ANNUAL REPORT
OF
PROGRAM ACTIVITIES

NATIONAL INSTITUTES OF HEALTH

1958

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 Disease and Blindness
Report of program activities
181

ANNUAL REPORT - 1958

NINDB Office of the
Director Project Reports

Title	Chief Investigator
The Director's Report	Bailey
Direct Training	Wipf
Collaborative Research	Masland
Epidemiology Branch	Kurland
Biometrics Branch	Goldstein
Extramural Programs	Seger
I. Research Grants	Seger
II. Field Investigations and Pilot Projects	Seger
III. Graduate Training Grants	Seger
IV. Special Traineeship Program	Hartman
V. Research Fellowships	Hartman
VI. Review and Approval of Grants	Seger

Clinical Research - 1958

Serial No.	Title	Principal Investigator
10(c)	Study of Mechanisms of Transmitter Liberation at Presynaptic Nerve Endings----------	del Castillo

Medical Neurology Branch

Summary---------- Shy

Individual Project Report

Serial No.	Title	Principal Investigator
11(c)	The Use of a Monomaine Oxidase Inhibitor (JB-516) as an Anticonvulsant Medication for Centrencephalic Seizures----------	Prockop Smith
12(c)	Clinical Pathological Correlative Study of the Nervous System in Orthorostatic Hypotension----------	Drager
13(c)	The Histopathological and Chemical Investigations of Neuromuscular Disorders-----	Shy Wanko
14(c)	Pathological Study of Intramuscular Motor and Sensory Nerve Endings in the Normal and in Neuromuscular Diseases----------	Haase
15(c)	The Physiology and Pharmacology in Myasthenia Gravis----------	Shy
16(c)	A Study of Progressive Parenchymatous Degeneration of the Central Nervous System----	Smith Drager
17(c)	Spirochetes and viral antigens and their relation to the spinal fluid and blood of multiple sclerotic patients----------	Korengold
18(c)	Investigations in Localization of Cerebral Neoplasia by Isotopic Detection------	Shy
19(c)	Metal Chelates as Possible Contrast Media for Myelography----------	Ci Chiro Rubin
20(c)	Blood and tissue cholinesterases in neuromuscular blockade----------	Irwin

Serial No.	Title	Principal Investigator
21(c)	The action of neuromuscular blocking drugs on directly stimulated innervated and denervated muscle----------	Irwin
22(c)	Study of muscle cholinesterase and its inhibitors----------	Irwin
23(c)	A study of naturally occurring choline esters.----------	Irwin
24(c)	A study to determine the effects of depolarizing drugs on muscle enzymes----------	Irwin
25(c)	Electrolyte and Energy Metabolism in Normal and Epileptogenic Cerebral Cortex in Vitro---------- -------	Tower
26(c)	Comparative Biochemistry of Smooth Muscle and Striated Muscle----------	Horvath
27(c)	Amino Acid Metabolism in Normal and Epileptogenic Cerebral Cortex in Vitro----------	Tower
28(c)	Clinical Evaluation of Various Amino Acids and Related Compounds in Control of Seizures including Studies of their Metabolism in Vivo-----	Tower
29(c)	The Relation of Pyridoxine (Vitamin B_6) to Certain Seizure States----------	McKhann
30(c)	The Metabolism of γ-Aminobutyric Acid in Neural Tissue----------	McKhann
31(c)	Microchemical Determination of Acetylcholine----------	Curtis
32(c)	Physico-chemical studies of Human Cerebrospinal Fluid----------	Curtis
33(c)	The Surface-Chemical Behavior of Urine in Relation to its Surface-Active Macromolecular Constituents------	Curtis

Serial No.	Title	Principal Investigator
34(c)	Cerebral Protein Metabolism and Turnover in Tissue Slices incubated in vitro.	Wherrett
35(c)	Distribution of Actin and Tropomyosin in Normal and Diseased Muscle	Horvath
36(c)	Alterations of Actomyosin Tensile Strength and Muscle Proteins in Neuromuscular Diseases	Proctor Shy

Ophthalmology Branch

Summary---------- von Sallman

Individual Project Report

Serial No.	Title	Principal Investigator
37(c)	Studies on Diet and Drug Induced Experimental Cataract	von Sallmann
38(c)	Study of Submicroscopic Structures of Lens Tissue Componets by Phase Contrast Microscopy	von Sallmann Caravaggio Aronson
39(c)	Studies on Central Nervous System Control of Intraocular Pressure. (Anatomy of Posterior Ciliary Nerves)	von Sallmann Grimes
40(c)	Study of Afferent Electric Impulses Induced by Intraocular Pressure Changes	Lele
41(c)	Effects of Muscle Relaxants on IOP and Extraocular Striate Muscles	Macri
42(c)	Study on the Pharmacodynamics of Various Agents Affecting Intraocular Pressure	Macri

Serial No.	Title	Principal Investigator
43(c)	Clinical Glaucoma Study----------	Paton von Sallmann
44(c)	The Ocular Diagnosis and Treatment of Ocular Complications of Primary Familial Amyloidosis----------	Kaufman van Alphen
45(c)	Study of Toxoplasmosis and Its Therapy----------	Kaufman
46(c)	Thyroid Hormone Turnover in Uveitis---------	O'Rourke
47(c)	Detection of Ocular Tumor by Isotope Tracer Methods----------	O'Rourke
48(c)	Immunological Relations in Ocular Tissues----------	van Alphen
49(c)	Basic Factors in Refraction Anomalies-------	van Alphen
50(c)	Intraocular Angiography----------	Cohan
51(c)	Electron Microscope Studies on Epithelium, Capsule and the Fibers of the Lens and on the Epithelium of the Ciliary Body and the Optic Nerve----------	Wanko
52(c)	Electron Microscope Studies on Biopsies of Human Muscle Diseases----------	Wanko Shy
53(c)	Study of Submicroscopic Structures of Ocular Pigment Cells. (Staining of the Living Tissue Culture Pigment Cell by Acridine Orange)----------	Wolf Aronson
54(c)	A Study of the Proteins of the Lens---------	Resnik
55(c)	An Investigation of the Enzymatic Systems Present in the Lens, Cornea and Aqueous Humor and their Relation to *in vivo* Tissue Metabolism----------	Kuhlman
56(c)	Electrophysiology of the Eye----------	Fuortes
57(c)	ERG Reactions of Pure-Cone Mammalian Retinae----------	Tansley Copenhaver

Serial No.	Title	Principal Investigator
70(c)	Neuromuscular Transmission in Hypothermia----------	Li
71(c)	Effect of Cooling on Conduction of Impulses in Cranial and Peripheral Nerves----------	Ortiz
72(c)	Study of Pharmaceutic Agents Acting on Various Cortical and Subcortical Structures of the Brain----------	Ortiz
73(c)	Properties of Cultured Nerve and Muscle Cells----------	Li, Klatzo and Baldwin
74(c)	Pinocytosis of Labelled Proteins in Tissue Culture----------	Klatzo
75(c)	The Localization of Myosin in Human Striated Muscle by Fluorescent Antibody----------	Klatzo
76(c)	Study of Pathology of Kuru Disease----------	Klatzo
77(c)	Study of Regeneration in the Central Nervous System----------	Ortiz-Galvan
78(c)	Histochemical and Electrophysiological Observations on the Muscle Fibers Grown in Vitro----------	Engel
79(c)	A New Method for Quantitative Study of Precipitin Reaction----------	Miquel
80(c)	The Relationship between Edema, Blood-Brain-Barrier and Tissue Elements in Experimental Brain Injury----------	Klatzo
81(c)	Study of the Effects of Hypothermia on Injured and Normal Brain Tissue----------	Laskowski
82(c)	The Investigation of the Site, Type and Extent of Lesions Involving the CNS in Cerebral Palsy and Allied Conditions--------	Dekaban

Serial No.	Title	Principal Investigator
83(c)	Maternal Condition During Pregnancy and the Course of Birth in Relation to Neurological Abnormalities in the Infants and Pathologic Lesions in Products of Abortion----------	Dekaban
84(c)	Pathological Lesions in the Central Nervous System Occurring During Prenatal, Intranatal and Early Early Postnatal Life----------	Dekaban
85(c)	The Incidence and the Type of the Central Nervous System Abnormalities Encountered in Offspring Born to Diabetic Mothers----------	Dekaban Baird
86(c)	Measurements of External and Internal Orbital Distance in Males and Females from Birth to Adulthood----------	Dekaban
87(c)	Preparation of the Horizons of the Normal Development of the CNS in Mice and Experimental Production of Congenital Malformations of the CNS----------	Dekaban
88(c)	Effect of "fear-provoking" stimuli on visual discrimination in primates-------	Lansdell
89(c)	Psychological Evaluation of Temporal Lobe Disease----------	Lansdell
90(c)	Body Temperature in Chimpanzees with Bilateral Temporal Lobe Damage----------	Blevins
91(c)	Fluothane Studies----------	Hall
92(c)	Hypothermia in Neuroanesthesiology---------	Hall
93(c)	Succinyl Choline in Awake Craniotomy-------	Hall
94(c)	The Effect of Hypertonic Urea Solution on Intracranial Pressure----------	Pritchard

Basic Research

Introduction---------- Livingston

Laboratory of Neuroanatomical Sciences

Summary---------- Windle

Individual Project Report

Serial No.	Title	Principal Investigator
NINDB-NA-DR-1	Development of Intrinsic Structures of the Human Brain----------	Windle Guth
NINDB-NA-DR-2	Histogenesis of normal and dystrophic retinas in mice----------	Sidman
NINDB-NA-DR-3	Histogenesis in the embryonic mammalian nervous system----------	Sidman
NINDB-NA-DR-4	Regeneration in the central nervous system----------	Windle
NINDB-NA-DR-5	Functional and Structural Changes in Reserpinized Animals----------	Windle
NINDB-NA-DR-6	Neuronal specificity in the autonomic nervous system----------	Guth
NINDB-NA-DR-7	Heterogeneous Reinnervation of the Diaphragm----------	Guth
NINDB-NA-DR-8	Experimental Analysis of the nerve fiber-taste bud relationship----------	Guth
NINDB-NA-DR-9	Nervous System Pathology in Macaca Mulatta after Asphyxia Neonatorum----------	Ranck Windle
NINDB-NA-DR-10	The significance of the acridine orange staining of neurons in vitro and in vivo----------	Wolf
NINDB-NA-DR-11	Structure and chemistry of photoreceptor cells----------	Sidman

Serial No.	Title	Principal Investigator
NINDB-NA-DR-12	Development of new histochemical methods----------	Sidman Feder
NINDB-NA-DR-13	Behavior and social organization of rhesus monkeys on Cayo Santiago, Puerto Rico----------	Altmann
NINDB-NA-DR-14	Physical measurements of rhesus monkeys from birth to old age----------	Altmann
NINDB-NA-DR-15	Technique of neurological examination of the monkey (Macaca mulatta)----------	Ranck
NINDB-NA-DR-16	Normal reproductive function in the rhesus monkey----------	Jacobson
NINDB-NA-DR-17	Maturation in infant rhesus monkeys; and care required for rearing them----------	Jacobson
NINDB-NA-DR-18	The intrinsic nerve supply to the endometrium in cat, guinea pig, monkey and man----------	Jacobson
NINDB-NA-DR-19	Neurological deficits of asphyxia neonatorum in macaca mulatta----------	Windle Ranck Combs Jacobson
NINDB-NA-DR-20	Psychological effects of asphyxia neonatorum in rhesus monkeys----------	Bailey Saxon
NINDB-NA-DR-21	Psychological and histopathological deficits of asphyxia neonatorum in guinea pigs----------	Bailey
NINDB-NA-DR-22	Centers and pathways involved in induced cerebellar seizures----------	Combs
NINDB-NA-NC-1	Ultrastructure of the nervous system--------	Palay
NINDB-NA-NC-2	Enzymatic reactions of gamma-aminobutyrate (γ-AB) catalyzed by brain tissue----------	Albers

[illegible]	[illegible]	[illegible]
[illegible]MA-MC-4	Quantitative histochemical distribution of glutamic decarboxylase in the nervous system----------	Albers
NIMDB-MA-MC-4	Micro-radiometric measurement of decarboxylase reactions----------	Albers
[illegible]MDB-MA-MC-5	A fluorimetric micromethod for the determination of succinic semialdehyde------	Albers
NIMDB-MA-MC-6	Neurosecretion in the rodent----------	Brightman
[illegible]MDB-MA-MC-7	Extraneuronal cholinesterase of the vertebrate central nervous system----------	Brightman
[illegible]DB-MA-EP-1	Pathogenetical factors in the development of myeopathies----------	Cammermeyer
[illegible]DB-MA-EP-2	Distribution of fat in the epidural space in mammals----------	Cammermeyer
[illegible]MDB-MA-EP-3	Structure of brains of monkeys in which the pituitary gland had been irradiated with high-energy deuterons----------	Cammermeyer
NIMDB-MA-EM-1	A study of the auditory afferent and efferent systems----------	Rasmussen
[illegible]MDB-MA-EM-2	A correlative histopathologic and genetic study of the hearing mechanism in a strain of congential deaf guinea pigs----------	Rasmussen
[illegible]MDB-MA-EM-3	An experimental study of the medial longitudinal fasciculus of the brain stem and spinal cord----------	Massopust
[illegible]DB-MA-EM-4	Neuronal connections and the functional significance of the interpeduncular nucleus----------	Massopust
[illegible]MDB-MA-EM-5	The comparative anatomy of the efferent cochlear bundle in selected submmalian vertebrates; and experimental study----------	Rasmussen

Serial No.	Title	Principal Investigator
NINDB-NA-FN-6	A study of an efferent component of the vestibular nerve arising from the medulla oblongata	Rasmussen

Laboratory of Biophysics

Summary---------- Cole

Individual Project Report

Serial No.	Title	Principal Investigator
NINDB-B-1	Ionic Permeabilities of the Squid Giant Axon Membrane	Cole
NINDB-B-2	Ionic Permeabilities of Nerve Membranes Theoretical Investigations	FitzHugh
NINDB-B-3	Correlation of Acetylcholinesterase Inhibition with Nerve Action	Whitcomb
NINDB-B-4	Membrane Potentials of a Lobster Giant Axon	Dalton
NINDB-B-5	Ionic Permeabilities of Nodal Membrane	Moore del Castillo

Laboratory of Neurophysiology

Summary---------- Marshall

Individual Project Report

Serial No.	Title	Principal Investigator
NINDB-NP-SS-1a	The chemistry of neural activity	Tasaki Spyropoulos
NINDB-NP-SS-3	Investigation of the sensory mechanism	Tasaki
NINDB-NP-SS-4	Physiological studies on the nervous elements in tissue culture	Chang Tasaki
NINDB-NP-SC-3	Generation of impulses in spinal motoneurons	Frank Fuortes
NINDB-NP-SC-4	Effects of locally applied drugs on single spinal motoneurons	Frank Paton

NATIONAL INSTITUTE OF NEUROLOGICAL DISEASES AND BLINDNESS

Office of the Director

Estimated Obligations - F. Y. 1959

	Direct	Reimbursement	Total
Administration	$128,000	0	$128,000
Publications and Reports (Information Office)	79,000	$16,000	95,000
Direct Training	39,500	0	39,000

ANNUAL REPORT

Calendar Year 1958

National Institute of Neurological Diseases and Blindness
National Institutes of Health

The Director's Report

The 1958 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; and the Introductory Annual Report of the Director of Basic Research, Dr. R. B. Livingston. It also contains summary reports of branch and laboratory chiefs and the Report of Dr. Richard L. Masland, Assistant Director of the National Institute of Neurological Diseases and Blindness, who heads the Institute's program in collaborative research.

