goldfish, and toad or in the glia, e.g., cat and rooster. There is no obvious phyletic pattern of distribution.

Significance: The close spatial relationship between neurons and glia, and the occurrence of pseudocholinesterases in glia or endothelium, suggest a modulating influence of such enzymes on nervous activity.

Proposed course of project: The project has been completed and published.

Part B included: Yes

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Brightman, M. W. and R. W. Albers: Species differences in the distribution of extraneuronal cholinesterases within the vertebrate central nervous system. J. Neurochem., 4:244-250, 1959.

Honors and Awards relating to this project: None

Serial No. NINDB-NA-NC-8
1. Neuroanatomical Sciences
2. Section on Neurocytology
3. Bethesda, Maryland
4.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Metabolism of the nervous system: quantitative and cytochemical studies.

Principal Investigator: R. W. Albers

Other Investigators: L. Embree

Cooperating Units: Section on Clinical Neurochemistry, NINDB; Section on Enzymes and Cellular Biochemistry, NIAMD.

Man Years (calendar year 1959):
Total: 1.4
Professional: 1.1
Other: .3

Project Description:

Objectives: To assess the functional significance of special aspects of biochemical pathways as they occur in the nervous system.

Methods employed: Quantitative histochemical techniques; isotopic studies; chromatography; cytological fractionation.

Major findings: Methods have been developed for the study of the rate of conversion of C^{14}-glutamate into C^{14}-γ-aminobutyrate *in vivo*. This has included methods for processing tissues, separating the isotopically labelled metabolites and determining their specific activities.

Studies have been carried out on mice, measuring the rate of this conversion in brain. Both amino acids become labelled very rapidly. The specific activity of γ-aminobutyrate attains 50 per cent of the specific activity of the glutamate in less than 90 seconds. Calculations based on this type of experiment lead to a rate of metabolism that would require an improbable

Part A Project Description

rate of cerebral oxygen consumption for the mouse even if all of the cerebral oxygen consumption were linked to γ-amino-butyrate metabolism Because of this finding several experiments are being carried out to investigate possible sources of error in this type of measurement.

Dr. L. Embree has been associated with the section as a Research Associate since July 1. He is currently conducting some experiments in the area of succinic semialdehyde metabolism.

An investigation of the types of transaminases occurring in brain was carried out with Dr. Wm. Jakoby of NIAMD. This study has helped to explain the unusually rapid incorporation of C^{14} from glucose into brain glutamic acid. A study was also carried out with Dr. Jakoby which elucidates the structural requirements of substrates for the enzyme, glutamic-γ-aminobutyr transaminase.

A review of the literature concerning the distribution of γ-aminobutyrate and related enzyme systems was prepared and presented before a conference on "Inhibition in the Central Nervous System and γ-Aminobutyric Acid."

Significance: The basic problem may be formulated as follows:

(1) Relatively large quantities of γ-animobutyric acid (γ-AB) exist in the central nervous system. This is a unique situation not found in other mammalian tissues.

(2) The only known direct biochemical precursor of γ-AB, glutamic acid, is also found in uniquely high concentration in the central nervous system.

(3) The enzyme which catalyzes the formation of γ-AB from glutamate appears to be localized to the gray matter of the central nervous system, probably to neuronal elements, and is not found in other mammalian tissues.

(4) The metabolism of γ-AB by the central nervous system is intimately linked to pyridoxine metabolism by virtue of the two enzymes, glutamic decarboxylase and glutamic-γ-amino-butyrate transaminase.

Part A Project Description

(5) The metabolism of γ-AB by the central nervous system is closely linked to cerebral oxidative metabolism by virtue of the enzyme, succinic semialdehyde dehydrogenase (see NINDB-NA-NC-2 (1958)).

(6) Evidence has been obtained relating cerebral pyridoxine metabolism to cerebral oxidative metabolism.

(7) Qualitative observations have been published by other investigators indicating that γ-AB is metabolized rapidly.

(8) The sum of the known reactions occurring in brain tissue metabolizing γ-AB is
α-ketoglutarate + DPN $\longrightarrow$ succinic acid + DPNH + CO_2

From the foregoing, we have hypothesized that glutamate and γ-AB might function as intermediates in the oxidation of α-ketoglutarate by brain and that this might be a pathway of major quantitative importance in cerebral oxidative metabolism.

The high concentrations of both γ-AB and glutamate in the central nervous system have led other investigators to propose a more direct influence of these amino acids upon the excitability of certain neurons, either as a chemical mediator of synaptic transmission or as 'modulator' substances. Pharmacological activities have been found for both molecules in various neural perparations.

It should be noted that the two hypotheses are not mutually exclusive. However, some of the evidence which has been adduced in favor of the "neurohumoral" hypothesis might also be considered as secondary to effects on cerebral oxidative metabolism.

Measurement of the quantitative contribution of γ-AB oxidation to total cerebral oxygen consumption is a direct test of the hypothesis, which would have major significance both to our concept of cerebral energy metabolism and as a stimulus to research which might relate defects in these unique cerebral enzyme reactions to neurological disease processes.

Part A Project Description

Proposed course of project: Data obtained in the present experiments will be extended and compared with that obtained by other techniques to establish its validity or to determine the sources of inconsistency. Experiments to study the possible existence of a relatively small pool of rapidly metabolizing glutamic acid are planned.

Further studies on succinic semialdehyde dehydrogenase, including further purification and characterization, are contemplated.

Part B included: Yes

Serial No. [illegible]

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Sokoloff, L., Lassen, N. A., McKhann, G. M., Tower, [illegible] Albers, R. W.: Effects of pyridoxine withdrawal cerebral circulation and metabolism in a pyridox dependent child. Nature, 178:751-753 (1959)

Salvador, R. A. and Albers, R. W.: The distribution glutamic-γ-aminobutyric transaminase in the nervous system of the rhesus monkey. J. Biol. Chem., 234: 924-925 (1959).

Albers, R. W. and Brady, R. O.: The distribution of glutamic decarboxylase in the nervous system of the rhesus monkey. J. Biol. Chem., 234:926-928 (1959)

Honors and Awards relating to this project: None

Serial No. NINDB-NA-SP-1
1. Neuroanatomical Sciences
2. Section on Experimental Neuropathology
3. Bethesda, Maryland
4.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Pathogenetical factors in the development of myelopathies.

Principal Investigator: Jan Cammermeyer

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1959):
Total: 2.4
Professional: .8
Other: 1.6

Project Description:

Objectives: (a) To obtain detailed information about normal histological and physical characteristics of the spinal cord to disclose factors involved in maintenance of tissue tension and blood circulation. (b) To assess histological characteristics of varied functional conditions of neurons and neuroglia. (c) To correlate cytological characteristics of the spinal cord with different degrees of functional abnormalities. (d) To clarify the mechanism of cell damage and progressive nature of tissue changes within the spinal cord.

Methods employed: Standard conditions of fixation and histological preparation of the brain and spinal cord.

Major findings: As part of a long-term project to study factors concerned with preservation of spinal cord function, the following observations were made:

(a) The vessels of the central nervous system are surrounded with numerous connective tissue fibers and these fibers pass from one vessel to another thus forming an anchorage for the vessels. These intervascular connections are noted in most animal species, most strikingly in the pigeon but not in the opossum. They are most numerous in the spinal cord.

Part A Project Description

(b) An analysis of the entire histological material made it possible to establish the principles for proper fixation, autopsy and histological staining in order to obtain even appearance of neurons, neuroglia, vessels and fibers. The most important fact was the revelation that the autopsy must start after a delay of several hours after the perfusion. The necessary delay varies with the fixative used, for a rapid fixative as the Heidenhain's Susa solution four hours is enough, but for a slow fixative as 10 per cent formalin even after a lapse of 24 hours the autopsy should be prepared with greatest care.

(c) Study of the neuroglial nuclei in normal animal material revealed:

Astrocyte nuclei vary in shape and size with region and animal species. The largest cats have the largest nuclei as measured in longitudinal sections of the spinal cord.

Oligodendrocyte nuclei vary in size but their average size is constant through the spinal cord and in cats of different size. Their appearance is the same in most animals with the exception of the guinea pig, hamster, squirrel and pigeon. In these species no distinction could be made between oligodendrocyte and astrocyte nuclei.

(d) Microembolization obtained by intravenous injection of oil in the dog resulted in a series of focal damage, one of which consisted of a minute pericapillary condensation of tissue and another of an aggregation of surviving oligodendrocyte nuclei after the neurons had disappeared.

(e) The oligodendrocyte nuclei formed clusters along vessels near neurons and points of vascular arborization in all 13 normal animal species studied. None appeared perineuronally or interfascicularly arranged.

Significance: (a) The demonstration of numerous intervascular strands of connective tissue fibers in different animal species is significant for interpretation of experimental results to avoid an erroneous diagnosis of vascular fibrosis. That such a system of fibers under pathological conditions may influence the blood flow in the small vessels is formulated as a hypothesis to be verified in future experiments.

Part A Project Description (cont'd)

(b) The demonstration that dark neurons are avoided by proper autopsy procedure is of significance for settling the discussion about the artifactual nature of these cells. As a consequence the entire concept about the role of dark neurons in cell physiology and cell pathology has to be revised.

(c) The demonstration that astrocyte and oligodendrocyte nuclei are not readily distinguished in all species is of importance for those concerned with these cells' function. Only such species should be used where an exact cell classification can be made.

(d) Fat emboli elicit cerebral changes of similar nature in dog and man. This is true for the size and shape of lesions and the speed of reactive changes. Of considerable interest is a striking mobilization of iron. The intensity of fibrin reaction was less in dog than man. Another fact was the high frequency of hydrocephalus. The latter is typical of normal dog material too.

(e) The study on the distribution of oligodendrocytes in normal material has shed light on a poorly understood problem, namely what are the factors regulating the intrinsic blood flow of the brain. For the first time the role of oligodendrocytes has been invoked.

Proposed course of project: To continue the study on the distribution of neuroglia cells by means of a model. The chinchilla is used because of the conspicuous difference between nuclear types.

To analyse the appearance of neurons and neuroglia in normal material after the animals have been forced to perform a certain amount of work. Mice, cats and monkeys are used because of the significance of using animals with varied rate of metabolism. Awaiting histological preparation.