Since the reports by Drs. Seger, Shy, Livingston, and Masland represent comprehensive commentaries on their 1958 scientific activities, they shall not be referred to further except when they relate to new trends or changes in program emphasis. The Director's Report, therefore, shall confine itself ... the main to new trends and developments in program emphasis and to special important events of 1958 which are relevant to the Institute's overall mission.

DIGEST OF EXTRAMURAL PROGRAMMING HIGHLIGHTS

At the end of the calendar year, 1958, there were 849 active research grants as compared to 670 at the end of 1957, or an increase of 26.8 percent. Of the total number of research grant awards, 26 percent were devoted to sensory disorders. Two thirds of this 26 per cent were related to studies of vision, and one third to studies of speech, hearing and equilibrium, and to studies of smell, taste, touch, and pain. A noteworthy change in programming was the lifting by the National Advisory Council of the arbitrary five-year ceiling for the support of research grants.

The program in field investigations and pilot project grants, though only initiated in 1957, now has 64 active projects, totaling $4,329,196. Ninety two percent of the funds expended in this program support multi-institutional, collaborative and cooperative studies. The largest of these is the collaborative study of cerebral palsy, mental retardation, and other neurological deficits of infancy and childhood. In this study, the NINDB functions as a coordination

center and central laboratory for 16 other institutions, in a study involving the examination of 40,000 pregnant mothers and infants. The cooperative study of intracranial aneurysms reached a full complement in 1958. Twenty cooperating institutions are in the study at an annual cost of $200,000. The cooperative anticoagulant therapy study in cerebrovascular disease must be conducted one more year before complete data will be available. This involves six institutions at the cost of $54,000 per annum.

A geomedical collaborative epidemiological study of selected neurological disorders in South Carolina, Nova Scotia, and Japan will be completed in 1959. New collaborative enterprises scheduled for 1959 include a glaucoma detection evaluation study in collaboration with the Chronic Disease Division of the Bureau of State Services, and a project of the Institute's Biometrics Branch with scientists of the American Academy of Ophthalmology and Otolaryngology.

Closely associated with the support of research programs within the extramural branch is NINDB's program for the development of future investigators. Early in the development of the research grants programs, it became evident that the lack of trained investigators was a bottleneck in our research effort. As initially set up, the training program concentrated on the training of clinical scientists and academicians in neurology and ophthalmology. This was important since it was recognized that research in the neurological sciences would require a "core of clinicians" well versed in the basic sciences who could serve in several capacities: as coordinators for clinical research, as teachers within university centers, and as leaders in neurology and ophthalmology to recruit and stimulate both basic and clinical research relating to neurological and sensory disorders.

At the end of the calendar year, 1958, the number of grant-supported programs for graduate training in the field of neurological disorders was 55. These programs were supported at a level of approximately $1,600,000 or at an average of $29,091 per program. One hundred and ninety-six trainees were in training for a career in clinical neurology during the period ending June 30, 1958. At present, 249 individuals are in training and 61 are expected to graduate in June, 1959. According to the present rate, 80 to 90 trainees will complete their training as specialists in clinical neurology each year.

The sharp research focus on neurologic disorders of early life has revealed the dearth of well-trained pediatric neurologists. The NINDB program for the training of pediatric neurologists is

still in a cradling stage. During 1958, one new training program was organized, bringing the total active programs in this area to three. These programs are being supported at the level of $63,768, or an average of $21,256 per program. At present there are 10 trainees in the program of pediatric neurology. At least 20 new programs are needed in this field.

In 1958, a greater program emphasis was given to the training of scientists in the neurologic basic sciences. These programs increased from seven in 1957, to 25 in 1958. This increase included eight new programs in neuropathology, three in neuroanatomy, three in neurophysiology, one in neuropharmacology, and three in neurochemistry. These grants now total 25 in number--in the amount of $675,458.

In 1958, the graduate training programs for developing future investigators in ophthalmology saw an increase of programs from 35 to 38. These programs provide a total of 296 trainees with approximately 87 potential teacher-investigators finishing residency training every year. Training programs in otolaryngology also increased from six to 18. The present number of trainees in this program total 116, of whom 25 are in the third year, nine in the fourth, and one in the fifth. It is expected that this program will produce between 20 and 30 potential teacher-investigators each year.

Another new area of program emphasis in training is the development of a training grant program in sensory physiology. This is a postdoctoral program designed to train basic scientists in the physiology of the special senses. Four applications for training programs in this area, in the amount of $249,475 will be submitted for review by the National Advisory Council at their March meeting.

In 1958, there was a substantial advance in the development of the program of Special Traineeships. A total of $905,750 was awarded to 125 trainees, an increase of approximately 50 percent over 1957. In the face of an acute need for neuropathologists, neurochemists, and those trained for research in the neurological deficits of the young, a special traineeship program contributed materially to training in these gap areas. The 1958 support in these areas was twice that of 1957 with 16 awards in neuropathology, seven in neurochemistry, and 18 in pediatric neurology.

An appraisal of the year's training activities indicates that the number of clinical training programs directed to neurologic disorders has reached a temporary plateau which will remain fixed

until more trainees under the program mature into teachers. Meanwhile, program emphasis is turning to training teacher-investigators in pediatric neurology, a serious gap area, and in the sciences basic to neurologic disorders. There also is an opportunity for the directors of clinical programs to bring their activities in closer contact with the basic sciences by developing clinical basic divisions under their own auspices, such as in clinical neuropathology, clinical EEG and neurophysiology, clinical neurochemistry, and related specialties.

The training programs directed toward ophthalmic disorders are progressing slowly without indication of a precipitous rise. The development of a genuine research interest on the part of ophthalmologists, with notable exceptions, has been comparatively slow. Programs in otolaryngology are on the march but of limited potential at present. The program in sensory physiology is in an advanced planning stage.

The multidisciplinary approach of the NINDB's collaborative projects has created a need for additional training for clinical specialists in their own specialty, or in related clinical specialties, or in the basic sciences. This important need is being met by the availability of special traineeships which has been one of the crowning achievements of the NINDB's training program.

INTRAMURAL GUIDELINES

During 1958, the National Institute of Neurological Diseases and Blindness continued its multidisciplinary clinical and basic project studies.

The clinical program advanced considerably in its research on neuromuscular, physiological, chemical, ophthalmological, pathological, and surgical problems in the field of neurological and sensory disorders. The clinical area has always operated under the guiding principle that increased knowledge of diseases of the nervous system depends upon the application of basic techniques to the study of disease. Accordingly, the personnel of this program have been trained not only in the clinical sciences, but also have had additional postgraduate training in the basic sciences, such as chemistry, mathematics, biophysics, anatomy, pathology, and other basic disciplines.

Another basic premise of this program is that the investigator must be free to shift his activities in any direction in which his research carries him. This gives to the program a

multidisciplinary trend. A similar trend also may be observed on an international scale. Here, even among scientists trained as physiologists and chemists, the newer techniques of electron microscopy and isotopic work is being applied.

The NINDB's clinical investigative program is reaching an optimal level of functioning within the present limits of the NIH. It is believed, however, that a clinical program in otology must be developed at some time in the future.

The basic research program is a segment of an established and powerful world center in scientific disciplines basic to neurology. Their guiding principle is to create and manipulate those concepts which will lead to a more fundamental understanding of the nervous system and its functions. During the next ten years, the biophysics, physical chemistry, and chemical structure of nerve membranes, axonal and synaptic, will undoubtedly be much better understood. Included in its present program are biophysical, neuroanatomical, neurophysiological, and neurochemical research. Predicted developments for the future lie in the fields of physical biochemistry and sensory mechanisms. The first of these will be devoted to cogent areas of genetics, cellular physiology, and theoretical chemistry; the second will pursue the analysis of sensory systems with special emphasis on mechanisms involved in the central control of perception.

SCIENTIFIC COUNSELORS

In October, 1958, the NINDB's Board of Scientific Counselors held its second meeting in Bethesda. The purpose of this group is to advise the Director, NINDB, and the Director, NIH, in matters pertaining to the intramural programs in both clinical and basic research. Another important function of the counselors is to monitor and make periodic reviews of the policies and activities of the intramural programs. Dr. Richard L. Masland, Assistant Director of NINDB, serves as a point of contact between the Board of Counselors and the Institute Director. The members of the NINDB's Board of Scientific Counselors are: Hallowell Davis, Chairman of the Central Institute for the Deaf; Raymond D. Adams, Chief of Neurology Service, Massachusetts General Hospital; Howard Curtis, Brookhaven National Laboratory; Algernon B. Reece of the Institute of Ophthalmology, College of Physicians and Surgeons; Roger J. Rossiter, Biochemist, University of Ontario; and A. Earl Walker, Professor of Neurological Surgery, Johns Hopkins University.

In their deliberations, the counselors indicated that the NINDB had two especially unique opportunities for justifying its existence as a national institute. The first is its program of collaborative projects and collaborative research in which the

Institute serves as a coordination center and a central laboratory for multidisciplinary and multi-institutional enterprises. It is the only institution in the country that could fill this role. The second is the Institute's program of clinical investigations, through which an all-out effort is being made to explore the potentialities of a particular technical method in relation to a definite clinical problem. NINDB alone, according to the Board, had the facilities for prosecuting research of this type.

The counselors also observed that the Bayne-Jones recommendation of no further expansion of the National Institutes of Health intramurally was instituted at an unfortunate time for NINDB. In their opinion, a new building would be most desirable to give adequate facilities for the present intramural staff. It would also allow a more efficient arrangement of space presently assigned to other activities in order that these could be more efficiently concentrated.

Among the several program gap areas discussed by the group, the most apparent and widest gap concerned studies in otology and the auditory and equilibratory functions of the inner ear. The counselors took full cognizance of the sterling basic research now being conducted by Drs. Tasaki, Rasmussen, and Gernandt, but strongly recommended the organization of a clinical division in otology as soon as adequate space and competent personnel became available.

Finally, the counselors were interested in the question of adequate representation of neuropathology as an essential foundation of clinical investigation at NIH. The opportunity of NINDB to provide a central neuropathological laboratory, to assist all institutes having an interest in systemic conditions involving the brain, and to service certain cooperating institutions in their collaborative projects, was considered both unique and challenging. Consequently, the counselors recommended that a discussion of the place of neuropathology in the organization and operation of the Institute be surveyed and made a topic of discussion at their next meeting.

LANDMARKS IN COLLABORATIVE RESEARCH

Since the activation of the Institute in 1951, there has been a trend toward more collaborative and cooperative types of research programs to meet certain special problems. The solution of these problems called for a greater collaboration between the Institute's intramural and extramural programs as well as greater cooperation between institutions on multidisciplinary levels. These problems arose because many neurological and sensory

syndromes appear to stem from a multiplicity of causes, giving rise to the problem of procuring adequate samples. The variation in the geographic and climatic prevalence of certain neurological and sensory disorders emphasizes the need of collaborative studies in geographic neuropathology, both national and international. The Institute's program is related to several specialized medical disciplines which operate as distinct specialties as a matter of practical expediency in medical practice, but which, from a research standpoint, overlap to such a degree that they can be regarded as merely different points on the same assembly line.[1] The rapid postwar development of older, and the introduction of newer disciplines, in basic and neurological sciences, add to these Institute problems.

These special problems gave rise to a need for a cooperative plan to establish coordination centers and to consolidate these program aims on institutional, geographic, and disciplinary levels. The earliest examples of Institute cooperative projects on an institutional level came in 1952. These included the cooperative project in retrolental fibroplasia, the collaborative project for the evaluation of glutamine and asparagine in epileptic seizures, and the epidemiological studies in amyotrophic lateral sclerosis on Guam. More recent examples of cooperative projects on a multi-institutional level are the evaluation of therapy for intracranial aneurysms and the evaluation of the administration of anticoagulants in cerebrovascular disease.

The NINDB's largest collaborative project is the one in cerebral palsy and other neurological and sensory deficits of early life. The present status of this multi-institutional and multidisciplinary project is described by Dr. Masland in his section of the annual report. It has become increasingly evident that in this type of research the Institute can plan an important role, if not its most important role, by serving as an integrating force in these collaborative projects. More specifically in this respect, it serves as a focal point for the planning and mapping of collaborative projects as well as a central laboratory for the biostatistical collation of data and for the examination and correlation of pathological specimens.

This developing trend in collaborative research has influenced the relative amount of funds expended in 1958 on individual research projects, as compared to the amount expended on collaborative and cooperative field investigations. As Dr. Seger points out, 51 percent of the research budget in 1957 was used for the support of individual research projects as compared to 47 percent of the total research budget in calendar year 1958. This shifting emphasis raises the question as to whether or not the growth of collaborative and cooperative field investigations, or the planned type of research, is being achieved at the expense of the individual

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1. Referred to our clinical disciplines of neurology and neurosurgery, ophthalmology and otolaryngology, and their respective counterparts in the basic sciences.

initiative and creativity of the individual project type of research. I think not. There is evidence at hand that planned collaborative research is providing leads for the development of more individual research projects. In other words, the collaborative and cooperative programs serve as a supplement to, and not as a replacement of, individual research projects. Serendipity, therefore, will be preserved.

INTERNATIONAL RESEARCH

An unmistakable trend in medical research is a growing gravitation toward greater international cooperation. This was evident over the past two years in the programs of international scientists' assemblies. More recently, it has been expressed along political lines. A section of President Eisenhower's January 9, 1958, message to the Congress on the State of the Union, emphasized the value and opportunities of international cooperation in medical research. Later in the spring of the same year, Congressman John E. Fogarty and Senator Lister Hill presented a joint resolution to both Houses calling for greater cooperation in international research to provide more building blocks for the accumulation of scientific knowledge and to serve as an instrument of peace.

In December, 1958, Senator Hubert Humphrey (D-Minn.) visited Western Europe, and the USSR principally, as Chairman of a Government Operations Subcommittee, to make a study of problems pertaining to international health, research, assistance, and rehabilitation. During his visit to Moscow, the Senator and Soviet Premier Nikita Khrushchev disagreed on many things but they agreed that "the world is hungry for some evidence of Soviet-American collaboration" and the best way to start is with medical research. Highlighted by Senator Humphrey is the need for international cooperation to conquer cancer, heart disease, and the killing and crippling neurological disorders of early life, arising from disease or injury during the perinatal period.

In Senate Resolution 361, Senator Humphrey invited President Eisenhower to extend to other nations of the world, through the World Health Organization, an invitation to meet and discuss the feasibility of designating an International Health and Medical Research Year.

GEOGRAPHIC AND CLIMATIC DISTRIBUTION OF DISEASE

International studies in the geographic and climatic distribution of disease often provide clues of a general nature which lead to further and more fundamental investigations of

the disease process involved. The value of such studies as those of yellow fever in Central America, and pellagra in the Southeastern United States, recently have been accentuated by the dramatic discovery of Allison regarding the significance of sickle cell anemia with relation to malaria in the tropical regions of Africa and Asia.

The increasing emphasis on chronic diseases of the nervous system has stressed the need for world epidemiological research programs in chronic neurologic and sensory disorders. Recent studies, for example, of geographical variations in the frequency of multiple sclerosis show that this disease is more prevalent in temperate than tropical climates, but the clinical manifestations are the same in all climates. Recent Institute studies in the distribution of a new disease called "kuru" among a tribe of savages in New Guinea afford an opportunity to study this type of neurologic disease under control conditions.

GEOGRAPHIC NEUROPATHOLOGY

These types of studies may be included under the title of "geographic neuropathology" or "geomedicine" which refers to the evaluation of the frequency of diseases, their pathology, and their relationship to associated genetic and environmental factors in diverse geographic regions and populations. It includes geographic variations in the manifestations of illness as well as total incidence and the prevalence figures.

THE WORLD FEDERATION OF NEUROLOGY

Symbolic of international cooperation in medical research was the formation of a World Federation of Neurology during the First International Congress of Neurological Sciences, held in Brussels in July, 1957. This newly created body is a federation of national neurological societies throughout the world. The chief objectives of the Federation are: (1) the dissemination and exchange of new scientific knowledge in clinical neurology and neurological science on a world-wide basis; (2) the stimulation and encouragement of international cooperation in neurologic research; (3) the organization of international congresses and symposia; and (4) the development and exchange of fellowships in neurology and neurological science.

Most important of all, however, are the Federation's functions as a coordinating mechanism and central clearing station for the stimulation, formulation, and integration of international collaborative research projects. In this respect,

the Federation is uniquely equipped. It is a Federation of national professional societies representing the academic pinnacles of neurology throughout the world. The Federation is nongovernmental and does not depend on fund raising for the administration of its routine affairs which are underwritten with the proceeds of annual dues from the national delegates of the many societies throughout the world, of which it is composed. Because of these factors, communications are free and easy between the delegates when the question of organizing symposia or collaborative projects is involved. The Federation hopes, however, to work in close liaison with the World Health Organization and other government agencies for the advancement of medical research.

Suggested among the primary problems to be attacked by the World Federation of Neurology are: (1) the establishment of an international reporting system and standards of nomenclature and classification of disease processes; (2) the identification of situations in various geographical regions which would lead to study problems of international geographic pathology on a world-wide scale. This preliminary planning involves the detection and classification of population isolates--classification of climatic, cultural, and economic factors within the regions to be studied; and (3) the establishment of geomedical studies to attack the major neurological disorders of mankind such as studies of perinatal morbidity similar to the present collaborative investigations of NINDB in this field.

The research program of the WFN has, in reality, already begun. A grant has been awarded the Federation to be used in the planning and conduct of an international symposium on the neuropathology of the encephalitides. This symposium, to be held in Antwerp early in 1959, will serve as a precursor for the establishment of an international reporting system and the development for standards of nomenclature and classification of disease processes.

A grant also has been made to the Federation for the implementation of research, especially along the lines of collaborative and cooperative studies in the cerebrovascular diseases and perinatal morbidity. The project in cerebrovascular disease is based on the preliminary findings of Baker and his associates at the University of Minnesota. These illustrate that the nature, frequency, and severity of degenerative changes in cerebrovascular disease differ in cerebral arteries of different sizes. For example, the larger arteries of the circle of Willis, the small intracerebral (150-500 microns in diameter), and the intracerebral arterioles (150 microns or below), differ in the type of degenerative changes they undergo in cerebrovascular disease. Moreover, the involvement of arteries of a certain caliber is not necessarily

associated with the involvement of the arteries of a different caliber. Finally, the degree of correlation between the arteries of different sizes varies with a number of factors: the pathological changes of underlying brain parenchyma, the age of the patient, the presence or absence of hypertension, and other factors. These variations in the reaction of cerebral arteries of different sizes provide a unique opportunity to establish leads by means of comparative studies in different geographic regions throughout the world. Thus, the effects of climate, race, cultural environment, diet, somatic disease and other factors can be measured.

We believe that this project can be easily and quickly developed through several selected centers throughout the world, particularly in Belgium, Brazil, Argentina, France, Japan, United States, Australia, and Mexico. We anticipate such a study, similar to the cooperative aneurysm study now underway in the United States, could be completed in a period of two years.

Geomedical studies directed toward discovery of geographic distribution of perinatal morbidity as it relates to the central nervous system are already underway in Ireland, the Netherlands, and Sweden. The protocol of the NINDB's collaborative cerebral palsy project is being used as a guide in the further organization of these studies and as a guide by the European group for the collection of their data. Eventually, the findings of the European scientists will be collated and correlated with those of the NINDB's collaborative project through the coordinating mechanisms of the World Federation of Neurology.

At present, about 40 major nations of the world have joined the World Federation of Neurology. Its current officers are: Drs. Ludo van Bogaert (Belgium), President; Macdonald Critchley (United Kingdom) and Auguste Tournay (France), Vice Presidents; Pearce Bailey (United States), Secretary-Treasurer General; and Richard L. Masland (United States), Assistant Secretary-Treasurer General. The Federation's executive office is at the Institut Bunge, 59, rue Phillipe Williot, Berchem-Anvers, Belgium. The administrative offices are at the National Institute of Neurological Diseases and Blindness, Bethesda 14, Maryland.

COMPARATIVE NEUROPATHOLOGY OF MAN AND ANIMALS

A generally conceded weakness in a comprehensive attack on neurologic disorders is the present lack of a systematic comparative neuropathology of spontaneous diseases of animals, both domesticated and wild. Further, what sparse literature there is on the subject is widely scattered and difficult to

find. At the University of Bern in Switzerland, a research and coordination center is in the making to bridge the gap between veterinary and human neurology and to establish an information exchange center of animal neuropathology. Heading this enterprise at the University of Bern, are R. Fankhauser, Professor of Animal Neuropathology, and E. Frauchiger, Professor of Comparative Neurology and alternate delegate (Switzerland) to the World Federation of Neurology. Backed by fifteen years of experience, these two Swiss investigators are organizing a project which calls for the protracted clinical studies of domesticated-wild animals suffering from neurological disorders. These studies include complete postmortems with the application of modern techniques for the examination of cerebrospinal fluids, and investigations in neuroradiology and electroencephalography. Their research also extends to studies of the embryology of the central nervous system and spontaneous congenital cerebral malformations arising in the perinatal period. The Institute of Comparative Neurology at the University of Bern also is tooling up as an information exchange center for the review and distribution of publications, histologic sections or paraffin blocs, photographs and slides.

The Bern project furnishes another example of international cooperation through neurologic research. Here it concerns the United States and Switzerland through the sponsorship of the project by the National Institute of Neurological Diseases and Blindness and the Swiss National Fund for the Advancement of Scientific Research.

MEETINGS AND SYMPOSIA

During the calendar year, 1958, several important meetings and symposia were held in Bethesda under NINDB auspices. Among these was a symposium on the Electrophysiology of the Visual System, organized by Dr. M. G. F. Fuortes; a meeting of the Eastern Section of the Association for Research in Ophthalmology (January 17 - 18); a conference on Graduate Training in Clinical Ophthalmology (January 26); and a symposium on the History and Prospects of Neurochemistry (April 19), in cooperation with the National Institute of Mental Health.

The proceedings of the symposium on the Electrophysiology of the Visual System, edited by Dr. M. G. F. Fuortes, has been published as a supplement to the September, 1958, issue of the AMERICAN JOURNAL OF OPHTHALMOLOGY. The proceedings of the other meetings are in press.

The proceedings of two symposia held in 1957 were published in 1958 as special supplements to NEUROLOGY: "The Sequelae of the Arthropod-Borne Encephalitides," edited by Drs. Pearce Bailey and A. B. Baker; and "A Classification and Outline of Cerebrovascular Disease," a report by an Ad Hoc Committee established by the National Advisory Council of NINDB, Clark Millikan, Chairman. In addition, in 1958, five books were published by members of the NINDB staff and collaborators. These are: "Temporal Lobe Epilepsy," "New Research Techniques of Neuroanatomy," "Biology of Neuroglia," "Neurological and Psychological Deficits of Asphyxia Neonatorum," "External Collimation Detection of Intracranial Neoplasia with Unstable Nuclides," and "The Epileptic Seizure."

In addition to the meetings and symposia held in Bethesda, members of the NINDB staff participated in many important meetings held elsewhere in the United States and abroad. Some mention of these has been made in other parts of the total annual report. This section will mention only important meetings abroad in which the Office of the NINDB Director was officially concerned.

NINDB MISSION TO RUSSIA

At its June, 1958, meeting, the National Institute of Neurological Diseases and Blindness Council endorsed a proposal by the Director, NINDB, to organize a neurologic mission to Russia and authorized him to take the necessary administrative steps.

By November, the mission had been formed and on November 18, they left Washington by air for Moscow via Paris. The members of the NINDB mission were: Drs. Francis M. Forster, Council Member; Clinton N. Woolsey, Council Member; Louis S. Goodman, former Council Member; Henry W. Woltman; Paul I. Yakovlev; and Karl Frank. The purpose of the mission was to observe the nature and conduct of research activities in the physiology and pharmacology of the nervous system in the U.S.S.R. The mission organized in collaboration with the Public Health Service and the Department of State was the first of its kind in the field of neurology and was made possible by a January, 1958, agreement between the United States and the Union of Soviet Socialist Republics to exchange missions in various fields.

Dr. Forster, Chairman of the mission, already has submitted a preliminary report of impressions perceived in the Soviet Union (November 18 - December 18, 1958). The report concerns training of Soviet physicians and scientists; the

organization and orientation of Soviet research; physical facilities and research equipment; types of research personnel; and a consideration of Soviet research and development. Among other things, Dr. Forster said: "Neurological research is given the highest priority because of the Soviet concept that the central nervous system is the central control of all bodily processes and must be regarded as the ultimate target in biologic research."

Among the Soviet methodologies which impressed the U. S. delegates were: "the application of descriptive anatomy to research, active studies in developmental morphology and the phylogeny and ontogeny of the central and autonomic nervous systems, the preservation of a historical approach in the training of scientists which provides a stimulating background for further achievements, and the acute awareness by Soviet scientists of things going on elsewhere and their determination to assimilate and improve them." Near the end of the report, Dr. Forster recommended: "that research in the United States in neurophysiology and neuropharmacology be supported even more generously than it has been; that scientific literature in English translation be made more available to American scientists; that the program of scientific exchange missions be promoted further; and that a program of exchange fellowships be developed."

Dr. Forster adds a final note when he writes, "If one appreciates that the majority of Soviet research institutes are postwar in origin, and that their application to modern research techniques is relatively recent, one cannot be complacent about their potential for rapid progress in the future."

A return Russian mission will visit the United States early in 1959. The members of the visiting Soviet team are: S. V. Anichkov (Pharmacologist and Physiologist specializing in the study of chemoreceptors); V. S. Rusinov (Physiologist, specializing in EEG and electroretinography); and V. V. Zakusov (Pharmacologist and Chemotherapist).

BABINSKI CENTENARY

The NINDB Director was appointed by the American Neurological Association and the American Academy of Neurology to represent the United States at the celebration of the centenary of the famous French neurologist, Joseph Babinski (1857-1932). A delegate from the Municipal Council of Paris opened the inaugural sessions of the centenary in the amphitheater of the École des Infirmières of the Salpêtrière, where in 1882 the world's first professorial chair in neurology was created for J.-M. Charcot.

After the opening remarks, there were presentations by Professor Raymond Garcin, President of the French Society of Neurology, and by official representatives of 18 countries on the life and works of Babinski. These papers have been published in the Révue Neurologique.

On June 3, the day following the centenary, the French Society of Neurology conducted its Twenty-second International Neurological Reunion at the Salpêtrière, with President Garcin and A. Tournay presiding. Still in tribute to Babinski, the morning sessions of the reunion were devoted to a symposium on the cerebellum. The International Reunion continued on June 5, with a program of free communications, interrupted at noon for a convocation at the Hôpitaux de la Pitié, to affix a medallion of Babinski on the outside wall of his old neurologic service (le Pavillon Benjamin Dalessert). The ceremonies were conducted by A. Tournay, who espied and singled out from the audience, in a moving scene, Mademoiselle Alips, Babinski's faithful and devoted chief nurse.