To study the reaction of glia vascular complex in experiments with antimetabolites, nutritional deficiency, anemia, microemboli, etc. Material of this nature has already been assembled and histological preparation is under way.

Part B included: Yes

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Cammermeyer, J.: A comparative study of the intervascular connective tissue fibers of the central nervous system. J. Comp. Neurol. (In press).

Cammermeyer, J.: An evaluation of the significance of the "dark" neurons. Ergeb. Anatomie (In press).

Cammermeyer, J.: Differences in shape and size of neuroglial nuclei in the spinal cord due to individual, regional and technical variations. Acta Anatomica (In press).

Cammermeyer, J., and R. L. Swank: Acute cerebral changes in experimental canine fat embolism. Exp. Neur., 1: 214-232 (1959).

Cammermeyer, J.: Is the perivascular oligodendrocyte another element controlling the blood supply to neurons? 7th Conference on Microcirculatory Physiology and Pathology. Trans. Microcirc. Conf. (In press).

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Introduction of a new chemical fixative for tissues.

Principal Investigator: Mignon Malm

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1959):
Total: 1.4
Professional: 1.0
Other: .4

Project Description:

Objectives: To find a suitable fixative for preparing autoradiograms from the central nervous system after S35-methionine injection and perfusion fixation. To introduce as a fixative a compound whose properties may be assumed to be suitable for this purpose. To investigate the effectiveness of the compound as a tissue fixative in general and a fixative for nerve tissue in particular.

Methods employed: Animals were anesthetized by chloroform and fixed by intravascular perfusion preceded by washing out of blood with Travenol. Brain, spinal cord and a number of other organs were removed and processed for embedding in paraffin. Blocks were sectioned and slides prepared. Approximately a dozen different microtechnics for demonstrating structures inside cell bodies as well as relations between cells and their processes were tried.

Major findings: Tissues from mainly rats, about 10 weeks old, and from a few guinea pigs, 2 days old, have been examined

Part A Project Description

The compound acts as a primary fixative. No addition of indifferent salts has been made. A standard concentration for the fixative has been worked out. The time required for rendering complete fixation of the brain was established by removal of brains at different times after perfusion. Washing of tissues after fixation is not necessary. Tissues can be transferred directly into 95 per cent ethanol.

The reaction of the fixative with proteins, lipids and carbohydrates in vitro has been studied.

The histological picture seen in paraffin sections shows the following: No artificial spaces occur around cells or vessels. The cytoplasm is homogeneously fixed. The shape of nuclei is well preserved, the nuclear membrane is clearly shown. The nucleolus is very distinct. Chromosomes are well fixed.

Significance: A new chemical primary fixative has been introduced with very good fixation properties. Especially conspicuous are the good results obtained with silver-technics on nerve tissue. The fixative is not volatile and thus agreeable to work with; the compound is inexpensive.

Proposed course of project: A manuscript on the new fixative will be submitted to an anatomical journal.

Part B included: No

Serial No. NINDB-NA-FN-1
1. Neuroanatomical Sciences
2. Section on Functional Neuroanatomy
3. Bethesda, Maryland
4.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: A study of the auditory afferent and efferent systems.

Principal Investigator: Grant L. Rasmussen

Other Investigators: Richard Gacek

Cooperating Units: None

Man Years (calendar year 1959):
Total: 1.4
Professional: .6
Other: .8

Project Description:

Objectives: To continue to explore and reveal unknown anatomical neuronal connections of the afferent and efferent auditory system; to learn something about the anatomical and functional interrelationships of these two systems.

(1) The question of tone or frequency conduction localized within the ascending pathway is poorly understood. One of the major objectives is to determine the point to point interneuronal hook-up that evidently exists between the organ of Corti and the cochlear nucleus and the projections from the latter to the superior olivary complex and to the higher auditory nuclei. (2) Also to learn more about the intrinsic and extrinsic recurrent or feed-back connections of the cochlear nucleus and higher nuclear levels.

Methods employed: The newer and more effective techniques are chiefly depended upon for demonstration of axonal, preterminal (Nauta-Gygax method) and terminal or synaptic degeneration (Rasmussen method), as well as Golgi preparations. The employment of the experimental techniques

Part A Project Description (cont'd)

on subjects in which small isolated lesions are properly placed makes it possible to reveal important complexly arranged neuronal connections. The Golgi method will be used as supplement to the other techniques.

Major findings: The study of the (1) afferent and (2) efferent nervous connections of the cochlear nucleus has disclosed the following information. The spatial arrangement and termination of the cochlear afferents related to the different turns of the cochlea (cat and chinchilla) has been completed. The information obtained from the study of the experimental material has been transferred to a three dimensional model of the nucleus constructed of plexiglass. This permits one to visualize more clearly the "unrolling" arrangement of the cochlear nerve fibers within the nucleus in respect to the coils of the cochlea. The apical neurons transmitting the lowest frequencies terminate most lateral and ventral; the middle and basal progressively more medial and dorsal in position. The efferent connections enter the nucleus via (1) the dorsal acoustic stria, (2) intermediate acoustic stria and (3) the caudal trapezoid body. The first originates from the nuclei of the lateral lemniscus and terminates in the dorsal nucleus of the lateral lemniscus; the second one connects the S-shape olivary segment with the ventral cochlear nucleus and the third collects fibers from the inferior colliculus and dorsal nucleus of the lateral lemniscus and terminates predominantly in the dorsal cochlear nucleus of the same side as described in 1958. The first two mentioned efferent connections are of recent discovery.

An uncrossed component of the efferent cochlear bundle has come to light. It arises from the principal olivary nucleus, unites with the crossed limb of the olivocochlear bundle before leaving the brain and proceeds together to the cochlea. The termination of the homolateral component is related more to the apical cochlear turns than is the crossed component.

The new observations dealing with the efferent fibers of the cochlear nerve and cochlear nucleus have been prepared for publication and will appear as a chapter in a monograph "Neural Mechanisms of the Auditory and Vestibular Systems", publisher, Charles C Thomas.

Part A Project Descrip[illegible]

Significance: Anatomical knowledge [illegible] relationships existing at the synaptic level between the afferent and efferent auditory system is basic to an understanding of the neuromechanism of hearing. Such information is essential for a foundation upon which to base physiological experiments for testing the functional significance, particularly of the descending conduction system.

Proposed course of project: To extend study of [illegible] afferent and efferent auditory system to the superior olivary complex and higher auditory levels.

Part B included: No

Serial No. NINDB-MA-FN-5
1. Neuroanatomical Sciences
2. Section on Functional Neuroanatomy
3. Bethesda, Maryland
4.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: The comparative anatomy of the efferent cochlear bundle in selected submammalian vertebrates; an experimental study.

Principal Investigator: Robert L. Boord

Other Investigators: Grant L. Rasmussen

Cooperating Units: This project was initiated July 1, 1958 under the auspices of Public Health Service Research Fellowship #9003 under the sponsorship and guidance of Dr. Gordon M Ramm, Department of Zoology, University of Maryland, and Dr. Grant L. Rasmussen, this laboratory.

Man Years (calendar year 1959):
Total: 1.3
Professional: 1.1
Other: .2

Project Description:

Objectives: (1) To establish the presence of an efferent cochlear bundle in submammalian vertebrates. (2) To study its central and peripheral anatomic relationships to the acoustic nerve complex.

Methods employed: The surgical midline incisions in the medulla from the dorsal approach, at the level of the facial genua, where fibers of the efferent cochlear bundle have been definitely established to cross in mammals. Following the placement of favorable lesions, serial sections of the brain stem and inner ears are prepared by degeneration methods. The Nauta-Gygax technique for degenerating axons is applied to intramedullary material and the Sudan black and Marchi methods

Part A Project Description (cont'd)

are employed on the peripheral portion of the acoustic nerve.

Major findings: An efferent component of the avian and reptilian cochlear nerve has been discovered by experimental degeneration methods. This efferent bundle is similar in topographic relationships to the olivocochlear bundle previously described in mammals. These cochlear efferents decussate dorsal to the medial longitudinal fasciculi, traverse the facial genu on either side of the brain stem and join the vestibular root. Peripherally the efferent fascicles traverse the vestibular ganglion and gain access to the cochlear nerve via the vestibulocochlear anastomosis. In the pigeon efferent fibers are traceable as far as the cochlear sensory epithelium and the sensory epithelium of the lagena. Although observations are less complete for the caiman at this time, it is presumed that the distribution of the efferents to the organ of Corti is similar to that of the pigeon.

Significance: The disclosure of an efferent cochlear bundle in the bird and reptile adds support to the contention that the efferent innervation of the cochlea plays an important role in audition and suggests that the efferent cochlear bundle is a part of the necessary neural equipment involved in the normal hearing process. Furthermore, the less highly differentiated cochlea of these submammalian forms is more favorable for determining the ultimate termination of these efferents, i.e., whether or not they terminate on the receptor cells along with afferent fibers.

Proposed course of the project: This project, under the present fellowship, will be terminated December 31, 1959 and the results used in the preparation of a dissertation for the degree of Doctor of Philosophy. It is expected that work will continue in submammalian forms along lines designed; (1) to determine the origin and termination of efferent fibers, and (2) to study afferent and efferent connections of the auditory system.

Part B included: No

Serial No. NINDB-NA-FN-6
1. Neuroanatomical Sciences
2. Section on Functional Neuroanatomy
3. Bethesda, Maryland
4.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Further studies on the efferent component of the vestibular nerve.

Principal Investigator: R. R. Gacek

Other Investigators: G. L. Rasmussen

Cooperating Units: None

Man Years (calendar year 1959):
Total: 1.7
Professional: 1.5
Other: .2

Project Description:

Objectives: (1) To determine the manner in which the efferent vestibular fibers terminate, i.e., whether or not they end on the vestibular hair cells as the afferent vestibular supply does. (2) To locate the exact cells of origin of the efferent vestibular fibers.

Methods employed: The Sudan Black technique previously described by Rasmussen (1953) for study of Wallerian degeneration of nerves of the petrous bones is employed subsequent to production of lesions in the vestibular nuclei and other regions of the hindbrain. Serial sections of the vestibular root and all its peripheral branches including the receptor epithelium are studied in a large series of experimental animals (cats and chinchillas). Protargol silver, Glees' ammoniacal silver, and Nauta-Gygax axonal degeneration methods are employed to demonstrate the ultimate termination of the efferents.