Both the Babinski Centenary and the International Neurological Reunion were organized by the French Society of Neurology and held under the patronage of the French Minister of Foreign Affairs, the French Minister of Public Health, and the President of the Municipal Council of Paris.

NEUROPHYSIOLOGICAL WEEK IN PARIS

Again in France, the NINDB Director had the privilege to preside at one of the sessions of the annual neurophysiological week of the Salpêtrière in Paris, under the Presidency of Professor Th. Alajouanine and the Secretary-Generalship of Antoine Rémond. The central theme of the scientific sessions (October 20 - 25, 1958) was "Sensory Integration." After an introduction by Professor Alajouanine on the evolution of ideas on sensory integration in neurology, some 28 neurologists and neurologic scientists from France and other countries participated in the presentations and discussions. Some of the contributors had just returned from a series of neurophysiological meetings in Moscow, Kiev, and Tbilis.

NEUROLOGY UNIT INAUGURATED IN MEXICO CITY

On November 18, 1958, the NINDB Director travelled as an official guest of the Mexican Government for the inauguration of a Neurology-Neurosurgery Unit of the Mexico City General Hospital. The official ceremonies were opened by the President

of the Mexican Republic, Don Adolfo Ruiz Cortines, accompanied by Dr. Ignacio Morones Prieto, the incumbent Secretary of Health and Public Welfare. The scientific program began with an address by Dr. Clemente Robles, the head of the new neurology unit, who traced the history of Mexican medical advances from the time of the first field hospital established by the Conquistador, Hernan Cortes, through the development of the present General Hospital which was founded by Profirio Diaz in 1911. He concluded by stating that the opening of the new Neurology-Neurosurgery Unit, the only one of its kind in the country, represented an important forward step in the study and treatment of neurological diseases in Mexico. Following other speeches by Dr. Leonides Guadarrama, Director of the General Hospital, and by President Cortines himself, the President and his retinue toured the Neurology-Neurosurgery Unit. The new unit has 42 beds for adult patients, two small pediatric wards, and surgical, EEG, and x-ray suites. It is furnished with new and modern equipment and ready for occupancy by patients. The cost of construction was 3 million pesos. Present at the inaugural ceremonies were official guests from other countries. Others from the U.S.A. were Derek Denny-Brown, John F. French, and Webb Haymaker; from France, Professor and Madame Paul Dell and Dr. Antoine Rémond; from Chile, Raul Hernandez Peon; and from Cuba, Dr. C. M. Ramirez Corria. The day following the inaugural ceremonies (November 19), the visiting neurologists and neurosurgeons joined their Mexican colleagues in a three-day scientific program held in the General Hospital and at the National University. Among the Mexican participants were Drs. Luis Saenz-Arroyo, Manuel Velasco-Svarez (President of the Mexican Society of Neurosurgery), Hernando Guzman West (President of the newly formed Mexican Society of Neurology and Psychiatry), Roberto Gamboa Acosta, Ramon del Cueto, Guillermo Santin, Mariano Vazquez, and Armando Ortiz-Galvan, currently a Research Associate of Maitland Baldwin at NINDB, who, with Jose Humberto Mateos, were of great help in guiding the visiting groups.

THE INFORMATION OFFICE

An important arm of the Director's Office is the Information Office, headed by Ruth Dudley. This unit functions as a general information and distribution center for the dissemination of NINDB news and reports to the outside world.

The work of the Information Office this past year has included the preparation and distribution of brochures, reports, articles, speeches, exhibits, and press releases. The office has also arranged press conferences, press interviews, and picture stories. It has edited many manuscripts and has answered many inquiries of all types.

Among the brochures completed in 1958 are: The Research Attack Against Cerebral Palsy, Multiple Sclerosis--Hope Through Research, NINDB Conference Programs, Highlights of Progress in Research on Neurologic Disorders, and Who's Who in NINDB. Nearing completion are three other brochures: Little Strokes, Parkinsonism, and a brochure about the Institute.

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, voluntary health agencies, and World Health Organization. In 1958, these included: Highlights of Research and Program Developments, Past Foreign Contributions to the Field of Neurology and Blindness, NINDB's Puerto Rico Project, Progress Reports on Electromyography and the Structure of the Synapse for use of the NINDB Advisory Council, NINDB portions of the NIH and PHS booklets, research accomplishments of NINDB for the Voice of America, WHO, and UNESCO.

Numerous interviews were arranged for press and magazine science writers, including the Associated Press, United Press, International, Scripps-Howard, Medical News, New York Times, and such magazines as Fortune, Time, Scope, Farm Journal, and Parents Magazine. Seventeen general and three individual press releases were prepared and distributed to the scientific press, wire services, and other science outlets. Background materials were made available by the Information Office for the production of two films (Conquest and Year of Birth).

During 1958, the Information Office replied to 788 letters of inquiry; mailed out 1500 individual pamphlets (bulk orders not included); and replied to 1700 telephone inquiries from the public. Finally, the Information Office edited and cleared many articles and scientific manuscripts, and gave assistance in the preparation and distribution of three scientific exhibits and accompanying literature for NINDB investigators.

ANNUAL REPORT
Calendar Year, 1958
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 offers an introduction to the more complex and protracted epidemiological problems of neurological and sensory disorders and chronic diseases.

In addition, the Institute is supporting the training of an officer who has gone to the Institute for Cellular Chemistry in 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. He will also be working on the oxidation of lipids by means of certain dyes and techniques available only in that Institute in Munich.

Another officer is undergoing training in neurological disorders at the Columbia-Presbyterian Medical Center, in New York, so that he will become a more effective epidemiologist. Additional training for a biophysicist and a physiologist is being planned. Further staff training in biometry and epidemiology is contemplated.

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

An Institute Committee has been formed to develop a complete program in in-service training, under the new Training Act passed by the Congress during 1958.

NATIONAL INSTITUTE OF NEUROLOGICAL DISEASES AND BLINDNESS

Research

Collaborative and Cooperative Projects
(including Project Services Branch)

Estimated Obligations for FY 1959

Total: $576,000
Direct: 478,200
Reimbursements: 97,800

ANNUAL REPORT
Calendar Year, 1958
Collaborative Research
National Institute of Neurological
Diseases and Blindness
National Institutes of Health

The most recent phase of the development of the Institute's total program has been the creation of the collaborative research area. The responsibility of this area is the coordination of certain types of research which are carried on with difficulty within a single institution. Most basic laboratory research and much clinical research can be carried on effectively in isolated laboratories or clinics. However, research which involves the collation of data from large numbers of individuals, or the comparison of health or sickness in one community as compared to another, requires a coordinated program, and frequently involves the collaboration of a number of institutions. The conduct of such work ordinarily requires the skill of the epidemiologist and the statistician. Therefore, within the past few years, the Institute has launched a broad program whose objective is to provide the necessary coordination for programs of epidemiological investigations and for other areas of research which cannot be carried out without difficulty within any single research center. Because of the very nature of such epidemiological investigations which presently involve wide areas of the United States, it is evident that their fullest expression eventually will require the extension of these activities to a worldwide basis.

One of the first investigations carried on within this program was the study of the cause of retrolental fibroplasia. Within a short period of time, an investigation concerning prematures, carried on simultaneously within a number of research centers, was able to verify the problem as the administration of oxygen and the duration of the infants. At the present time, important epidemiological investigations of multiple sclerosis are being conducted by this program. These have involved the collection of data from Canada, United States, and more recently, Japan. Important studies on the island of Guam, also, serve to demonstrate the genetic basis of one form of amyotrophic lateral sclerosis. For assisting in the conduct of such investigations, the collaborative research area has developed an epidemiology branch and a biostatistics branch.

The most recent program now developing within this area is the collaborative project for the study of perinatal pathology. This program, requiring the collaboration of 15 different institutions, is relying on the intramural program to provide the central statistical and coordinating services. In order to achieve uniformity, both for procedure and for the type of information being obtained, a coordinating staff is being developed in a project services branch. It is the responsibility of this group to establish training programs for

collaborators, to prepare the procedural manuals, to visit the collaborating institutions for the purpose of assisting in the establishment of standard procedures, and finally, to collect, assemble, and process the data forwarded to the central office.

In this study, we have been concerned in the past year with two important steps; namely, the recruitment and training of personnel for the conduct of the study within the collaborating institutions and in the central office; and the development of a definitive protocol for examination of mother and child, recording the observations, and processing the recorded data. These requirements have been met by a "pretest period", during which over 1200 women have been examined in the study, and through which personnel have become experienced, methods of examination have been developed, and knowledge has been gained as to the types of information which can be obtained.

The major problem encountered during this year stems from the fact there are not available the basic methods of interviewing and examination which are required if one is to obtain valid and reliable data in a vast study of this sort. These methods had to be developed before the study could be launched, yet during this recruitment phase, the personnel were not available within the central office. For this reason, service contracts with established groups have been utilized as a means of rapid development of specific phases of the study. Largely through this means, as of January 1, 1959, the actual study of cases according to a sound protocol can be undertaken. This protocol will include a thorough socio-economic and genetic history of the gravida, data from her initial and return prenatal examinations, and detailed observations during the course of labor and delivery. For the child there has been developed a series of meticulous neonatal examinations, including a special neurological examination; a developmental examination at eight months and a repeat neurological at 12 months. Subsequent examinations remain to be developed. Special studies will include serological examination of the gravida for virus, these studies to be conducted within the laboratories of NIAID. Special embryological studies of abortuses will be conducted at Harvard, Johns Hopkins, and Brown University where special laboratories have been established for this purpose. The Neuropathological examinations will be conducted at the Warren Museum, Harvard University, until such time as central facilities can be developed at Bethesda.

At the present time the collaborative project has achieved an encouraging degree of cohesiveness and mutual understanding, through which group action has become very effective. The basis for proceeding actively with the study has been established. Its productivity will depend to a large extent on our ability to bring into the central group the additional investigators of vision and

imagination to develop the fullest exploitation of the tremendous opportunity which the new research facility presents.

The collaborative research area appropriately provides a bridge between the extramural and the intramural programs. A similar situation exists in respect to the laboratory of perinatal pathology located in Puerto Rico. The work of this laboratory, concerned with the reproductive physiology of the Rhesus monkey, parallels very closely the interest of the collaborative project on perinatal pathology in humans. In this instance, the laboratory is related directly to the basic research laboratories of the intramural program, but because of its relationship to the University of Puerto Rico, it is properly regarded as a collaborative undertaking. Here again, the intramural program is providing the services and coordination required by an outside research organization.

Pathological studies of the brains of patients dying in New Guinea from "Kuru" may provide information regarding the nature of the process responsible for this mysterious malady. Reports from the field indicate that within a small area the incidence of this disorder is extremely high. It is still not known whether there is a genetic basis for this disorder, whether it represents an infectious disease, or is the result of some toxic agent to which the natives of this particular area are subjected. Definite changes in the nervous system have been demonstrated by pathological means. Extensive investigations will be required to determine the nature of this process and whether it has implications in other less common disorders seen in this country. Numerous aspects of the disease, including its peculiar age and sex predilection, its tendency to unique pathological reaction in the brain, indicate that solution of the "Kuru" problem would provide a considerable advance in our understanding of other disease processes in the nervous system.

NATIONAL INSTITUTE OF NEUROLOGICAL DISEASES AND BLINDNESS

Research

Epidemiology Branch

Estimated Obligations for FY 1959

Total: $109,000
Direct: 90,500
Reimbursements: 18,500

CRP-2

ANNUAL REPORT - 1958
PHS - NIH
NINDB - EPIDEMIOLOGY BRANCH

Reorganization during the year resulted in the transfer of the Epidemiology Branch to the new Collaborative Research Program. This is in keeping with the cooperative services and collaborative type of research in which the Branch has been active. Our present program aims to develop a nucleus of highly trained and experienced personnel both in epidemiology and in genetics who can function well within the clinical categories for which the Institute is responsible. These personnel will operate primarily on a cooperative basis with other Institutes and agencies or, when invited, in collaboration with extramural projects.

Organization of the Branch into Sections on Genetics, Neurologic Epidemiology, Ophthalmologic and Otologic Epidemiology has proceeded slowly due to lack of personnel trained in both epidemiology and the clinical specialties; provision of limited clinical training for physicians oriented in epidemiologic methodology appears to be the practical solution to this problem.

The recruitment of Robert S. Krooth, M. D., Ph. D., represents an important step in the development of the proposed Section of Genetics within the Branch. The proposed Head for the Section in Neurologic Epidemiology will complete his training in neurology in 1960 and in the interim the Branch Chief will continue in his activity. The planned cooperative studies on phlyctenulosis and mastoiditis with the Arctic Health Research Center and on a uveitis and strabismus collaborative study with the Biometry Branch have provided further stimulus for recruitment of trainee epidemiologists for the proposed Ophthalmologic Epidemiology Section.

The Epidemiology Branch of the Communicable Disease Center and this Branch continue in a successful cooperative effort. Dr. Siedler, on assignment to our Branch, has participated in several of our research projects while at the same time remaining available for Epidemic Intelligence Service calls. (See project descriptions.) The Asian Influenza collaborative study which he supervises 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.

The Guam Field Station continues to represent a low-cost, highly productive research operation and an excellent training facility for our medical officers. The clinical type of studies of the highly prevalent neurological disorders as approached by our clinically-oriented epidemiologist (Dr. Pieper who left the Service after a successful tour on Guam to complete his training in neurology) are giving way to a series of genetically-oriented mathematical and laboratory type of field investigations under Dr. Krooth. 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 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.

During the year several major population surveys were completed. The results of the intensive survey in Rochester, Minnesota, in collaboration with the Mayo Clinic has been published. This report is expected to provide baseline incidence and prevalence statistics for numerous neurological disorders for many years to come. The local population surveys under Dr. Alter, in collaboration with staffs at the Medical College of South Carolina, in Charleston, and Dalhousie University, Halifax, Nova Scotia, have been completed and the reports are being readied for publication. Dr. Alter, after two years in the field, has been assigned for a year of training at the Neurological Institute in New York.

Several other projects of limited scope were completed and reported. These included the study of Sequelae of Japanese B and Mumps Encephalitis on Guam, a review of the Epidemiologic and Genetic Factors of Parkinsonism, and a genetic and physiologic report on Mirror Movements with Dr. Robert Cohn of the Naval Hospital, Bethesda. The progress of the long-term cooperative study on the Natural History of Multiple Sclerosis and Retrobulbar Neuritis with respect to Multiple Sclerosis is continuing with the National Research Council and Veterans' Administration. This study should be completed next year.

Dr. Myrianthopoulos, geneticist, has aided in the completion of a study of skeletal deformities in motor system disease and continues in his extensive twin study on multiple sclerosis. He has developed a new program aimed at clarifying genetic factors in parkinsonism and some of the genetic factors in cerebromacular degenerative disorders. In cooperation with the Laboratory of Blood Products, NIH, the association of blood groups with amyotrophic lateral sclerosis is being evaluated. Several other studies of more limited scope on Huntington's chorea and peroneal muscular atrophy are also being pursued.

New projects cover various fields, particularly in chronic neurologic disease. One of the most encouraging steps forward in our struggle with amyotrophic lateral sclerosis (ALS) resulted from the detection of a previously unrecognized amorphous intracutaneous substance in ALS patients by Dr. Harold Fullmer, of NIDR. Attempts at histochemical identification, if successful, promise to open an entirely new field of exploration in the study of the degenerative lower motor neurone disorders. The availability of specimens from our field station on Guam has been particularly valuable in this project.

This Branch has a responsibility in developing standardized procedures and reporting methods which will allow for broad population comparisons in several geomedical research programs in neurology. These includs preparations for the forthcoming symposium at the International Congress of Neurology (1961) and advisory status to surveys now under way in several other countries.

Problems of nomenclature and efforts to revise classification of neurologic disorders for hospital use and for international mortality comparisons are expected to become an important program next year. The Branch is now represented on the Neurology Section of the American Medical Association "Standard Nomenclature."

Other activities which are not strictly of an intramural research nature are services to the Medical Advisory Boards of the Canadian and the National Multiple Sclerosis Societies, and the Washington, D. C. Chapter of the NMSS; The Committee on Nomenclature and Biometrics of the American Academy of Neurology; and limited teaching responsibilities by staff members in the local universities.

Dr. Myrianthopoulos has continued in instruction and consultation of genetics for clinical associates at the Clinical Center and for students at George Washington University. The D. C. Heredity Counseling Clinic which he established at George Washington University has met with increasing success and serves as a useful case material source for research and clinical experience and at the same time supplies a heretofore unavailable service for this region.

There are two factors at present serving to interfere with the full development of our program which is needed in the Institute. The first is the lack of trained manpower referred to at the beginning of this report. The second is the shortage of satisfactory space which has resulted in frequent changes in office assignment (now totaling 7 moves in 42 months). These moves have been so disruptive that we have not been able to use, with maximum efficiency, the few highly trained specialists available to us in this field; nor are the latest assignments to office-type facilities away from NIH likely to improve our opportunities for recruitment of scarce professional personnel or young physicians who would otherwise be well inclined towards our program. The continued location of this Branch in a building geographically removed from the Clinical Center, where laboratories, library facilities and patients are available, will seriously reduce the efficiency and productive capabilities of this Branch.

ORP -2

ANNUAL REPORT - 1958
PHS - NIH
NINDB - EPIDEMIOLOGY BRANCH

SUMMARY

During the calendar year 1958, the Branch became part of the new Collaborative Research program. One of its major activities in descriptive epidemiology has been completed with a series of reports on the incidence and prevalence of neurological disorders in the United States. A series of genetic and epidemiologic projects of limited scope in neurology and ophthalmology has also been completed. Collaborative projects on the teratogenic effect of Asian influenza and the natural history of multiple sclerosis are expected to be completed during the next calendar year. The Guam Field Station continues as an important activity in the Branch and has provided numerous opportunities for epidemiologic, genetic, clinical and pathological investigations of neurological, ophthalmological and other disorders. The joint project with NIDR, begun late this year following the detection of a previously unrecognized intracutaneous substance in ALS patients, promises to be a major program in forthcoming months as efforts are made to identify the material, to determine its significance in the pathogenesis of ALS and to evaluate its specificity and sensitivity as a diagnostic aid.

The genetics research program is now well established and an increasingly successful genetic counselling program has been developed in cooperation with George Washington University. The organization of a Section of Genetics is expected in the near future.

Other responsibilities of the Branch include a program concerned with international geomedical research of diseases of the nervous system and forthcoming revision by the section of neurology of the AMA Standard Nomenclature.

Preliminary steps were taken to develop a new activity on phlyctenulosis and deafness with the Arctic Health Research Center in Anchorage.

Repeated moves (average 1 each 6 months!) continue to disrupt the operation of the program. Space poorly suited to the needs of the Branch imposes a serious problem for recruitment of capable personnel.

PUBLICATIONS

1. Kurland, Leonard T., Sachs, David, Kerpelman, Larry C., and Davis, F. Sterling, Jr.: Evaluation of the "Phosphenator" Device: For the Detection of Increased Intraocular Pressure. American Journal of Ophthalmology, Vol. 45, No. 2, February, 1958.

2. Cohn, Robert, and Kurland, L. T.: Synkinesia, Transaction of American Neurologic Association, June 1958.

3. Siedler, Howard D., Nicholl, Willard, and Kurland, Leonard T.: The Prevalence and Incidence of Multiple Sclerosis in Missoula County, Montana. The Journal-Lancet, Vol. 78, No. 8, pp 358-360., August 1958.

4. Pieper, Samuel J. L., and Kurland, L. T.: Sequelae of Japanese B and Mumps Encephalitis. Amer. J. of Tropical Medicine and Hygiene, Vol. 7, No. 5, pp 481-490, September 1958.

5. Kurland, L. T., and Myrianthopoulos, N. T.: Skeletal Abnormalities With Motor System Disease. Neurology, Vol. 8, No. 10, pp 727-733, October 1958.

6. Kurland, Leonard T.: Descriptive Epidemiology of Selected Neurologic and Myopathic Disorders With Particular Reference to A Survey in Rochester, Minnesota. Journal of Chronic Diseases, 8:378-418, October 1958.

7. Kurland, Leonard T.: The Frequency of Intracranial and Intraspinal Neoplasms in the Resident Population of Rochester, Minn. Journal of Neurosurgery, Vol. XV, No. 6, p 627, November 1958.

8. Parkinsonism - William Fields, Editor. Kurland, L. T.: Chapter I - Parkinsonism. Epidemiology: Incidence, Geographic Distribution and Genetic Considerations. Charles C. Thomas, Springfield, Illinois, 1958.

9. Mackay, R. P. and Myrianthopoulos, N. C.: Multiple Sclerosis in Twins and Their Relatives. Preliminary Report on a Genetic and Clinical Study. Trans. Am. Neurol. Assn., 1958. Also accepted for publication by the AMA Arch. Neurol. Psychiat.

REPORTS (Unpublished data)

10. Alter, Milton., Allison, R. S., Talbert, R., Godden, J., and Kurland, L. T.: Epidemiologic Investigations of Multiple Sclerosis and other Neurological Diseases in Charleston County, S. C., and Halifax, N. S. Series of three reports.

11. Alter, Milton and Talbert, R.: Myasthenia Gravis in Twins.

12. Siedler, Howard D.: Paralytic Poliomyelitis and Aseptic Meningitis Syndrome in Washington, D. C., and Surrounding Counties During 1957.

13. Myrianthopoulos, N., Pieper, S. J. L., Kurland, L. T.: The ABO and Rh Blood Groups Among the Chamorros of Guam.

14. Myrianthopoulos, N., Rowley, P., and Kurland, L. T.: Huntington's Chorea in Monozygotic Twins.

15. Fullmer, Harold M., Kurland, L. T., and Siedler, H. D.: A Cutaneous Mucopolysaccharide in Amyotrophic Lateral Sclerosis.

16. Kurland, Leonard T.: The Incidence and Prevalence of Convulsive Disorders in A Small Urban Community.

17. Pieper, S. J. L., Fields, W. S., and Kurland, L. T.: Failure of Amyotrophic Lateral Sclerosis to Respond to Intrathecal Steroid and Vitamin B12 Therapy and to the Use of Poly-alcoholic Hydrocarbons.

ELECTED TO MEMBERSHIP

International Society of Geographic Pathology, October, 1958. Dr. Kurland.

Committee on Nomenclature, Neurology Section, AMA Standard Nomenclature, September 1958. Dr. Kurland.

Neurology Instructor (Genetics), George Washington University, 1958-59, Evening Classes. Dr. Myrianthopoulos.

PHS-NIH
Individual Project Report
Calendar Year 1958

PART A.

1. Project Title: Epidemiologic Investigations of Neurological and Ophthalmological Disorders and Other Conditions of Unusual Prevalence in Guam and Other Islands of Micronesia

Principal Investigator: Leonard T. Kurland

Other Investigators: Robert S. Krooth, Geneticist, NINDB
Samuel J. L. Pieper, Jr., Epidemiologist, NINDB
Nathan Malamud, Professor of Neurology, Langley Porter Neuropsychiatric Institute, San Francisco, California
William Fields, Professor of Neurology, Baylor University School of Medicine, Houston, Texas
Robert J. Huebner, Chief, Lab. of Infectious Diseases, NIAID
Leon Jacobs, Head, Section on Protozoal Diseases, Laboratory of Tropical Diseases, NIAID

Cooperating Units: Division of Medical Services, Government of Guam
Laboratory of Infectious Diseases, and Laboratory of Tropical Diseases, NIAID

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

Project Description:

a. Amyotrophic Lateral Sclerosis: A general point survey is underway to ascertain all affected in a specified population on the Island of Guam to determine the extent of familial aggregation of cases. The completion of a registry and the preliminary analysis may clarify the extent of genetic factors and reasons for the high incidence in the population. The survey will determine the feasibility of the proposed long term anterospective program.

Other studies on the ALS patients and comparisons with members of the unaffected population include cutaneous histochemistry (see project 8) and studies aimed at identifying genetic markers. These include urine and spinal fluid chromotography, serum protein studies and blood typing of the native population (see project 12).

Therapeutic studies have been completed on intrathecal steroid and Vitamin B12. These served the purpose of forestalling the publication of a preliminary report by Dr. Fields on the supposed value of this

treatment. Our study demonstrated the treatment as valueless among the patients on Guam and enabled Dr. Fields to reevaluate the status of his patients in Houston and to redirect his report.

A study of Inositol and Sorbitol failed to demonstrate any therapeutic value by these drugs in ALS.

b. Parkinsonism: This disorder appears to be more prevalent in this population than in corresponding populations of the Continental United States. The mean age at onset is less than that in the United States. There is a preponderance of males, there is a frequent association with ALS in the same patient or in family members, and there is evidence of appreciable diffuse brain damage and intellectual dysfunction in the patients. Post encephalitic disease has been suspected as well as a variant of amyotrophic lateral sclerosis. Serological, virus isolation, and further clinical pathology, genetic and epidemiologic studies are now under way to clarify these issues.

c. Sequelae of Japanese B and Mumps Encephalitis: A follow-up nine years after the epidemic has been completed. Forty-six persons (most infants and children) were known to be affected; there were 43 survivors of the epidemic. No parkinsonism was observed. 7.4 per cent of the patients with JBE died and 40 per cent (11 per cent severe - mental retardation or paralysis) had sequelae. 22 per cent of these with ME had evidence of slight neurological damage.

d. Toxoplasmosis and Chorioretinitis: A survey of the incidence of chorioretinitis has been completed for a sample of the population on Truk and Guam. Blood from humans and animals has been collected for toxoplasmosis antibody titration. The results are now being analyzed.

e. Diaphyseal Aclasia: This rare disease is also called multiple exostoses. The present plan is to study frequency at post mortem or frequency among orthopedic admissions among the natives. One of the most recent projects on Guam, the high incidence observed by Dr. Krooth, will be followed by appropriate genetic analysis.

PART B.

Publications other than abstracts from this project:

Pieper, Samuel J. L., and Kurland, Leonard T.: Sequelae of Japanese B and Mumps Encephalitis. Amer. J. of Trop. Med. and Hygiene, Vol. 7, No. 5, September 1958

PHS-NIH
Individual Project Report
Calendar Year 1958

PART A

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

Principal Investigator: Milton Alter

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

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

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

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.

PART B. None

PHS-NIH
Individual Project Report
Calendar Year 1958

PART A

3. Project Title: The Prevalence and Incidence of Multiple Sclerosis in Missoula County, Montana, and Duxbury, Massachusetts (Collaborative Project)

Principal Investigator: Howard Siedler

Other Investigators: Walter Deacon, Duxbury, Massachusetts
Leo Alexander, Boston, Massachusetts
Willard Nicholl, Western Montana Clinic, Missoula, Montana
Leonard T. Kurland

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

Project Description:

Missoula County, Montana. A study of the frequency of multiple sclerosis in Missoula County, Montana was conducted to determine whether the clinical impression that multiple sclerosis was unduly prevalent in this area was valid. The average annual incidence rate for Missoula County was found to correspond to rates for other cities of comparable climate. It is concluded that the prevalence and incidence of M.S. in Missoula County, Montana are consistent with the pattern of rather uniform frequency rates of the disease in widely separated populations living in comparable regions of climate in the temperate zone of North America.

Duxbury, Massachusetts. 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:

Siedler, Howard D., Nicholl, Willard, and Kurland, Leonard T.: The Prevalence and Incidence of Multiple Sclerosis in Missoula County, Montana. The Journal Lancet, Vol. 78, No. 8, August 1958.

PHS-NIH
Individual Project Report
Calendar Year 1958

PART A

4. 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, 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. This finding will be reported; the main objective of the investigation cannot be achieved in view of this low incidence of M. S.

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 1958

PART A

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

Principal Investigator: Howard D. Siedler

Other Investigator: Leonard T. Kurland and H. Goldstein, Biometrics Branch, NINDB

Cooperating Units: 15 Cooperating Clinics, Universities and Health Departments.

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

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. Sera from mothers of affected offspring and controls will be evaluated. A consolidated report of the participating groups will probably be made next year.