The method of Brodal, which is based on the phenomenon of retrograde cell changes, is used to demonstrate the cell bodies of the efferent fibers.

Part A Project Description (cont'd)

Major findings: No major findings concerning the termination or exact cells of origin of the efferent vestibular fibers can be reported at this time. Original observations dealing with the origin of the vestibular efferent fibers and their course and distribution in the labyrinth have been prepared for publication and will appear as a chapter in a monograph, Neural Mechanisms of the Auditory and Vestibular Systems

Significance: Experimental neuroanatomical description of the termination of the efferent vestibular systems is needed to verify the fact that endings possessing characteristics of centrifugal fibers have been described in the vestibular neuroepithelium by electron microscopic and histochemical means. Elucidation of the ultimate termination of these efferents will be of particular use in physiological study of this efferent system which may regulate sensory input, as the cochlear efferent bundle does in the cochlea.

Proposed course of project: Study of the ultimate termination of the efferents in the vestibular end-organs will be continued using appropriate experimental neuroanatomical techniques.

Part B included: No

Serial No. NINDB-NA-FN-7
1. Neuroanatomical Sciences
2. Section on Functional Neuroanatomy
3. Bethesda, Maryland
4. New

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: An experimental study of the ascending and descending fiber connections between corpus geniculatum mediale and auditory cortex.

Principal Investigator: Grant L. Rasmussen

Other Investigators: Jost B. Walther

Cooperating Units: The National Academy of Sciences by awarding an appointment under its Visiting Research Scientists Program to Jost Bernhard Walther.

Man Years (calendar year 1959):
Total: 2.0
Professional: 1.2
Other: .8

Project Description:

Objectives: An attempt (a) to improve the knowledge about the auditory connections ascending from the thalamus; (b) to explore descending connections from cortex areas known or suggested to be auditory in function; (c) to learn the pattern of innervation of "sustaining" auditory cortical projection areas.

Methods employed: Lesions are placed at various locations in (a) the medial geniculate body and (b) the classical auditory and other cortex areas of the cat. The resulting axonal degeneration after 6 days' survival time is stained in serial sections with Nauta's technique.

Major findings: Histological sections of 17 experimental animals have been processed and studied thus far but the observations and series of experiments are too incomplete to offer any significant observations at this time.

Series No. NINDB-NA-PP-
1. Neuroanatomical Sciences
2. Section on Perinatal Physiology
3. San Juan, Puerto Rico
4. Same as NINDB-NA-DR-13
1958

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Ecological study of rhesus monkeys on Cayo Santiago, Puerto Rico

Principal Investigator: C. B. Koford

Other Investigators: None

Cooperating Units: University of Puerto Rico School of Medicine, San Juan

Man Years (calendar year 1959):
Total: 2.1
Professional: 1.0
Other: 1.1

Project Description:

Objectives: To study monkeys in natural social groupings and their relations to a seminatural environment. To establish whether a supply of experimental animals can be produced more economically in a free-ranging colony than in a laboratory colony.

Methods employed: The principal method of study is systematic year around observation. Periodically several monkeys are trapped for examination, measurement and marking. Approximately 85 per cent of the animals are distinctively tattooed As far as possible, the reproductive history, group association, and other important facts in the life of each animal are recorded for analysis.

Major findings: In 1956, 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 per cent per year. Five females have lived more

Serial No. NINDB-MA-PP-2
1. Neuroanatomical Sciences
2. Section on Perinatal Physiology
3. San Juan, Puerto Rico
4. Same as NINDB-MA-DR-2
1958

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Normal reproductive function in the rhesus monkey.

Principal Investigators: Howard N. Jacobson and R. F. Vollman

Other Investigators: None

Cooperating Units: University of Puerto Rico Medical School and USPHS Clinic, San Juan.

Man Years (calendar year 1959):
Total: 1.8
Professional: 1.0
Other: .8

Project Description:

Objectives: To obtain data on menstruation in monkeys under standard conditions. To observe whether or not seasonal variations occur. To study spontaneous fluctuations of the menstrual cycle. To obtain data on conceptions in the monkey and determine whether or not seasonal changes in conception rate occur in a climate of essentially one season.

Methods employed: (a) The animals are fed a standard superior diet and are inspected daily for evidence of menstrual bleeding. Matings are presently confined to what is considered the most optimal portion of the menstrual cycle. Care is exercised in choosing couples for mating and incompatibilities are watched for and avoided when known. Minimum gentle handling of monkeys is routine. (b) Statistical analysis of menstrual records; calculation of parameters for the length and variability of the menstrual

Part A Project Description (cont'd)

cycles observed in Bethesda and San Juan; testing for significance of possible differences; analysis of a possible correlation between the degree of irregularity of the menstrual cycles as found in Bethesda and San Juan; analysis for an annual fluctuation in the variability of the menstrual cycles. (c) Statistical analysis of mating records; the distribution of fertile and sterile matings to the days of the menstrual cycle will be summarized. The hypothesis will be tested according to which the fertile days vary in a positive and linear correlation with the length of the menstrual cycle.

Major findings: The menstrual cycle of individual monkeys is subject to wide variations. These variations are spread throughout the year. There is no systematic relation between regularity of cycles and fertility. Matings in this same colony show that conception occurs throughout the year. This is in contrast to most published reports. The colony as a whole is remarkably fertile, part of which is tentatively attributed to the care with which the matings are performed. In a recent three-month period during a season when relative infertility had previously been observed in this colony 17 out of 43 monkeys conceived. Of these 17 monkeys 9 conceived following the first mating which was performed on the 11th day of the menstrual cycle.

Significance: The results bear on the future development of the colony for optimal reproductive performance and for rearing of a healthy stock of monkeys. Most of the hypotheses concerning reproduction in the human have their origin in observations on primates. Our data indicate that seasonal variation in fertility is not a major factor in the rate of conception. Further, with careful supervision of a monkey colony forty per cent of selected females may be expected to conceive.

Proposed course of project: To continue to study factors influencing reproduction in the monkey.

Part B included: No

Serial No NINDB-NA-PP-3
1. Neuroanatomical Sciences
2. Section on Perinatal Physiology
3. San Juan, Puerto Rico
4. Same as NINDB-NA-DR-17 1958

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Maturation in infant rhesus monkeys and care required for rearing them.

Principal Investigator: Howard N. Jacobson

Other Investigators: None

Cooperating Units: University of Puerto Rico Medical School and USPHS Clinic, San Juan, P R

Man Years (calendar year 1959):
Total: 1.1
Professional: .2
Other: .9

Project Description:

Objectives: To collect data on normal cage reared infant rhesus monkeys in order to describe growth and development and the kinds of variations encountered. To establish the standards of care required for the rearing of infants damaged at fetal or newborn asphyxiations.

Methods employed: A nursery patterned in many details after those in hospital use is maintained for the care of infant monkeys. Records are kept of daily weights, milk intake, temperature, and respiration rate. Heart rate is recorded electrically. Grasp reflex is measured routinely. The maturation of the ability of self-feed is assessed.

Major findings: Normal ranges have been established for birth weights of infants delivered both vaginally and by cesarean section. Ranges have also been established for weights heart rate, temperature, respiration rate and dentition throughout the first two months of life. Food requirements and methods of feeding were established and also methods were developed for the regulation of body temperature.

Part A. Project Description (cont'd)

This knowledge has been applied to the care of infants damaged by either fetal or newborn asphyxiation. In a series of 10 technically successful asphyxiation-resuscitation experiments 9 babies were maintained in good health. One of these babies required hand care for nearly 10 months. Many of these infants required prolonged assistance in learning to self-feed.

Significance: These normal values should prove useful to others establishing primate colonies. They are also essential in assessing the standards of care of this nursery in comparison to others. Further, these studies show that it is possible to raise severely handicapped infant monkeys in a state of good health.

Proposed course of project: To continue collection of data and improve standards of nursery care.

Part B included: No

Serial No NINDB-NA-PP-4
1. Neuroanatomical Sciences
2 Section on Perinatal Physiology
3. San Juan, Puerto Rico
4. Same as NINDB-NA-DR-18 1958

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: The intrinsic nerve supply to the endometrium in cat and monkey.

Principal Investigator: Howard N. Jacobson

Other Investigators: None

Cooperating Units: University of Puerto Rico, Medical School and USPHS Clinic, San Juan

Man Years (calendar year 1959):
Total: 1.0
Professional: .2
Other: .8

Project Description:

Objectives: To determine whether the primate has an intrinsic nerve supply in the endometrium similar to that found in the cat. If such is found then to determine the course and distribution of these nerves.

Methods employed: The endometrium of adult cats and rhesus monkeys was stained by the methods of Dastur and of Weddel using methylene blue dye. The dye was injected both intra-arterially and locally into the uterus of lightly anesthetized animals. Whole mounts of tissues were prepared.

Major findings: The endometrium of the monkey is densely supplied with fine terminal nerves. This verifies the earlier work reported in cats. These nerves pass from the muscularis of the uterus into the endometrium and are found approaching the surface endometrium. In suitable sections the nerves may be seen arborizing around secretory glands and there is some evidence that they pass to individual cells. The nerves may also be seen penetrating into the vicinity of the arterioles of the endometrium.

Part A Project Description (cont'd)

Significance: This is an unequivocal example of the participation of the nervous system in an area which is usually considered to be exclusively controlled by hormones. The physiological significance of these nerves may help elucidate mechanisms of uterine bleeding, and may perhaps be involved in such things as nidation.

Proposed course of project: To continue the histological studies to determine whether the human uterus is similarly supplied with nerves; to devise means of studying the functional significance of these nerves.

Part B included: No

Serial No. NINDB-NA-PP-5
1. Neuroanatomical Sciences
2. Section on Perinatal Physiology
3. San Juan, Puerto Rico
4. Same as NINDB-NA-DR-15, DR DR-19, 1958

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Neurological evaluation and electroencephalographic studies in the monkey (Macaca mulatta) asphyxiated at birth.

Principal Investigator: Maria I. R. Ramirez de Arellano (consultant)

Other Investigators: C. M. Combs and Max Ramirez de Arellano

Cooperating Units: University of Puerto Rico Medical School and USPHS Clinic, San Juan

Man Years (calendar year 1959):
Total: .9
Professional: .5
Other: .4

Project Description:

Objectives: To determine the neurological deficit resulting from asphyxia neonatorum in monkeys by means of neurological examination and electroencephalographic studies.