PART B. None

PHS-NIH
Individual Project Report
Calendar Year 1958

PART A

6. Project Title: Descriptive Epidemiology of Selected Neurological and Myopathic Disorders With Particular Reference to A Survey in Rochester, Minnesota

Principal Investigator: Leonard T. Kurland

Cooperating Units: Sections of Neurology and Biometry, Mayo Clinic, Rochester, Minnesota

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

Project Description:

Statistics from a number of selected sources, particularly those surveyed by members of the Epidemiology Branch, NINDB, were compiled and presented; the need for further descriptive and definitive epidemiologic or other investigations were also considered. New data from a recent survey in Rochester, Minnesota were also presented. The discussion for each of the subjects covered in the paper was oriented toward the use of the available statistical and genetic data to provide some foundation for further clinical, laboratory or definitive type of epidemiologic research.

PART B.

Publications other than abstracts from this project:

Kurland, Leonard T.: Descriptive Epidemiology of Selected Neurologic and Myopathic Disorders with Particular Reference to A Survey in Rochester, Minnesota. Journal of Chronic Diseases 8:378-418, October, 1958.

Kurland, Leonard T.: The Frequency of Intracranial and Intraspinal Neoplasms in the Resident Population of Rochester, Minn. Journal of Neurosurgery, Vol. XV, No. 6, p 627, November 1958.

PHS-NIH
Individual Project Report
Calendar Year 1958

PART A

7. Project Title: Mirror Movements and the General Phenomenon of Synkinesia

Principal Investigator: Robert Cohn, U. S. Naval Hospital, Bethesda, Md.

Other Investigator: Leonard T. Kurland

Man Years: Total - 1/16
Professional - 1/32
Other - 1/32

Project Description:

Mirror movements and other aberrant synkinetic actions may give insight into the normal functional organization of the motor system. This survey was undertaken when four members of a single family who show mirror movements were observed. An isolated case of "spontaneous" mirror activity, one case of acquired mirror movements and one case of acquired synkinesia were also studied. These uncontrollable movements appear to be the result of a functionally decreased control of a motor system which normally operates in parallel with the pyramidal tract system. It is suggested that the inherited defect observed in the first four cases was due to a developmental defect in the reticular system.

PART B.

Publications.

Cohn, Robert; Kurland, Leonard T.: Mirror Movements and the General Phenomenon of Synkinesia. Presented at the American Neurological Association, Atlantic City, June 1958.

ORP-2 NINDB - Epidemiology Branch

PHS-NIH
Individual Project Report
Calendar Year 1958

PART A

8. Project Title: A Cutaneous Mucopolysaccharide in Amyotrophic Lateral Sclerosis

Principal Investigator: Harold M. Fullmer, NIDR

Other Investigators: Leonard T. Kurland
Howard D. Siedler

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

Project Description:

The papillary layer of the dermis in formalin-fixed sections of abdominal skin in amyotrophic lateral sclerosis patients has been found to contain an extracellular amorphous material which stains with aldehyde fuchsin after paracetic acid oxidation and with the Hale stain (Rinehart modification). The material remains unstained with azure A at pH^4, it is digested by testicular hyaluronidase and by glucuronidase. On the basis of these findings it is believed to be a neutral mucopolysaccharide, or mucoprotein. It is possible that the substance represents a heretofore unrecognized step in the abnormal metabolism which is believed to be present in motor neurone disease.

PART B. None

PHS-NIH
Individual Project Report
Calendar Year 1958

PART A

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

Principal Investigator: **Howard D. Siedler, on assignment to NINDB from CDC. (Cooperative Project)**

Cooperating Agencies: **D. C. Health Department**

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

Project Description:

A report of studies concerning paralytic poliomyelitis and aseptic meningitis syndrome in Washington, D. C. and surrounding counties in 1957. 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. None

[illegible]
[illegible] Local Project Report
[illegible] Year 1958

PART A

10. Project Title. Parkinsonism - Epidemiology: Incidence, Geographic Distribution and Genetic Considerations

Principal Investigator: Leonard T. Kurland

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

Project Description:

This epidemiologic study of parkinsonism included an analysis of mortality statistics in the United States and Canada, a morbidity survey in Rochester, Minnesota, and an evaluation of several reports on possible etiologic mechanisms. Prevalence and incidence data reveal that parkinsonism is one of the most prevalent of the chronic neurologic disorders. About 300,000 cases are believed to be active in the United States at present. Studies of concordance for parkinsonism in twins and of the possible constitutional factors in reactions to ataraxic compounds were suggested as steps in defining the relative roles of genetics and exogenous factors in the various forms of parkinsonism. Ultimate prevention and control of the "ideopathic" forms of parkinsonism await the identification of a "chronic metabolic defect" which is believed to be present and which is likely to be of a genetic nature.

PART B.

Publications other than abstracts from this project:

Kurland, L. T. Chapter I - Parkinsonism. Epidemiology: Incidence, Geographic Distribution and Genetic Considerations. Charles C. Thomas, Springfield, Illinois. 1958.

PHS-NIH
Individual Project Report
Calendar Year 1958

PART A

11. 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 is coming to an end by December 31, 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. Trans. Am. Neurol. Assn., 1958.

Also accepted for publication by the A.M.A. Arch. Neurol. Psychiat.

PHS-NIH
Individual Project Report
Calendar Year 1958

PART A

12. 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, and many practicing neurologists are participating in this project by providing blood and saliva specimens for analysis. A small number of specimens from Guam has also been received.

PART B. None

PHS-NIH
Individual Project Report
Calendar Year 1958

PART A

13. 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.

PART B. None

PHS-NIH
Individual Project Report
Calendar Year 1958

PART A

14. Project Title: A Survey of Schizophrenics Among the Relatives of Schizophrenic 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 (13) and to determine the mode of inheritance of schizophrenia. This project is a by-product of project No. 13 and has the advantage of overcoming some of the biases involved in selecting an adequate sample.

PART B. None

PHS-NIH
Individual Project Report
Calendar Year 1958

PART A

15. 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. When data for 1957 become available, these will be added to the already existing ones and then analyzed.

PART B. None

PHS-NIH
Individual Project Report
Calendar Year 1958

PART A

16. 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.

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

PART B. None

PHS-NIH
Individual Project Report
Calendar Year 1958

PART A

17. Project Title: Skeletal Abnormalities With Motor System Disease

Principal Investigator: L. T. Kurland

Other Investigator: N. Myrianthopoulos

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

Project Description:

A detailed study of congenital skeletal defects in two families in which amyotrophic lateral sclerosis is transmitted in a dominant fashion, to determine whether an association, genetic or otherwise, exists between these anomalies and motor neurone disease.

PART B

Kurland, L. T., and Myrianthopoulos, N. C.: Skeletal Abnormalities With Motor System Disease. Neurology 8:727-733, 1958.

PHS-NIH
Individual Project Report
Calendar Year 1958

PART A

18. 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.

Present Status: The study has been completed and a report is being prepared for publication.

PART B. None

PHS-NIH
Individual Project Report
Calendar Year 1958

PART A

19. 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:

A study to investigate the possibility of detecting the heterozygous carriers in the infantile and juvenile forms of cerebroretinal degeneration, by changes in the peripheral blood

PART B. None

PHS-NIH
Individual Project Report
Calendar Year 1958

PART A

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

Principal Investigator: N. 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.

PART B. None

NATIONAL INSTITUTE OF NEUROLOGICAL DISEASES AND BLINDNESS

Research

Biometrics Branch

Estimated Obligations for FY 1959

Total: $165,000
Direct: 137,000
Reimbursements: 28,000

ANNUAL REPORT
Calendar Year 1958
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 1958 may roughly be divided into four major areas:

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

2. Other collaborative studies.

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

4. 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 1958, as well as proposed future objectives in each of the above four 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 project programs will contribute enough pregnancies over five year 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, medical history, socio-economic, prenatal, etc.) to outcome of pregnancy. In January 1958, pretest forms covering the various aspects (prenatal, labor, delivery, neonatal, etc.) were distributed to each of the participating institutions. The purpose of the pretest was to train local personnel and to determine whether the types of data requested could feasibly and reliably be secured. As the forms were completed, they were 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. These evaluations were taken up with each institution separately. It should be mentioned that in the above-mentioned trial run only the forms were being pretested. There was no attempt to pretest case selection or methodological procedures. From the data received, tabulations pertaining to certain characteristics of the obstetrical patient population of these institutions were compiled. In addition, statistical evaluations were made of data secured in areas of special interest, such as an anlysis of the data received in neuromuscular examinations of 166 babies at one of the collaborating institutions. During the course of the study, it became evident that the pretest forms would need considerable modification before they were suitable for the full-scale investigation. As a result, statisticians of the Branch worked in close cooperation with obstetrical, pediatric, and psychological consultants in the Project Services Branch, Collaborative Division, and with the staff of the Bureau of Social Science Research, Washington, D.C., in devising forms that would secure more meaningful and reliable data in the obstetrical, pediatric, and socio-economic-genetic areas and that would also be amenable to coding and tabulating procedures. Consideration was also given to revising hospital methodology for the collection of the data. In connection with the Branch's participation in giving consultation on standardization sampling procedures for the Bayley Test, Branch members designed an abbreviated version of socio-economic data sheets for use by psychologists during the "standardization." This would provide data on the characteristics of those included in the standardization and would allow a comparison of the socio-economic data of patients included for standardization with those who for various reasons refuse to participate.

It is planned to make the revised forms in all aspects available in January 1959 to all institutions in the project who are sufficiently well organized to be able to start the study at that time. Furthermore, it is hoped that some information on population characteristics and pregnancy outcome, evident at delivery, may be secured by each institution from reviewing its past hospital records so that decisions pertaining to modification of sampling procedures for each institution may be made by the Branch. The development of data on expected incidence of maternal characteristics and fetal pathology will be of great help in serving as a basis for modification of the sampling design. The Biometrics Branch is prepared to assist in the development of such data. In this connection, it should be stated that a source document and related punch card format and codes have been devised for one of the collaborating hospitals. It is anticipated that this would replace their present administrative punch card and would serve both administrative and research purposes. Furthermore, it would make it possible and relatively easy for this hospital to provide to the Biometrics Branch population and background data needed for the Collaborative Project.

During the course of the year the Branch Chief attended meetings of the Advisory Board of the Collaborative Project and the Ad Hoc Review Board of which he is an ex-officio member. He and other Branch staff members attended meetings of the Project Directors, as well as conferences and workshops devoted to considerations of the protocols dealing with the following aspects of the Project: (1) Socio-economic-genetic, (2) prenatal, (3) emotional evaluation of gravida, (4) labor and delivery, (5) pediatric, (6) neurological, (7) psychological, and (8) statistical. In addition, numerous meetings of small, working subcommittees were attended. Practically every institution in the Collaborative Project was visited at least once by Branch staff -- either as members of Project Site Visit Committees, or as statistical consultants on procedure in any of the above aspects. The purpose of these visits was to obtain information regarding the characteristics of the hospital populations, routine procedures for all hospital obstetrical patients, the way in which the Collaborative Project patients fitted into the hospital routine, and other specific problems of concern, such as selection of obstetrical patients for study, selection of children for standardization of the Bayley psychological test, processing of patients, completion and processing of study records, and other problems of a statistical nature with regard to the various aspects of the study.

Assistance in the formulation of study design and in sampling considerations was given by the Branch to a study at the University of California, under contract with the NINDB, of the Bayley Test with reference to its efficiency in detecting neurological damage at the eighth month of age.

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

Members of the Branch presented a paper on 'Statistical Aspects of the Collaborative Project," at a conference on The Epidemiological Approach to Problems of Pregnancy Wastage, held at Arden House, Harriman, New York, in March 1958. Another paper, dealing with the detectability of differences in incidence rates of various neonatal defects resulting from populations of gravidas with specific maternal complications, was prepared for presentation jointly with the Assistant Director, NINDB, at the annual meeting of the American Public Health Association, St. Louis, Missouri, in October 1958.

A multitude of 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; (2) the preparation of appropriate codes and manuals of forms and procedures for each aspect of the study; (3) the development of adequate methodological tests of reliability and validity of the data secured; (Some of these tests will precede the inauguration of the study and others will be conducted concurrently with it.) (4) preliminary planning for data analysis.

The Branch is involved in two studies, in which it is offering 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, the Biometrics Branch will be involved as the central statistical agency. Visits have been made by Branch members to several of the collaborating centers in this study. Codes have been set up and procedures for processing these forms from institutions have been established. Consultation relating to the sampling of serological specimens in the study has been given. The tabulation and analysis of these data will become a responsibility of the Branch. In the other study, Branch members have provided consultation in the formulation of a study design to the Director of Research and Statistics, Baltimore City Health Department, and his associates, in conjunction with a proposed research study, "Smoking and Prematurity." The purpose of this study is to determine the incidence of prematurity among offspring of gravida who smoke and among those who do not smoke, and to include within these groups other covariables, such as work history, education, blood grouping, and personality characteristics.

Staff of the Branch have consulted with the Director, Research and Statistics, Health Insurance Plan of New York City (HIP), and his associate, concerning the possibilities of cooperation with NINDB. The HIP is currently conducting a prospective pregnancy study based on completed report forms received on services rendered to members of the Plan. The purpose of the investigation is to study pregnancy loss, congenital anomalies, and morbidity in early childhood as related to maternal morbidity and other conditions prior to and during the antenatal period, and for a two-year period following birth. As a result of several conferences between the Biometrics Branch and HIP staff members to determine how the HIP study and the Collaborative Project could reinforce each other and mutually take advantage of both research programs, methods of liaison and areas of cooperation were established.

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 of disease may be increased. 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. The inauguration of an extensive phase program in this New York City area has been undertaken by the Columbia University School of Public Health and Administrative Medicine. The Branch Chief has consulted with officers of that school in order to help coordinate the respective efforts of the agencies concerned.

Additional studies, valuable to the conduct of the extensive phase, may be executed by utilizing copies of punch cards of all births and fetal deaths, purchased by contract from cooperating State and local health departments in the cities concerned. Such a study of fetal wastage in New York City is currently underway 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 may come to light as a result of this investigation. Attempts will be made to undertake similar or related studies during the coming year in other cities in which collaborating institutions are located.

2. Other collaborative studies.

(a) Collaborative Study of Etiology of Uveitis.