Methods employed: Standardized neurological examinations are performed periodically from the time of birth on. A motion picture record of the examination is made at established periods. Electroencephalographic recordings are also done periodically from the time of birth on. Stainless steel needles applied to the scalp are used. Both monopolar, using ears as reference, and bipolar recordings are made. Photic stimulation is used as an activation procedure. Both neurological and electroencephalographic studies are carried out on monkeys asphyxiated at birth (technique used in this laboratory) and on control animals.

Part A Project Description (cont'd)

Major findings: Neurological deficit of considerable degree and persistent nature has been observed in the asphyxiated monkeys. There has been little tendency for lateralized motor deficits. The general pattern has been one of choreo-athetoid movements and a marked lag on the development of isolated movements such as pawing, reaching, and picking up small objects. The electroencephalogram of most asphyxiated monkeys did not differ greatly from the normal. Most marked finding is depression of the electrical activity during the first five or six days.

Significance: A prospective type of study in primates (Macaca mulatta) has enabled us to study the neurological deficits due to brain damage as a result of asphyxia in the newborn.

Proposed course of project: To complete the study.

Part B included: No

Serial No. NINDB-NA-PP-6
1. Neuroanatomical Sciences
2. Section on Perinatal Physiology
3. San Juan, Puerto Rico
4. Same as NINDB-NA-DR-20 1958

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Psychological effects of asphyxia neonatorum in rhesus monkeys.

Principal Investigator: Sue V. Saxon

Other Investigators: None

Cooperating Units: USPHS Clinic, San Juan

Man Years (calendar year 1959):
Total: 1.6
Professional: .7
Other: .9

Project Description:

Objectives: To evaluate the behavior of rhesus monkeys after asphyxia neonatorum as compared with the behavior of comparable normal controls. Primary emphasis has been placed on learning abilities.

Methods employed: A test battery has been developed for both asphyxiated and normal animals. Testing starts at 60 days of age and continues throughout the first two years of life. The battery includes measures of learning ability, both in simple and difficult learning situations, tests of attention, perseveration, activity, emotionality, and general reactivity to surroundings. Standard psychological testing equipment is used as well as strictly observational techniques.

Major findings: No significant differences in learning ability have been found between groups (5 normals; 5 asphyxiates). Also no significant correlation exists between length of asphyxiation and object discrimination learning. However, normal controls have been found to be significantly more emotional than asphyxiated subjects.

Part A Project Description (cont'd)

Significance: The majority of the tests are still in progress, so group comparisons can not be discussed thoroughly as yet.

Proposed course of the project: The present test battery will be continued until all animals have completed it. Additional tests planned for the near future will be conditioning procedures to evaluate some of the sensory abilities of the animals. Also, more sensitive measures of attention or responsiveness to environment will be initiated.

Part B included: No

Serial No. NINDB-NA [illegible]-7
1. Neuroanatomical Sciences
2. Section on Perinatal Physiology
3. San Juan, Puerto Rico
4. New

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Evoked potential evidence for connections from the cerebellar hemispheres to the sigmoid gyri.

Principal Investigators: C. M. Combs and S. V. Saxon

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1959):
Total: 1.3
Professional: .5
Other: .8

Project Description:

Objectives: To find whether using the evoked potential method a connection could be proven to exist between the cerebellar hemispheres and the sigmoid gyri as was clearly implied by physiological studies.

Methods employed: Data were obtained from 40 cats either anesthetized with Nembutal or maintained on curare-like drugs. Single shock electric stimuli were given to the exposed surface of crus I, crus II, paramedian lobule, tuber vermis, or culmen of the cerebellar cortex. Evoked activity was recorded with cathode-ray oscillograph from the exposed surfaces of the ipsilateral and contralateral anterior and posterior sigmoid gyri.

Major findings: The largest cortical responses were found in the intermediate one third of the contralateral anterior sigmoid gyrus. Smaller potentials were consistently obtained from the lateral part of the contralateral posterior

Part A Project Description (cont'd)

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. Within this region, the largest responses resulted from stimulation of the anterior two thirds of the medial one third of crus I. No potentials could be evoked with stimulation of the lateral parts of crus I and II, tuber vermis or the medial one half of culmen. The results were as readily obtained in the Nembutalized as in the curarized preparations.

Significance: Although evidence has existed for many years that repetitive or pharmacologic stimulation of the cerebellar hemispheres could exert a distinct effect on the contralateral motor cortex (anterior sigmoid gyrus), previous to this study no investigators had been able to find any indication of this with the evoked-potential method.

Proposed course of project: The project is finished on the cat, but it is felt that these findings should be tested in the monkey.

Part B included: Yes

Serial No. NINDB-NA-PP-7

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Combs, C. M. and S. V. Saxon: Evoked potential evidence for connections from the cerebellar hemispheres to the sigmoid gyri. Experimental Neurology (in press).

Honors and Awards relating to this project: None

Serial No. NINDB-NA-PP-8
1. Neuroanatomical Sciences
2. Section on Perinatal Physiology
3. San Juan, Puerto Rico
4. New

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Multiple alpha-rhythm cortical responses resulting from single shock cerebellar stimulation.

Principal Investigators: C. M. Combs and J. M. Dennery

Other Investigators: None

Cooperating Units: None

Man Years:
Total: 1.3
Professional: .6
Other: .7

Project Description:

Objectives: To determine which cerebellar areas will produce a multiple alpha-rhythm cortical response when stimulated; to determine the cortical areas where these responses may be obtained; to establish whether these responses use the same pathways as those which have been described as resulting from stimulation of various other neural structures.

Methods employed: A variety of experimental preparations have been tested in cats in order to find the one most suitable to the carrying out of this project. After much experimentation it has been found that cats deeply anesthetized with Nembutal are most satisfactory. In these animals single shock electrical stimuli are given to the exposed surfaces of crus I, culmen, or tuber vermis of the cerebellum. Simultaneous electroencephalographic recordings are made from various areas of the bilaterally exposed cerebral cortex.

Major findings: The preliminary findings in this study indicate that single shock stimulation of cerebellar crus I will induce in the Nembutalized preparation a bilateral multiple alpha-rhythm response in the cerebral cortex. It is

most pronounced in the contralateral ectosylvian and motor cortex. Many interesting details which have been seen must be confirmed with subsequent experimentation.

Significance: The significance of this study will probably center about the mechanism and pathways whereby the cerebellar hemispheres can induce this multiple cerebral response.

Proposed course of project. To continue as described above in order to follow up these preliminary findings

Part B included: No

Serial No. NINDB-NA-PF-9
1. Neuroanatomical Sciences
2. Section on Perinatal Physiology
3. San Juan, Puerto Rico
4. New

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: An evoked potential study of connections between the diencephalon and the sigmoid gyri.

Principal Investigators: J. M. Dennery and C. M. Combs

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1959):
Total: 1.8
Professional: 1.0
Other: .8

Project Description:

Objectives: To find those areas of the diencephalon which when stimulated would evoke a potential change in the ipsilateral anterior and posterior sigmoid gyri. This should give an indication of the diencephalic regions which can serve to relay impulses to the motor-sensory cortex (sigmoid gyri).

Methods employed: Data have been obtained from 77 cats either anesthetized with Nembutal or maintained on curare-like drugs. In 17 of these animals electrolytic lesions had been placed in the red nucleus and medial lemniscus at least one month prior to the experiments described. Single shock electric stimuli were given through juxtaposed bipolar electrodes which were moved stereotactically millimeter by millimeter throughout the diencephalon. Consequent electrical activity was recorded with surface electrodes on the exposed ipsilateral anterior and posterior sigmoid gyri and visualized, after amplification, on a cathode ray-oscillograph.

Part A Project Description (cont'd)

Major findings: There were no qualitative differences between the diencephalic areas which when stimulated evoked a response in the anterior sigmoid gyrus and those which evoked responses in the posterior sigmoid gyrus. In the animals which had had no previous lesions the active diencephalic areas were: n. reticularis; ventroposterolateral nucleus; ventroposteromedial nucleus; n. ventralis pars medialis; n. centralis pars lateralis; fields of forel; zona incerta; red nucleus; mesencephalic reticular substance. These structures essentially include the sensory pathway and the course of the brachium conjunctivum through the diencephalon.

In the 17 cats which had had previous mesencephalic destruction of the brachium conjunctivum and the medial lemniscus, the same structures responded with the exception of the anterior part of the red nucleus, the fields of forel, the zona incerta, and the mesencephalic tegmentum.

Significance: The results of this mapping procedure will aid in designing further experiments to establish the precise diencephalic structures involved in the cerebellar influence on the motor cortex.

Proposed course of project: To fill in a few hiatuses that are in the data and prepare for publication.

Part B included: No

Serial No. NINDB-NA-PF - (3)
1. Neuroanatomical Sciences
2. Section on Perinatal Physiology
3. San Juan, Puerto [illegible]
4. New

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Physiological studies of fetal and newborn monkeys.

Principal Investigator: Geoffrey S. Dawes (consultant)

Other Investigators: Howard N. Jacobson, Joan C. Mott and Heather Shelley-Hoc ton

Cooperating Units: Nuffield Institute for Medical Research, Oxford, England, and USPHS Clinic San Juan, P. R.

Man Years (calendar year 1959):
Total: 1.6
Professional: .8
Other: .8

Project Description:

Objectives: The primary object of this work was to make physiological observations upon the cardiovascular and respiratory systems of fetal and newborn primates, and, in particular, to explore the feasibility of undertaking experiments upon the monkey in utero.

Methods employed: Various physiological methods were employed. Observations were made on 12 fetal rhesus monkeys, 6 newborn, 2 aged 1 to 2 years and on a few adult females.

Major findings: 1. The fetal environment. In fetuses the O_2 saturation of blood from the descending aorta (obtained by cannulating a femoral artery from a leg withdrawn from the uterus) averaged 56 per cent. The O_2 saturation of blood from a brachial artery (obtained by withdrawing an arm from the uterus through a separate incision) was consistently higher by about 8 per cent; values as high as 79

Part A Project Description (cont'd)

per cent have been observed. The blood lactate in fetuses was of the same order as that in newborn monkeys (7-17 mg/100 ml the blood glucose (mean 20 mg/100 ml) was less than in monkeys 8 to 12 days old (49 mg/100 ml). These observations suggest that the primate fetus normally has an adequate oxygen supply under pentobarbitone anesthesia; the oxygen content of the blood fell profoundly on delivery, as the uterus contracted, even though the placenta was not separated.

2. O_2 consumption after birth. In unanesthetized monkeys, within a few hours of natural delivery exposure to a cold environment (33° C or less) caused shivering and a rise in O_2 consumption. This effect was reduced or abolished when the O_2 content of the inspired air was decreased to 15 per cent or less.

The rate of O_2 consumption at an environmental temperature of 35° C (i.e., within the neutral zone) rose progressively during the 8 to 12 days following birth from about 10 to nearly 20 ml/kg/minute; these 6 monkeys had been reared in an incubator at 30° C.

The mean O_2 consumption of 8 anesthetized pregnant female monkeys (4.7-8.6 kg) was 6.2 ml/kg/minute, and that of 6 anesthetized monkeys 8 to 12 days old (0.4-0.56 kg) was 14.8 ml/kg/minute. The greater rate of O_2 consumption of the newborn monkeys was attributed to their relatively greater surface area/unit body weight (mean 1123 sq cm/kg as compared with 488 in adults), and this is probably the ultimate physical reason for the increase in O_2 consumption at 35° C whi takes place after birth.

3. Cardiovascular and respiratory reflexes in newborn monkeys. In monkeys 8 to 12 days old the Hering-Breuer inflation and deflation respiratory reflexes were present; they were abolished by cutting the vagi. Stimulation of the peripheral vagus caused a profound fall in heart rate and blood pressure. Occlusion of the carotid arteries caused a rise of blood pressure; denervation of the carotid sinuses caused a rise of pressure, and abolished the response to carotid occlusion. Thus the cardiovascular and respiratory reflexes investigated were all active.

Part A Project Description (cont'd)

4. Anatomical changes in the circulation after birth. In 6 monkeys 8 to 12 days old the ductus arteriosus was closed and would not admit a probe postmortem. The ductus venosus was also shut, and indeed very hard to find as compared with fetal specimens. The foramen ovale was, however anatomically patent; there were no macroscopic changes in the free edge of its valve.

5. Other physiological and biochemical changes. During gestation the blood pressure rose from about 33 mm Hg at 115 days to over 60 mm Hg in monkeys 8 to 12 days old. The ability to survive asphyxia (as measured by the time to the last gasp) fell from 25-30 minutes at 115 to 120 days gestation, to less than 10 minutes at birth. The carbohydrate content of the heart fell pari passu from about 20 mg/g to under 6 mg/g after birth. The carbohydrate content of the lungs fell from 115 days gestation onwards, that of the skeletal muscles fell after birth. There were ample glycogen reserves in the liver in both fetuses and newborn monkeys. These observations are very like those recorded in fetal and newborn lambs. However, unlike lambs the O_2 capacity of the blood showed no significant fall after birth.

6. Asphyxia or anoxia. There was a striking difference in the behavior of fetal monkeys whose umbilical cords were tied, as compared with newborn monkeys given nitrogen to breathe. In the fetus there was a rise of blood pressure lasting up to 10 minutes, no struggling, a steady fall of heart rate and a great increase in blood glucose. In the newborn there was a rapid and profound fall of blood pressure, followed by a short period of recovery during which the heart rate increased somewhat from a low level; there was struggling and the blood glucose fell. These differences require further study.

Significance: It is evident that there is much useful basic information to be obtained on the physiology of the primate fetus. The difference between the behavior of the uterus under experiment, as compared with that of the sheep, is particularly interesting; the primate uterus is thick and soon begins to contract vigorously. The facilities at San Juan, not only for producing mated monkeys of known gestation age, but also for rearing newborn monkeys (with 24 hour supervision and 2-3 hourly feeding schedules) are exceptionally good.

Part A Project Description (cont d)

There are also a number of specific conclusions which may be drawn from this work; thus, firstly it is evident that sampling cord blood after delivery is unlikely to give a realistic indication of the conditions of intrauterine life so far as respiratory metabolism is concerned. Moreover the results suggest that the primate fetus has a far better supply of O_2 than might be inferred from Barcroft's evocative phrase "Mount Everest in utero". Secondly, the O_2 consumption of newborn monkeys changes in the same way as does that of newborn lambs after birth, but the time relations of this change are quite different. They are similar to the few observations recorded in human infants and it is clear that this problem needs further investigation Thirdly, it is evident that some of the principal respiratory and cardiovascular reflexes are active and we are therefore in a better position to interpret the anatomical and physiolog changes which occur in the primate after birth. Fourthly, it i particularly interesting that there are a progressive series of changes in the carbohydrate reserves of many animals as gestation proceeds, and that similar changes occur in the monkey. The relevance of these changes to the ability to withstand anoxia or asphyxia at birth needs no emphasis. Finally, the discovery that the behavior of the fetus and the newborn differ during O_2 lack has already started a further series of investigations which are now being pursued in San Juan and in Oxford.

Proposed course of project: Studies are expected to be continued in August 1960, when the research team can again assemble.

Part B included: No

Serial No. NINDB-NA-PP-11
1. Neuroanatomical Sciences
2. Section on Perinatal Physiology
3. San Juan, Puerto Rico
4. New

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Tests of ovulation in the monkey

Principal Investigator: R. F. Vollman

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1959):
Total: 1.2
Professional: .5
Other: .7

Project Description:

Objectives: One of the important factors in breeding monkeys is the precision with which the time of ovulation may be estimated. So far, only daily palpation of the ovaries (Hartman) has been used systematically for this purpose. Methods approved in human sterility work should be tried in the monkey. The suitability and the degree of precision of clinical tests for ovulation should be ascertained in the monkey and tested by timed matings.

Methods employed:
1. Daily vaginal smears (Papanicolaou-Shorr)
2. Crystallization of the cervical mucus (Zondek)
3. Course of body temperature
4. Fluctuations in total and differential white cell count.

Major findings: Project started in August 1959, no results yet available.

Significance: It may be expected that through a more precise estimate of the time of ovulation the conception rate in the colony is to increase considerably. It might help to eliminate sterile females, as well as subfertile male monkeys, thus saving space, food and labor for fertile animals.

Part A Project Description (cont'd)

Proposed course of project: To adjust technical details of the tests mentioned above for routine performance under the present conditions of the colony. To perform trial matings over a period of at least twelve months and to evaluate the results.

Part B included: No

Serial No. MINDB-NA-PP-12
1. Neuroanatomical Sciences
2. Section on Perinatal Physiology
3. San Juan, Puerto Rico
4. New

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Responses of fetal and newborn monkey to asphyxiation and resuscitation.

Principal Investigator: William F. Windle and Staff

Other Investigators: None

Cooperating Units: University of Puerto Rico Medical School and USPHS Clinic, San Juan

Man Years (calendar year 1959):
Total: 2.0
Professional: .6
Other: 1.4

Project Description:

Objectives: To determine the physiological responses of both fetal and newborn monkeys to asphyxia. To establish criteria for determining when a given asphyxiation should be terminated in order to successfully resuscitate and maintain an infant monkey.

Methods employed: Fetal monkeys were asphyxiated by leaving them within the amniotic sac. They were resuscitated by intermittently inflating the lungs with oxygen from a rubber bag through an endotracheal tube. Activity, respiratory effort, and heart rate were recorded.

Newborn monkeys were asphyxiated by placing them in a glass jar through which nitrogen was flowing. The same method of resuscitation described above was used. Similarly, activity, respiratory effort and heart rate were recorded.

Major findings: Technically satisfactory asphyxiation-resuscitation were accomplished in 8 fetuses and 3 newborns.

Part A Project Description (cont'd)

The differences in the responses of fetal and newborn monkeys to asphyxiation observed during these studies fell into three groups. (a) The fetus was quiescent, except for respiratory efforts, whereas the newborn struggled, defecated and salivated. (b) The heart rate fell slowly in the fetus. In the newborn, in contrast, the heart rate fell abruptly and then rose slowly. (c) A fetus could be resuscitated 5 and one half minutes after its last gasp, whereas the newborn could not be resuscitated if asphyxiated for one minute after its last gasp.

Significance: The general term "asphyxia neonatorum" has been used to include both fetal and newborn asphyxiations. These findings, however, show that the responses of the fetus to asphyxia is different from that of the newborn. This information complements that of Dawes and his colleagues in acute experiments in that they have shown differences in blood pressure response, glycogen reserve in the heart, blood sugar between fetuses and newborns asphyxiated past the last gasp.

Proposed course of project: To attempt to combine the chronic experiment with survival with some of the techniques performed by Dawes and his colleagues in acute experiments.

Part B included: No

Summary Report of Laboratory of Biophysics

Calendar Year 1959

Kenneth S. Cole, Chief

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 FitzHugh, 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 measurement 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 percent of normal external divalent ion concentration increased the resting net sodium inflow to about three times the normal value.

Summary Report of Laboratory of Biophysics (cont'd)

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 blockade. Although both lines of investigation are interesting and promising the results are so increasingly divergent as to further decrease the probability of a causal 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.

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LABORATORY OF NEUROPHYSIOLOGY

Wade H. Marshall, Chief

The Spinal Cord Section, under the leadership of Karl Frank, is proceeding with analysis of fundamental work on generation of impulses in nerve cells. The work is immediately aimed at, determining with certainty that 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 only a part of the soma. Work is going forward to plot the 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 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 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, Nelson, Frank and Becker have shown that 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. It is probably due to abnormal sensitivity to hypoxia of the neurons injured by the electrode.

The Section on Special Senses, under the leadership of Dr. Tasaki, has made 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. Teorell from Uppsala, Franck from Darmstadt, Germany and Hime from Brookhaven. It has been recognized for some time that tracer data often contained inadequate

Further work has been done on the general symposium of the two stable state process in many kinds of reactive neurons or interface systems. Four cats have been found to demonstrate and study the two stable state and hyperpolarizing responses. The applications of this to mechanoreception have been discussed at an IUPAB acoustics symposium.

Further work on EMG and EOG spectroscopy is also planned.

The Section on Limbic Integration and Behavior, under the leadership of Dr. 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 observation, conditioning and learning studies, electrical examination of the CNS, biochemical lesions and neuroanatomical work.