At the request of the Executive Secretary-Treasurer of the American Academy of Ophthalmology and Otolaryngology, the Biometrics Branch was called upon to set up a collaborative retrospective study to evaluate possible etiological factors in the production of granulomatous and non-granulomatous uveitis. After consultation with members of the Academy's Committee on Field Investigation for the Use of Diagnostic Procedures and Therapy in Uveitis, a study design and study forms were devised by the Biometrics Branch and approved by members of the Committee. This study will secure data from 15-20 eye institutions on family history, patient's medical history and condition, exposure to rare diseases and infections, and laboratory findings with respect to blood tests, biopsies, skin tests and skin sensitivity to various streptococcal agents for approximately 3500 uveitis patients and 3500 controls over a five-year period. The American Academy of Ophthalmology and Otolaryngology has requested that this be a collaborative study involving the Biometrics Branch as central statistical agency. It is planned that, if the study is

approved by the Field Investigations Committee and by the Advisory Council, the Biometrics Branch will render consultation to each of the collaborating institutions with respect to case selection and data collection procedures. Completed data will be forwarded to the Biometrics Branch via the American Academy of Ophthalmology and Otolaryngology for statistical processing, tabulation, and analysis.

(b) Collaborative Study of Evaluation of Non-Surgical Treatment of Strabismus.

At the request of the Chairman of the Committee on Evaluation of the Non-Surgical Treatment of Strabismus of the American Academy of Ophthalmology and Otolaryngology, the Branch Chief reviewed with several members of the Committee a proposed study to evaluate such therapy. The need for a clinical trial set up on a "blind" basis with adequate controls and with evaluation made on an unbiased basis was stressed. It is planned to have a Committee meeting in the near future so that these facts might be brought to their attention and so that the necessary groundwork for collaboration, with the Biometrics Branch as central statistical agency, may be laid.

3. 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 1958 to outside investigators on other projects.

(a) Preparation of age-specific mortality tabulations on cerebrovascular accidents in the United States.

(b) Evaluation of study design of a retrospective study of etiology of cerebral palsy in Chicago, supported by an NIH grant, and consultation given to help remedy the flaws in the study design.

(c) 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.

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

Evaluation of the study design and merits of several projects submitted for research grants to the Easter Seal Research Foundation and to 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 the Causes of Mongolism and Other Congenital Defects,' 'Correlation between Clinical and Pathological Findings in Cerebral Palsy,' and 'Prepregnancy Investigation of the Emotional, Physical, Endocrinological, and Nutritional Factors Involved in Congenital Malformations, Premature Fetal Deaths, and Spontaneous Abortions.'

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 'National Institute on the Role of the Workshop in Rehabilitation," sponsored in April 1958, by the National Rehabilitation Association.

4. 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 1958 and the problems involved:

(a) Laboratory of Neuroanatomical Sciences.

Assistance in preparing data on the social behavior of free-running rhesus monkeys for machine calculations in order to obtain correlations between the occurrence of specified social "acts."

(b) Medical Neurology Branch

Statistical analysis of the effects of certain drugs on muscle reaction to electrical stimuli.

(c) Surgical Neurology Branch

Statistical aid in problems involving physical distribution of various types of cells in the pituitary gland.

Statistical analysis of the effect of various types of brain surgery on chimpanzee body temperature. Consultation given concerning ways to improve the study design.

(d) Ophthalmology Branch

Statistical help for a proposed correlation of physical parameters involved in human sight.

Statistical analysis of data on the blood level of a certain drug.

Statistical aid in analyzing data on the visual response of the human eye to light of different wave lengths.

Statistical analysis of data on the effect of different enzymes on several types of tissue from rats' eyes at selected ages.

(e) Epidemiology Branch

Statistical analysis and interpretation of incidence and prevalence rates for Parkinsonism, based on results of a survey conducted in Rochester, Minnesota. Also included was a comparison of life expectancy of cases of Parkinsonism with that of the U. S. population.

Statistical computation and analysis of age-adjusted death rates from various neurological diseases in foreign countries, in the total United States, and by State and region.

Statistical analysis in connection with an investigation of multiple sclerosis in a community with a high incidence rate.

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 developing 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 due to various biological, genetic, and environmental factors, will be undertaken cooperatively with the Institute's Epidemiology Branch. It is believed that, as the Branch grows and as knowledge of availability of its statistical assistance becomes more widespread, there will be increased utilization of its services.

C. RECRUITMENT

The Branch's budget for fiscal 1959 includes 20 positions (12 professional-statistical positions and 8 clerical-stenographic positions). To date, 8 of the 12 professional positions and 5 of the 8 clerical-stenographic positions have been filled. Efforts at recruitment are steadily being made. The employment offices of the American Public Health Association and of the American Statistical Association have been requested to lend their efforts in this direction. Letters have been written and interviews arranged whenever there has been a possibility of securing a promising candidate. However, the great shortage of qualified biostatisticians has, to date, made this a frustrating experience. The provision of additional space for the housing of needed staff and files has become an acute problem. In August 1958, the Biometrics Branch was moved off the NIH grounds to the Progress Building, Bethesda. While this move eased temporarily the need for additional space, it is believed that the disadvantage of being off the reservation will act to deter younger statisticians from seeking employment with the Branch. It is expected that the proposed additional move of the Branch to Silver Spring, Maryland, will only accentuate further these difficulties.

NATIONAL INSTITUTE OF NEUROLOGICAL DISEASES AND BLINDNESS

Extramural Programs

		Estimated Obligations for FY 1959
I	Research Grants	
II	Field Investigations and Pilot Projects	$16,334,000
III	Graduate Training Grants	4,075,000
IV	Special Traineeship Program	1,500,000
V	Research Fellowships	536,000
VI	Review and Approval	551,000

ANNUAL REPORT
Calendar Year, 1958
Extramural Programs Branch
National Institute of Neurological
Diseases and Blindness
National Institutes of Health

I RESEARCH GRANTS

1. Program developments

During the year a considerable expansion occurred in the number of research studies on neurological and sensory disorders, and in the basic neurological sciences fundamental to sound clinical investigation. This expansion was made possible by a marked increase in the budget. All research projects recommended for approval during the year have been paid. In addition, awards were made on several projects approved last year, but unpaid, then, because of lack of funds.

Numbers of grants: - As the year ended (December 1958) there were 849 active research grants. A year ago at this time there were 670 active research grants (a net increase of 26.8% occurred during the present year).

Budget distribution: - When the budget for research projects is broken down into broad program areas it is seen that 47% was used for support of individual research projects in neurological disorders such as epilepsy, multiple sclerosis, muscular dystrophy, cerebrovascular disorders, degenerative diseases, neurological deficits of the young, and others. The previous year such studies used 51% of the budget.

Support of projects on sensory disorders consumed 26% of the research grant funds this year; this was the same as in the previous year. About two-thirds of these funds were used for investigations of vision and its disorders such as glaucoma cataract, uveitis, and accidents, the other one-third was absorbed in studies in hearing, speech and equilibrium, and other sensory studies as smell, taste and touch including pain. The balance of the budget was used during the year for supporting field investigation studies.

Expanding programs: - During the past year research into the cerebrovascular disorders expanded rapidly. Nearly $800,000 went into its support, as compared with $400,000 last year and only a little more than $100,000 two years ago. These figures include the two cooperative studies on aneurysms and anticoagulants that began last year and which, this year, used $300,000. Nevertheless, it is evident that even aside from these cooperative studies a marked increase in interest in the area of cerebrovascular problems has occurred. Undoubtedly the two cooperative studies have stimulated much of this interest and are directly related to the rapid expansion in research grant support flowing into this area. Activity in this program will remain high in the future.

An active interest in multiple sclerosis and other demyelinating diseases is shown by support of research projects at a level of $700,000 during the year, as compared with $400,000 last year. Studies in neuromuscular disorders including muscular dystrophy increased to a level of $800,000 this year as compared with $500,000 last year.

Among the disorders of vision, a marked increase has occurred in studies related to glaucoma. During 1958 investigations in this field received support of about $400,000; this was double the level of a year ago. Further rapid expansion in this important area will undoubtedly occur because of the interest aroused by the field investigation study and the research conference that are scheduled to be supported next year. A similar sharp increase is noted in studies related to uveitis and infectious eye diseases.

Efforts are under way to promote studies in speech disorders as related to neurological deficits and mental retardation. This year saw 8 projects started with $100,000 support. Rapid expansion in the immediate future is anticipated.

2. Research developments: Contributions from specific awards

During 1958 reprints of 426 papers that appeared in Scientific Journals were placed on file by NINDB Grantees. These were supported by 296 research grants. With 670 grants active at the start of the year it is evident that nearly half of NINDB grantees published at least one paper during the year. Contributions from specific projects are listed in the following paragraphs, but without any attempt at covering all, or even the most important, discoveries.

During the year Dr. Heinrich Waelsch of Columbia University has reported extensively on his studies on metabolism of protein and amino acids in nervous system and brain. This important work is fundamental to the understanding of normal, as well as abnormal, activity of brain and nerve cells. The relation of tryptophan to serotonin and other CNS-active drugs exemplifies this interest. It has been shown that gamma amino butyric acid is formed by brain cells from glutamic acid and that during convulsions of the animal the ability of brain cells to promote this conversion is lessened or abolished. Is this changed metabolism a cause or effect of the convulsions? Clearly much more information is needed as to the function of amines and the role of amino acid metabolism in brain and nerve cell activity.

Dr. Eugene Kennedy of the U. of Chicago has expanded his fundamental work on the way the cells of the body produce cephalin, lecithin and other phospholipides important to nervous tissue composition and activity. He has worked out the pathways by which these fundamental units are put together, and has reported on the enzymes which are needed

for this. Several other grantees continue the laborious attempts at sorting out and identifying the various bizarre lipid components of nerve tissue. Noteworthy in this area of work are Dr. Jordi Folch-Pi of Boston who has reported on new lipoproteins from the nervous system, and Dr. E. Carter of U. of Illinois who continued studies on the composition of sphingosine and the various sphingolipids.

Dr. Larrabee of Johns Hopkins continued his fundamental studies of the metabolism and glycolysis of nerve cells. He has obtained evidence that the source of energy for nerve impulse in ganglion cells involves some component in addition to glucose. In this connection Dr. F. O. Schmitt of M.I.T. has re-emphasized the theory that the Schwan cells of the myelin sheath, far from being inert cells, are actively involved in supplying the energy necessary for ion transport and operation of the "sodium pump" in nerve impulse transmission.

During a symposium supported by another Institute, Dr. Denny-Brown of Harvard, an NINDB grantee, reported on the importance of nutrition to neuropathology. His review of the neurological disorders resulting from simple nutritional deficiencies in man and animals brought the following relations to mind: (a) Mental deterioration occurs from a deficiency of nicotinic acid and tryptophan (pellagra in humans). The structural relation between serotonin, several of the hallucogenic drugs and tryptophan were recalled. (b) An extreme neurological syndrome resulting from pernicious anemia, can be prevented by therapy with vitamin B12. (c) The epileptoid convulsions reported as a result of vitamin B6 deficiency in mice, rabbits and human infants. (d) The spinal cord lesions and collapse of voluntary muscles in vitamin B6 deficiencies in swine and rabbits. (e) The polyneuritis in humans and the involuntary rolling motion in rats deficient in vitamin B1 (thiamine). (f) Specific cells in the cerebellum of chicks fed diets lacking in vitamin E undergo necrosis; this is related to the encephalomalcia which can be prevented in chicks by adequate dietary vitamin E. (g) In rats a chronic deficiency of vitamin E results in pronounced lesions in the spinal cord; this is correlated with slowly developing paralysis or muscular dystrophy in these animals. In rabbits fed the diet deficient in vitamin E the muscular dystrophy proceeds explosively to fatal termination in a few weeks. (h) A deficiency of potassium in the diet of dogs, rats, rabbits and humans results in total flacid paralysis of striated muscle, similar to familial periodic paralysis in humans. (i) A dietary deficiency of choline, the parent substance of acetylcholine, and the major component of phosphelipids and sphingolipids, results in a muscle weakness and paralysis in rats, guinea pigs, and rabbits. (j) A partial lack of vitamin A as well as other diet essentials, individually, results in abnormal offspring of various kinds; for example, hydrocephalus can be produced at will by proper attention to the diet of pregnant rats. (k) A lack of copper in the diet of cattle or sheep

produces very marked neuromuscular disorders. Dr. Denny-Brown emphasized that in spite of the clear examples of close relationships, nutritional scientists as a group ignore neuropathology and few neurological investigations accord significant consideration to the nutritional component of etiology of neurological disorders.

Dr. Nachmansohn and his group at Columbia U. have succeeded in preparing fat-soluble derivatives of acetylcholine. These compounds act like acetylcholine in triggering smooth muscle contractions and in promoting synaptic transmissions of nerve impulse. However, since they are fat-soluble they penetrate cells, neuronal axons and myelin sheaths, and are not subject to inhibition by curare and other acetylcholine inhibitors at the motor-end plates and the neuromuscular junction. The possible and potential uses of such compounds may be very interesting.

Dr. Frank Morrell at the U. of Minnesota has continued his studies on the epileptic lesion and the conditioned reflex arc. Epilepsy was induced in monkeys by implanting a disk of alumina or by spraying a specific area of the brain with methylchloride. A study was then made on the ability of these animals to establish new conditioned reflex pathways, or to respond to reflex stimulae established before epilepsy. The epileptic animals were significantly slower than normals in both aspects. However, when the epileptogenic lesion in the brain was surgically removed, the ease of establishing the conditioned reflex arc was returned to normal. These studies indicate the pathways of certain legs of the reflex arc through specific brain areas. In addition this study re-emphasizes that the spontaneous volleys of impulses characteristic of epilepsy originate from brain cells whose metabolism has been altered in some way. Removal of these cells by surgery or other means frees the animal from the burden of these maverick cells. The nature of the abnormality in the specific cells is not known.

Drs. R. Meyers and W. Fry at the U. of Iowa are attempting to remove the specific centers of abnormal cells in the brains of humans suffering from epilepsy, chorea, parkinsonism and other movement disorders. They are doing this by killing the cells with focused beams of ultrasound waves. These studies are in a preliminary stage with reports of good success in about eight patients. Dr. Spiegel of Temple U. reports success in epileptic patients using implanted electrodes as the technique for killing the specific brain cells. Much of the success of these efforts depend on the ability to locate the desired area of the brain, and several stereotactic devices have been developed for this purpose.