An intensive work is being done on the functional localization of the genitalia in the brain of the squirrel monkey. Another specific project being conducted by Dr. Dougan and Maclean is aimed at [illegible] of the [illegible] between the highly excitable hippocampal system and other parts of the brain on the one hand and connection between the hippocampal system and endocrine neuroendocrine mechanisms. An intensive study is being made on the midline nuclei of the limbic system.

Dr. Ploog is conducting a valuable naturalistic observation study on a small group of squirrel monkeys which is remarkable for the information already 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. He was also instrumental in introducing the squirrel monkey to the [illegible]. This group is preparing an ethological atlas to aid in the laboratory use of the squirrel monkey. The variety of work done by this section is unique and the work is also unique as an example of good basic science with clear [illegible] 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. Kandel, Spencer, and 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 and differences with data 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 agrument for mechanisms of spreading cortical depression. This program is proceeding to gain some knowledge of actual integrative mechanisms of which the firing of the cell axon is the end point.

A valuable project was done using tracers to examine K ion release from the cortex by various chemical agents and spreading cortical depression.

Dr. Bodkin is proceeding with an interesting method of conducting analysis of sensory discrimination at cortical and thalamic levels.

Dr. Struwasser is proceeding with unitary extracellular analysis in the frog CNS. This work is aimed at study of the phenomena of attention, habituation, discrimination, extinction, etc. Incidental to this general program, a very useful method of extracellular stimulation was developed and a significant study made of stimulus parameters. This method is particularly useful for locating the cells not reacting in the near vicinity of the electrode. Dr. Struwasser is proceeding with a project using the dorsal root cells of the frog spinal cord in which cells and electrodes can be directly visualized. This project is expected to aid in clearing up an old controversy about the "gaint" extracellular positive spikes first seriously analyzed by Freygang. One group holds that these positive spikes indicate that a large part of the membrane of many cell types is not electrically excitable. Another group considers that this form of potential is recorded only from injured cells. The electrode is so close to the membrane that injury cannot be excluded so the argument needs more definite evidence. It is to be noted that several

Serial No. NINDB-NP-SS-5
1. Lab. of Neurophysiology
2. Special Senses Section
3. Marine Biological Lab., Woods Hole, Mass. and Bethesda, Md.
4. Combination of 1a, 3 and 4, 1958

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: The mechanism of nerve excitation.

Principal Investigators: I. Tasaki, T. Teorell, U.-Franck and C. Spyropoulos

Other Investigators: L. Nims, A. Bak, M. Ezzy and D. McKenzie

Cooperating Units: Departments of Biophysics and Physiology, Uppsala, Sweden; Dept. of Electrochemistry, Eduard-Zintl-Institute, Darmstadt, Germany; Brookhaven National Laboratory, Upton, Long Island, N. Y.

Man Years (calendar year 1959):
Total: 5
Professional: 2.0
Other: 3.0

Project Description:

Objectives: For a number of years, the primary objective of the Section on Special Senses has been the elucidation of the mechanism of excitation. This process constitutes the primary activity of the cellular elements of the nervous system. In addition, it is involved in many other well known biological processes such as receptor activity, muscle contraction, cell division and amoeboid movement. Experimental attempts during the last year at solution of this problem were spurred by collaboration of members of this section with outstanding scientists from other institutions, both in the United States and abroad. These collaborative scientists were Torsten Teprell of the University of Uppsala, Ulrich Franck from the

Eduard-Zintl-Institute in Darmstadt, Germany and Leslie Nims from the Brookhaven National Laboratory

Methods Employed: The method of intracellular multiple wire electrodes, the method of intracellular injection and the method of perfusion of the intracellular compartment were employed with the squid giant axon. Standard microelectrode techniques were employed in conjunction with the isolated dorsal root ganglion of the frog to study the effects of Ba^{++}. Standard radioactive tracer techniques and the method of Obrink and Ulfendahl (for simultaneous measurement of radioactive Na and K) were employed in conjunction with electrophysiological techniques to study tracer movements in the squid giant axon. The Obrink-Ulfendahl method was introduced into our laboratory by Prof. Teorell. These same radioactive tracer techniques in conjunction with a cation exchange membrane from Ionics, Inc. were employed to study tracer movements in artificial membranes. A rotating fractionator was employed for measuring tracer movements during the action potential.

Major Findings:

(1) Movements of tracers across membrane in general. The principles governing the movements of tracers across animate and inanimate membranes and their relation to the movement of the untagged species were defined by application of the modern theory of thermodynamics of irreversible processes. Experimental verification of the proposed principles underlying movements of tracers across membranes and their relation to the movement of untagged parent elements were obtained by the study of movements of tracers across cation exchange heterogenous membranes. It is found that, except in the case where the ratio of the concentration of the tagged species to that of the untagged species of the same element is the same on the two sides of the membrane, the movement of the tagged species does not give any information as to the movement of the untagged species. The physical information obtainable by the tracer technique was clarified.

(2) Movement of tracers across the nerve membrane

The tracers employed include K^{42}, Na^{24}, Ca^{45}, P^{32}, Cl^{36} H^3, etc. It was found that Na^{24} and K^{42} had a time constant of outward movement of the order of 50 hours, Na^{24} being somewhat faster than K^{42} at room temperature. The time constant of Ca^{45} is far more rapid, of the order of 6 - 8 minutes. Evidence was obtained, that an appreciable amount of Ca^{45} introduced into cell was bound. The movement of negative ions (chloride, phosphate and sulfate) was far slower than that of the positive ions. This provides the first biological experimental evidence that the nerve membrane has a fixed charge behaving like a cation exchange membrane. That biological membranes have such properties was proposed more than twenty years ago by Professor Teorell. The tracer whose movement was found to be fastest was tritiated water with a time constant of less than 30 seconds. The effect of a variety of agents was studied upon the movement of these tracers. These agents included repetitive suprathreshold and subthreshold stimulation, temperature, zero calcium in outside medium, zero sodium, high potassium, de- and hyperpolarization by applied currents, pH and narcotics. Stimulation increased the outflux of both Na^{24} and K^{42}, Na^{24} being favored. Low temperature increased the outflux of K^{42}. Low calcium increased the outflux of both Na^{24} and K^{42} the latter being favored. Stimulation did not appreciably affect the movement of the negative tracer ions. Potassium depolarization increased the outflux of both Na^{24} and K^{42}. Current depolarization increased the outflux of both Na^{24} and K^{42}, favoring the latter.

By the use of a rotating fractionator, it was possible to collect samples of sea water flowing by the giant axon at different intervals during the action potential. Preliminary results indicate that both K^{42} and Na^{24} flow out of the axon both at the beginning and at the end of the action potential. At neither

time did there appear to be any profound preference of one ion over the others.

(3) A mathematical formulation of the process of excidation in animate and inanimate excitable systems. In the past few years there has been developed in this laboratory the two-stable state concept of nerve excitation. During approximately the same interval of time, it has been becoming more apparent that excitation in living cells shows many of the same characteristics as certain inanimate systems. Two such well known systems are a redox system comprised of a metal in contact with an oxidizing agent and a system comprised of a membrane separating two solutions at different hydrostatic pressures. Today's outstanding authority on this system is one of our collaborators Professor Ulrich Franck and the discoverer of the redox system is Professor Torsten Teorell, our other colleague. Discussions during the past summer have led to a general agreement on a mathematical formulation of the excitation process apolicable to all these systems animate and inanimate.

(4) Other related developments.

a) It was found that dorsal root ganglion cells in the absence of Na^+ and in the presence of Ba^{++} give rise to action potentials which may be of the normal polarity but of an appreciably increased duration or of a reversed polarity. In the latter instance, the cell displays a resting potential of reversed polarity as well. Such behavior was anticipated from the two-state concept of excitation and provides the most striking illustration obtained to date of the "flip-flop" property of the membrane prescribed by the two-stable state concept.

b) A method was developed whereby a large portion of the cytoplasm could be continuously perfused with isotonic KCl for 20 - 30 minutes at a rate of 0.01 without marked impairment of the electrophysiological properties of the cell. This technique involves

c) It was found that when the normal squid axon is subjected to strong hyperpolarizing currents a "hyperpolarizing response" appears which shows a type of "refractoriness", a Ca^{++} dependence upon the presence of Ca^{++} but not upon Na^{+}.

d) Upon supercooling a squid giant axon (with the outside medium in the frozen state), it was found that the membrane potential fell rapidly to a very low value. At this point, responses of the hyperpolarizing variety could be elicited.

e) At the acoustical meeting held at NIH last summer a theory of mechanoreception which was based upon the two stable concept of excitation was presented.

f) In anticipation of continuing previous work on NMR and EPR Spectroscopy, one of us attended a workshop at Varian Corp. in Palo Alto, California designed to provide experience with these techniques.

g) During the past year, the chapter on "Excitation and Synaptic Transmission" of the Annual Review of Physiology was completed by members of our section.

Significance to Neurology Research: This investigation is expected to contribute to the understanding of the normal function of the nervous system.

Proposed Course of Project: It is our opinion that exploitation of the purely electrophysiological approach to the problem of excitation is nearly complete. At this time, we feel that the more promising avenues of research are those utilizing chemical, spectroscopic and mechanical or thermal transducer techniques. To this end, we intend to study, in the next few years, the process of excitation with such tools as interference microscopy, NMR spectroscopy, EPR spectroscopy, ultraviolet and visible light spectroscopy. Furthermore, we plan to make an attempt to measure mechanical and

thermal changes during the action potential. Professor Teorell from Sweden and Drs. Arvanitaki and Chalazonitis from France have had experience with some of these techniques in the past. We are contemplating inviting these scientists to collaborate with us on this general problem. Two years ago, we collaborated with Dr. Richard Sands at the University of Michigan on EPR spectroscopy. We plan in the next year to resume this study.

Publications other than abstracts from this project:

Tasaki, I. Physiological properties of the myelin sheath and of the node of Ranvier. Chapter VI in: The Biology of Myelin, Vol. IV, Progress in Neurobiology, Korey, S. R., Ed, New York, Hoeber-Harper, 1959, xxii, 405 pp.

Tasaki, I. and Spyropoulos, C. S. Stria vascularis as source of endocochlear potential. J. Neurophysiol., 1959, 22: 149-155.

Tasaki, I. and Bak, A. F. Voltage clamp beh vior of iron-nitric acid system as compared with that of nerve membrane. J. Gen. Physiol., 1959, 42: 899-915.

Tasaki, I. Conduction of the nerve impulse. Chapter III in: Handbook of Physiology, Section I: Neurophysiology, Vol. I., Field, J., Magoun, H. W. and Hall, V. E., eds., Washington, D. C., American Physiological Society, 1959, xiii, 779 pp.

Spyropoulos, C. S. Miniature responses under "voltage-clamp". Am. J. Physiol., 1959, 196: 783-790.

Spyropoulos, C. S. and Brady, R. O. Prolongation of response of node of Ranvier by metal ions. Science, 1959, 129: 1366-1367.

Tasaki, I. Demonstration of two stable states of the nerve membrane in potassium-rich media. J. Physiol. (London), in press.

Spyropoulos, C. S. and Ezzy, M. E. Nerve fiber activity in heavy water, Am. J. Physiol., 1959, 197: 808-812.

Tasaki, I. Afferent impulses in auditory nerve fibers and the mechanism of impulse initiation in the cochlea. (Presented at the Conf. on Neural Mechanisms of the Auditory and Vestibulary Systems, Bethesda, Md., June, 1959) To be published as a monograph by Charles C. Thomas, Springfield.

Part B: Honors, Awards and Publications (cont.)

Tasaki, I. Resting and action potentials of reversed polarity in frog nerve cell. Nature (in press)

Spyropoulos, C. S. Cytoplasmic pH. J. Neurochem. (in press)

Spyropoulos, C. S. and Tasaki, I. Conduction and transmission in the nervous system. Chapter in: Ann. Rev. Physiol. (in press)

Honors and Awards relating to this project:

Dr. Tasaki was invited to participate in the Conference on Neural Mechanims of the Auditory and Vestibulary Systems, Bethesda, Md., June, 1959.

Dr. Spyropoulos was made a member of the American Physiological Society.

Serial No. NINDB-NP-SC-3
1. Lab of Neurophysiology
2. Spinal Cord Section
3. Bethesda, Maryland
4. Continuation

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Generation of impulses in nerve cells.

Principal Investigators: K. Frank and M. G. F. Fuortes

Other Investigators: P. Nelson, W. Rall and M. Becker

Cooperating Units: Dr. M. G. F. Fuortes of the Ophthalmology Branch of NINDB has devoted considerable time to this project. Dr. W. Rall of the Office of Mathematical Research of NIAMD is providing important theoretical assistance.

Man Years: Patient Days: None
Total: 5.7
Professional: 2.5
Other: 3.2

Project Description:

Objectives: To determine which parts of the neuron contribute the various components of the recorded potentials, the time sequence of their activation and the quantitative aspects of the changes each part undergoes.

Methods Employed: (1) Voltage clamp technique. A technique has been developed for the introduction of concentric micropipettes inside a cat's spinal motoneuron. Measurement of the current through the outer pipette required to just maintain the potential of the inner pipette at any desired level permits the manipulation of the potential of the penetrated portion of the cell in the face of activity in various parts of the cell. This technique has required a detailed study of the control of size and shape of glass micropipettes by Mrs. Mary Becker. A device for nesting and advancing the concentric micropipettes has been developed and a contract let for its manufacture. (2) Single cell external fields. A second experimental approach is under way to determine the extracellular potentials produced in the volume conductor of

the spinal cord by activity in a single motoneuron. This requires stimulation in the ventral root of the axon from the single cell to be studied. A few ventral root fibers at a time are drawn up into a coarse micropipette under Ringer's solution. It is then possible, using a bridge circuit, to stimulate and record simultaneously the selected axon while mapping the potential field in the spinal cord with a microelectrode. Various types of electrodes and electrode arrays will be used for mapping the "A" and "A-B" type fields surrounding the motoneuron. It is anticipated that it will be necessary to use techniques developed in Project No. NINDB-NP-SC-6 for increasing signal to noise ratio in this study. (3) Marking technique Correlation of microelectrode position with the character of potentials recorded would provide valuable information on the roles of different neuron parts. Dr. P. Nelson has been primarily responsible for the development of a marking technique. He has also set up an isolated frog cord preparation for testing the marking technique.

Major Findings:

(1) Use of the voltage clamp technique applied to spinal motoneurons has led to the conclusion, based on certain assumptions, that the resistance measured between inside and outside the cell decreases by a factor of only 2 to 3 during activity. When the clamped region separates one part of the neuron from another, voltage changes in one part cannot affect the other. Thus the clamped area cannot lie between "A" and "B" regions, since A activity elicits B firing even in the presence of the clamp. These results are consistent with the earlier hypothesis that the "A" spike originates in the axon, but suggest that the "B" spike does not invade more than a part of the soma-dendritic membrane.

(2) Experiments to determine the potential field around a single motoneuron have been reported by Fatt. His conclusion that the cell soma is the source of the "A" spike and that the "B" spike represents conduction out the dendrites is in disagreement with the results of the voltage clamp technique and Freygang's interpretation of giant extracellular responses. It is therefore necessary to repeat Fatt's measurements with more elaborate techniques for recording the field potentials and for insuring that they are from only one cell.

Each of the techniques on which this experiment depends has now been worked out and tested separately. It remains to be seen whether they can be made to work simultaneously for a long enough time to complete a map of the fields.

(3) Mr. Nelson's marking technique employs concentric pipettes for both intra- and extracellular recordings. After recording cell activity, ferrocyanide in the inner pipette and ferric citrate in the outer are expelled by iontophoresis, producing a Prussian Blue reaction at the electrode tip. Blue spots 10-20 microns in diameter have been obtained in or near motoneuron somata in about five cases. These are marked by larger "signposts" on the cord surface. Agglutination of cord substance decreases the certainty of electrode position as the spot may move on withdrawal of the pipette. The method appears to be practical for identification of the cell penetrated but probably not for determining from which part of the cell the recording was made.

Significance to Research: This is the major project of the Spinal Cord Section. Results of these studies add to the fundamental knowledge of basic mechanisms of neuron physiology. Specifically, they may lead to a better understanding of the role of the dendrites and the manner of integration of presynaptic influences. A goal of major significance to neurology is the elucidation of mechanisms of repetitive impulse generation in neurons.

Proposed Course of Project: Voltage clamping studies are temporarily discontinued pending results from field measurements around cells and the results of Project No. NINDB-NP-SC-3. If these and other studies support the assumptions necessary to success with the voltage clamp technique, it will be continued to explore the nature of synaptic potentials.

Field measurements are just beginning and will be continued until conduction in dendrites has been tested or the method proved impractical.

A new experiment has been proposed by Dr. Rall for determining the relative importance of soma versus dendrites in contributing to the measured membrane resistance. This

[illegible] measurement of the current voltage phase shift in the passive motoneuron membrane at different frequencies and will probably be attempted by the Spinal Cord Section during the following year.

Part B included Yes X No

Publications other than abstracts from this project

Frank, K., Fuortes, M. G. F., and Nelson, P. G. Voltage clamp of motoneuron soma. Science (1959) 130: 38-39.

Frank, Karl. Identification and analysis of single unit activity in the central nervous system. Handbook of Physiology - Neurophysiology I (1959) Chapter X: 251-[illegible]

Frank, Karl and Sprague, James M. Direct contralateral inhibition in the lower sacral spinal cord. Exp. Neurol. (1961) 1: 43-63.

Frank, Karl. Basic mechanisms of synaptic transmission in the central nervous system. IRE Transactions on Medical Electronics (1959) ME-6: 85-88.

Honors relating to this project:

Dr. Frank was invited to be a member of the Medical Exchange Mission on the Physiology and Pharmacology of the Nervous System to the U.S.S.R.

Dr. Frank was asked to be an Associate Editor for the journal "Experimental Neurology"

Dr. Frank was invited to be a member of the Physiology Study Section. His term expires in 1963.

Drs. Frank and Fuortes were invited to write the chapter on Conduction and Transmission in the Nervous System for the Annual Review of Physiology.

Dr. Frank and Mrs. Becker were invited to write the chapter on Microelectrodes for the Handbook of Physical Methods in Biology.

Dr. Frank was invited to address the EEG Society at its annual meeting in Atlantic City, the Japanese Physiology Society in Tokyo, the John Curtin School of Medicine in Canberra and the Nobel Institute in Stockholm

Serial No. NINDB-NP-SC-6
1. Lab of Neurophysiology
2. Spinal Cord Section
3. Bethesda, Maryland
4. New

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Augmentation of signal to noise ratio.

Principal Investigator: K. Frank

Other Investigator: P. Nelson

Cooperating Units: Mr. R. Cox of the Laboratory of Clinical Science, NIMH, and Dr. R. FitzHugh of the Laboratory of Biophysics, NINDB, are contributing to this project.

Man Years: Patient Days: None
Total: .5
Professional: .4
Other: .1

Project Description:

Objectives: To find practical methods for summing recurrent signals in order to increase the ratio of signal to noise. If the signal repeats at the same time during each trial it will add linearly with the number of trials (epochs) while noise, being random, tends to cancel and increases only as the square root of the number of trials.

Methods Employed: The methods listed are not original with the Spinal Cord Section, but are being considered for their applicability to neurophysiological research. (1) The recurrent signal-plus noise is translated into digital form either in real time or from a slowed tape recording. Each epoch is divided into time slices and the digital signal plus noise in corresponding slices is summed for a number of epochs. This digital computer technique has been used successfully at M.I.T. and is practical for neurophysiological applications. Modification of a commercially available digital pulse height analyzer would provide an adequate solution at a cost of about $30,000. Inexpensive computers

for performing this specialized function are not likely to be developed in the near future. (2) Analog summing of signal-plus-noise by storage in a series of condensers each summing corresponding time slices, may be a practical solution using an operational amplifier and some form of high speed switch. Dr. FitzHugh has agreed to help with preliminary trials of this approach. (3) Mr. Cox is exploring a number of methods including the use of beam storage tubes. (4) A more cumbersome but inexpensive method has been devised by Mr. Cox and modified for use by the Spinal Cord Section. This method translates signal-plus-noise into variations in intensity of an r.f. modulated cathode ray tube beam and stores successive epochs as superimposed exposures on a film. Augmentation of signal to noise ratio by a factor of 10 appears practical, but the method suffers by not providing a continuous readout of the augmented signal as it develops. This is the method planned for use in recording single cell potential fields in Project No. NINDB-NP-SC-3.

Significance to Research: There are many applications of the principle of signal to noise ratio augmentation not only in neurophysiology but in biomedical research in general. Along with advances in data processing techniques in general, this particular technique will be of great importance as the exploration of electrical signs of nervous activity becomes more and more refined.

Proposed Course of Project: A number of possible solutions to the problem of augmentation of signal to noise remain to be explored. When this has been done, serious consideration must be given to the need for developing a practical instrument of wide applicability either within the NIH or by an outside development contract.

C. Improved Yes ___ No X

Serial No. NINDB-NP-SC-7
1. Lab of Neurophysiology
2. Spinal Cord Section
3. Bethesda, Maryland
4. New

PHS-NIH
Individual Project Report
Calendar Year 1950

Part A.

Project Title: The effect of anoxia on motoneuron membrane potential.

Principal Investigator: P. Nelson

Other Investigators: K. Frank and M. Becker

Cooperating Units: None

Man Years: Patient Days: None
Total: 1.0
Professional: .3
Other: .7

Project Description:

Objectives: To test reports by Kolmodin and Skoglund that membrane potential of motoneurons decreases in two steps during brief asphyxia.

Methods Employed: Trans-membrane potential of motoneurons was recorded with concentric micropipettes--one inside and one outside--during asphyxia by apnea and anoxia by nitrogen breathing.

Major Findings: Results of Kolmodin and Skoglund were not confirmed. Their changes are attributed to movement produced by blood pressure responses to asphyxia. When such movement was blocked by hexamethonium infusion, cell membrane potential was shown to be remarkably unaffected by long periods of anoxia.

Significance to Research: The possibility of a sensitive dependence of nerve cell membrane potential on oxygen tension has often been suggested. It is therefore important to show that at least in spinal motoneurons under the conditions of these experiments this popular view is false.

Part A. (cont.)

Proposed Course of Project: It is possible that the effect of oxygen lack might be demonstrated if the blood circulation could be interrupted so that metabolites such as potassium, if released from the cell, would not be immediately swept away. However, no practical experiment for testing this suggestion has yet been devised.

Part B included Yes___ No X

Serial No. NINDB-NP-SC-8
1. Lab of Neurophysiology
2. Spinal Cord Section
3. Bethesda, Maryland
4. New

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Generation of impulses in the Mauthner Cell of the goldfish.

Principal Investigator: T. Oikawa (Visiting scientist)

Cooperating Units: None

Man Years:
Total: .5
Professional: .5
Other: 0

Patient Days: None

Project Description:

Objectives: To use the large Mauthner cell of the goldfish as a model for testing ideas on mechanisms of impulse generation in spinal motoneurons.

Methods Employed: Intra- and extracellular recording of action potentials during anti- and orthodromic stimulation and direct excitation through the micropipette will be attempted. If possible, voltage clamp techniques will also be used. Marking techniques and histological studies will be an important aspect of the study.

Major Findings: Dr. T. Oikawa is a visiting scientist with the Spinal Cord Section for five months. Work on this project is just beginning.

Significance to Research: Results of studies on a giant nerve cell provide important guides for smaller cells just as studies on the giant axon of the squid have contributed so much to an understanding of conduction and excitation in all fibers.

Proposed Course of Project: Continuation of this project after Dr. Oikawa returns to Japan depends on the usefulness of the results obtained.

Part B included Yes___ No X

Serial No. NIMH-MP-BS-1
1. Laboratory of Neurophysiology
2. Section on Brain Stem
3. Bethesda, Maryland
4. Continuation of NIMDB-NC-13

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Vestibular Influences on Spinal Mechanisms

Principal Investigator: Bo E. Germandt, M.D.

Other Investigators: Magdolna Iranyi, M.D., Sid Gilman, M.D., and Robert B. Livingston, M.D.

Cooperating Units: None

Man Years
Total: 2.5
Professional: 2.5
Other: 0

Project Description:

Objectives: Our recognition of the spatial world in which we live and of our own bodies disposed within that space is derived from several channels of sensory information: visual, vestibular, proprioceptive, and visceral. These systems are involved in most of our perceptual, experiential, and motor activities. Proprioceptive and vestibular systems are interdependent in controlling posture and locomotion; streams of afferent impulses generated by receptors in each of these systems interact in supraspinal and spinal nervous structures and converge to influence the activity of the final common path, the motor units. The sensory organ of particular importance to the maintenance of equilibrium and orientation is the nonauditory, phylogenetically oldest portion of the inner ear, the labyrinth or vestibular apparatus. The objectives of the present investigations are to analyze the complex mutual interactions among activities mediated through the vestibular, segmental and intersegmental proprioceptive, and pyramidal systems, as reflected by alterations in spinal motor outflow.

Methods Employed: The method of Germandt and Anderssen for stimulation of the vestibular nerves in cats is employed. Responses are recorded from a variety of spinal nerves, ventral roots, and small filaments of the ventral roots. Additional input is initiated as applied to the cerebellum, cerebral motor cortex, and

Major Findings: Neurophysiologic evidence suggests that fibers within the vestibular nerve cross just below the obex and pass directly to ventral horn cells of the contralateral cervical cord without synaptic relay. This finding is supported by unpublished anatomic studies of Grant Rasmussen. There are significant differences in the configuration of responses obtained bilaterally from motor nerves of cervical and lumbosacral segments of the spinal cord. This implies important changes in organization along the neuraxis, with the vestibulospinal tract becoming less important at lower levels than reticulospinal (and/or relaying propriospinal) neurons. Activity in these tracts is reflected in individual components of the responses recorded from cervical levels. These components can be selectively influenced by particular techniques of stimulation, anesthesia, and asphyxia. Descending fibers of the medial longitudinal fasciculus make no important contributions to descending vestibulofugal activity.

Vestibular responses, particularly those recorded from cervical levels, are influenced by neck muscle proprioceptor stimulation. Ventroflexion of the head through 30° is sufficient to markedly diminish ipsilaterally recorded responses and to completely abolish the contralateral ones. Gradually increasing ventroflexion of the head selectively inhibits components attributed to reticulospinal impulses before there is any change in the component supposed to be due to vestibulospinal activity. This effect persists but is significantly reduced following complete cerebellectomy. A tonic inhibitory control of the cerebellum on the vestibular system is demonstrated by a marked augmentation of the vestibular responses following acute cerebellectomy. The responses undergo a further growth following removal of a large portion of the medial reticular formation, demonstrating that the reticular formation exercises inhibitory influences upon the vestibular system which are independent of the cerebellum.

Descending vestibular influences affect segmental spinal reflexes by an initial enhancement followed by a sharp depression and a subsequent period of re-enhancement. The re-enhancement disappears after cerebellectomy. The early facilitatory influences appear to be bilaterally reciprocally organized; the inhibitory influences appear to be bilaterally symmetrical. Both cervical and lumbosacral outflows yield two successive wave responses to vestibular stimulation. These are influenced bilaterally symmetrically in parallel fashion in both cervical and lumbosacral outflows. Projections responsible for a large spikelike wave in the radial nerve response are more direct

and imperious and not so extensively distributed as are those for the two wave responses. Ventral root responses to single shock vestibular stimulation reveal the firing of small motor units; more rapid vestibular stimulation recruits large motor units. Descending vestibular influences induce activity in lumbosacral motor units earlier (even though the descending path is longer), and have a more prolonged effect on segmental activity, than do propriospinal influences studied by Lloyd in the spinal cat by means of brachial plexus stimulation.

The importance of tonic and phasic suprasegmental control of spinal reflex activity is investigated. In decerebrate preparations, single shock activation of the descending vestibular or propriospinal mediating systems evokes a pattern of facilitatory and inhibitory excitability changes of lumbosacral ventral horn cells. The prominent inhibitory influence of intersegmental propriospinal activity can be eliminated by high spinal transection. The protracted inhibition of local and long spinal reflex activities following high frequency vestibular or brachial plexus stimulation is abolished by a small localized incision in the midline at the caudal limit of the medulla, interrupting continuity between medial reticular formation and spinal segments.

Significance to Science: The mechanisms underlying muscle tone, posture and locomotion are basic neurophysiological problems. Any increased knowledge of mechanisms of circuitry in the central nervous system and especially of integration between the classical and the diffusely projecting systems is of especial value.

Proposed Course of Project: It is proposed to pursue a continuing stepwise analysis of the mechanisms of vestibular integration.

Part B included: Yes

PHS - NIH
Individual Project Report
Calendar Year 1959

Part B: Publications

Gernandt, B.E., Iranyi, M., and Livingston, R.B. Vestibular influences on spinal mechanisms. Exper. Neurol., 1959, 1:248-273.

Gernandt, B.E. and Gilman, S. Descending vestibular activity and its modulation by proprioceptive, cerebellar, and reticular influences. Exper. Neurol., 1960, 1:274-304.

Honors and Awards: Presentation of this material at an international Symposium on Neural Mechanisms of the Auditory and Vestibular Systems at the National Institutes of Health by invitation.

Director of Basic Research, NIMH-NINDB October 30, 1959

Chief, Section on Lipid Chemistry, NINDB

Summary of Research Reports for 1959

Investigations have continued 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 the 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 co-factor 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.

Work on the formation of complex sphingolipids 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 gl... ...ceramide in Gaucher's disease.

We have prepared labeled psychosine (sphingosine ... ...ctoside) for investigating the biosynthesis of gang... the polyhexose sphingolipids present in ganglion cells of ... cortex. Preliminary experiments indicate that the ... synthesis of these compounds has been successfully dem... for the first time in cell-free preparations of brain t... We have also devised an ultrasensitive analytical method ... the quantitative determination of gangliosides. The ava... of this procedure is required for studying the metabol... gangliosides. We expect to undertake investigations of t... nature in cortical biopsy specimens obtained from patients ... Tay-Sachs disease. This condition is characterized by the ... accumulation of abnormally large quantities of gangli... ganglion cells.

A method has been devised 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 levels of 1 to 1.4 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 of a new class of intermediate 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 ... 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 ... holic antagonism between inositol and choline has been ... elucidated. These substances compete with each other ... syntheses of essential phospholipids in growing ...

Director of Basic Research - 10/30/59

Dr. 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.

Roscoe O. Brady, M.D.