A report on the use of preserved human eye tissue for transplantation in surgical cases, has been given by Dr. J. H. King of George Washington U. The cornea of eyes can best be preserved for later use by dehydrating them from pure glycerine, sealing, and storing in vacuo at room temperature, Dr. King finds. These can be used even after two

years of storage. Use in over 50 patients show that the preserved corneas are as good as fresh corneas used for similar transplantation. Dr. King also reports on methods for preserving vitreous humor for use in specialized problems of retinal detachments and other uses. Successful use of sclera and conjuctiva is also reported. Transplantation of lens has so far not been successful because of the opacity that invariably develops.

3. Major problems encountered

No major problems have been encountered during the year. Some concern was encountered relative to a change in rules governing use of research grant funds for foreign travel. As of July 1, 1958 the new policy on foreign travel required prior approval by the Advisory Council. This was more restrictive than the previous rule requiring approval only for travel to international meetings. The Council went on record favoring relaxation of the restriction rather than the reverse, but agreed to live with the new ruling for a trial period.

4. Changes and improvements in program

The year saw the lifting of the arbitrary five-year ceiling for support of research grants. Under the leadership of the Advisory Council, 12 grants were made with a period of support recommended up to eight years. Selection of recipients of this long term support was based on the productivity potential of the man as well as the breadth and significance of the project. Undoubtedly this type of support will contribute to stability and freedom of research in the broad area of interests in basic neurological sciences, represented by these twelve grants.

During the year a research grant was made to Massachusetts Institute of Technology for the total salary and ancillary support of a senior, mature investigator, Dr. McCulloch. Five years were recommended. Although made on the basis of a research project and under conditions that departed from no rules, nevertheless, this grant was widely interpreted as a "career investigator" grant by the consultants who reviewed the application. As such, it may serve as a prototype and precedent.

5. Program objectives for 1959

During the next year every effort will be made to maintain the proper balance between support flowing into the various programs of interest to NINDB. Insofar as possible attempts will be made to promote interest in certain areas that appear somewhat retarded; specifically, one such area is the neurological aspects of speech development in the young. If the Program Planning Committee of the Council is to carry out its important role of mapping areas for special program development in the neurological and sensory disorder field, it is important that research grant funds be available in flexible amounts. At least $15,000,000 will be necessary during the year for Research Grants.

6. Volume of applications
(not including Field Investigations)

Council Meeting		Considered		Recommended	
		No.	Amount	No.	Amount
March	1958	209	$3,673,515	149	$2,024,391 (55%)
June	1958	201	$3,625,030	119	$1,929,963 (53%)
November	1958	162	$2,764,719	111	$1,572,446 (57%)
Total		572	$10,063,264	379	$5,526,800 (55%)

7. Staff assignments

Dr. Edwin Hove (since March 13, 1958)

1958 ANNUAL REPORT
of the
National Institute of Neurological
Diseases and Blindness

II. FIELD INVESTIGATION AND PILOT PROJECT GRANTS

1. Program developments

The purpose of this program is to broaden the research grant-in-aid base and to facilitate the development, where need is indicated, of epidemiological studies, national surveys, cooperative and collaborative studies that call for a multi-institutional and often a multidisciplinary approach, as well as pilot projects established to work out leads, i.e., the most promising approach for further research. In many respects it is a program of applied research, in that it endeavors to enlist the aid of a certain segment of the Nation's scientific manpower in an effort to combine forces, utilize the information at hand, and obtain some answers relatively soon, instead of depending on the generally slow course of events. This program got underway in January 1957, and now consists of 64 projects in the amount of $4,329,196.

This program is one which has been established specifically to meet urgent research needs. In order to assure competent, unbiased, objective review of applications, two preliminary review committees have been established. The Field Investigations Committee, so broadly constituted that a particular specialty or area is usually represented by only one member, reviews all applications except those involved in the Collaborative Study of Cerebral Palsy and Neurological Deficits of Infancy and Childhood. Applications for this latter study are reviewed by the ad hoc Committee on Cerebral Palsy. Through this mechanism it is possible to bring to bear on the C.P. study, a concentration of expert advice necessitated by the breadth, size and intricacy of this particular research effort.

Although a number of single institution projects of a pilot nature are financed through this program, 92 per cent of the funds is expended in the support of cooperative and collaborative studies involving a number of institutions. To provide a means of differentiation between multi-institutional projects involving an expenditure of Institute direct operations funds and intramural personnel from those supported entirely from extramural funds, the term "collaborative" has been adopted for the former and the term "cooperative" for the latter. The term "cooperative" is, therefore, utilized to describe a multi-institutional project in which a number of outside institutions are cooperating with each other, with no counterpart Institute activity and supported wholly by extramural funds.

During 1958, the Collaborative Study of Cerebral Palsy and Neurological Deficits of Infancy and Childhood has almost reached optimum development. This study now involves 14 central institutions, with one more to be added in order that the study cover a total of 40,000 infants as required for validity. It is now being financed in the amount of $5,407,994. This is a long term multi-institutional multidisciplinary study from which little significant data is expected until follow-up is relatively complete in 1967 or 1968.

The Cooperative Study of Aneurysms and Acute Subarachnoid Hemorrhage, involving 20 institutions and financed in the amount of $200,000, reached full development in 1958. The purpose of this study is to amass a body of baseline data on the medical versus surgical treatment of aneurysms, utilizing a number of optional forms of treatment under conditions governed by a study protocol.

The geomedical Collaborative Epidemiological Study of Selected Neurological Deficits involving South Carolina, Nova Scotia and Japan, will be completed in 1959. Data from this study are not yet available.

The Cooperative Anticoagulant Therapy Study involving six institutions at a cost of $54,000 per annum has one more year to run before complete data will be available. At that time it may be indicated that a broader approach, testing a larger number of drugs, would be promising.

During the year, working with the Chronic Disease Division of the Bureau of State Services planning has been completed for a Collaborative Glaucoma Detection Evaluation Study to start early in 1959. This study, which has as its purpose the development of techniques and procedures leading to improvement in prevention and detection, will involve 5 research institutions, and cost approximately $150,000 to $175,000 per year for five years.

In addition, the Institute's Biometrics Branch, working with officials of the American Academy of Ophthalmology and Otolaryngology and research ophthalmologists, has developed an acceptable protocol for a uveitis study. This study to be entitled "The Collaborative Study of Etiology of Uveitis", will involve 20 institutions, cost about $250,000 per year and get underway in the fall of 1959.

The National Advisory Neurological Diseases and Blindness Council has made tentative plans to sponsor an annual series of conferences starting in 1961, on various aspects of glaucoma research. It is believed that these conferences and the Collaborative Glaucoma Detection Evaluation Study will provide considerable impetus to research in the field of vision.

Plans have been completed to hold a national working conference on the conservation of hearing to be jointly sponsored by the Childrens Bureau, the Chronic Disease Division of the BSS and this Institute,

during the last week in May 1959. The purpose of this conference is to delineate the various facets of the problem, map out the most promising attack and stimulate research efforts in a field that is sadly lacking in activity. A conservative estimate of the number of persons presently handicapped by impaired hearing is 10% or 17 million. In industrial communities, sample surveys place the figure as high as 20%.

2. Contributions resulting from particular awards

Since this program got underway in 1957, and since almost 100% of the expended funds involve multi-institutional studies, the shortest of which are three years, no research contribution is yet ready for announcement. It is known that several studies such as the cooperative aneurysm study, the cooperative anticoagulant study, the national survey of hearing in children and others are resulting in data which seem at this point to be highly significant. There is no doubt that this program will serve to open up many new research leads as well as contribute definite advances of marked significance. Specific citation, however, must be delayed at least one more year.

3. Major problems encountered

The Field Investigations program has grown from 51 projects in the amount of $1,961,415 in 1957 to 64 projects requiring $4,329,196 in 1958. (See attached table entitled "Field Investigations Program, Fiscal Years 1958 and 1959") Concurrently, other extramural programs have similarly expanded necessitating additional staff. Because of this pressure it has been decided to employ a full-time staff person to take immediate charge of this program instead of relying on the part-time efforts of the Chief, Extramural Programs Branch. A full-time staff individual, serving as Executive Secretary of the Field Investigations Committee, will be able to devote more time to grantees, keep the program under closer surveillance and thus have a more intimate knowledge of problems as they occur, and also be of greater aid to the Committee in carrying out its plans.

4. Program objectives for 1959

Program objectives for 1959 are (1) to get the collaborative glaucoma and uveitis studies established on a sound basis; (2) to develop a larger research program in the field of hearing; (3) to get underway a cooperative brain tumor chemotherapy project, and (4) to stimulate in foreign countries projects which because of their unique aspects will materially contribute to research progress in the U. S.

5. Volume of applications

Council Meeting	Reviewed No.	Reviewed Amount	Approved No.	Approved %	Approved Amount	Approved %
March 1958	9	329,719	5	55.6	217,536	66.0
June 1958	14	1,028,346	6	42.9	274,045	26.7
November 1958	22	1,455,155	14	63.6	1,003,311	68.9
TOTAL	45	2,813,220	25	55.6	1,494,892	53.1

6. Staff assignments

Dr. Seger, Acting Executive Secretary, Field Investigations and *ad hoc* Cerebral Palsy Committees.

FIELD INVESTIGATION PROGRAM

(Fiscal year basis)

	1958			1959 *		
	No.	Amount	% of $	No.	Amount	% of $
L DISORDERS	51	$1,961,415	100	64	$4,329,196	100
urological Deficits of Infancy & Childhood - Total	15	1,295,515	66.1	25	3,639,151	84.1
ollab. Study of C.P.)	(8)	(1,101,506)	(56.2)	(16)	(3,407,994)	(78.7)
rebral Vascular Disorders - Total	24	375,465	19.1	25	318,810	7.4
oop. Study of Aneuryam)	(17)	(253,033)	(12.9)	(17)	(188,514)	(4.4)
oop. Study of Anticoagulants)	(6)	(56,748)	(2.9)	(6)	(53,964)	(1.2)
llab. Epidem. Survey Selected Neurological Disorders	3	81,183	4.1	2	55,241	1.3
sorders of Aging	--	---	--	1	23,621	0.5
llepsy	2	22,607	1.2	1	19,607	0.5
ltiple Sclerosis & Other Demylinating Diseases	--	---	--	1	18,501	0.4
aring & Balance	3	111,992	5.7	3	100,552	2.3
tinopathy	--	---	--	1	19,103	0.4
trolental Fibroplasia	1	6,597	0.3	2	59,370	1.4
eitis, Keratitis & Other Inflamatory & Parasitic Diseases	1	43,056	2.2	2	54,935	1.3
eech	--	---	--	1	20,355	0.5
formation	1	10,000	0.5			
ministrative Grant	1	15,000	0.8			

Awarded to date

1958 Annual Report

of the

National Institute of Neurological Diseases and Blindness

III. Graduate Training Grants

A. Clinical Neurology and Pediatric Neurology

Graduate training grant aid is offered in Clinical Neurology and Pediatric Neurology to facilitate the establishment and development of postdoctoral training programs in each field. The primary purpose of these programs is to aid in the training of postdoctoral clinical personnel for careers as teacher-investigators in the field for which the grant is given. In essence, the grant-in-aid training program of the NINDB was primarily established to facilitate the research effort in the neurologic and sensory disease fields through the production of career investigators.

Training grants in Clinical Neurology provide stipend support in whole or in part for trainees chosen by the grantee institution. The November 1956 Council adopted a policy, effective July 1, 1957, limiting the stipend which could be paid a trainee in whole or in part from a training grant to $3,800, $4,200, and $4,600, for the first, second, and third years, plus $350 per dependent. This is a maximum limit that may not be exceeded without prior approval. Trainee stipend funds are not provided on training grants in Pediatric Neurology, since as will be indicated later, trainees in this field satisfy residency requirements in Pediatrics prior to entering into Pediatric Neurology training and therefore are eligible to apply individually for Special Traineeship support. Special Traineeship support is felt to be more appropriate since the awards are at a higher level than under a training grant and in line with the more advanced stage of Pediatric Neurology training.

Table I below, outlines the fiscal details of grant support in Clinical Neurology and Pediatric Neurology during Calendar Year 1958.

Table I

	Clinical Neurology		Pediatric Neurology		Total	
	Amount	No.	Amount	No.	Amount	No.
Active Grants--12/31/57	$1,559,255	55	$40,035	2	$1,599,290	57
Programs Awarded Initial Support	52,689	3	23,760	1	76,449	4
Programs Awarded New Period of Commitment	750,286	24	---	--	750,286	24
Disapproved for continuation support	---	5	---	--	---	5
Terminal Grants	99,733	4	---	--	99,733	4
Committed continuations	714,332	26	40,008	2	754,340	31
Active Grants--12/31/58	1,599,685	55	63,768	3	1,663,453	58
Increase during C. Y. 1958	$ 40,430	0	$23,733	1	$ 64,163	1

1. Programing Accomplishments

Clinical Neurology represents one of the areas of initial programing emphasis. Toward the end of calendar year 1957, the rate of growth of this program rapidly decreased. As can be seen from Table 1, a total of 55 active programs were receiving support on December 31, 1957. During calendar year 1958, three new programs were awarded training grant aid but at the same time five applications for continuation support were disapproved. Three of the latter terminated on June 30, 1958, with the result that at the end of the calendar year 1958, the number of grant supported programs remains at 55. These programs are supported at the level of approximately $1,600,000 or at an average of $29,091 per program. The largest grant is $59,000 and involves 15 trainees, the smallest is $12,000, for which as yet no trainees have been recruited.

Table II

	Academic Year 7/1/57 - 6/30/58				Academic Year 7/1/58 - 6/30/59			
	Year of training			All Trainees	Year of training			All Trainees
	1	2	3		1	2	3	
Clinical Neurology No. of Trainees	88	74	34	196	105	83	61	249
Average Stipends	$3,407	$3,753	$4,016	$3,522	$3,442	$3,678	$4,112	$3,803
Pediatric Neurology No. of Trainees	2	2	3	7	6	2	2	10
Average Stipend	SPECIAL TRAINEESHIPS							

As can be seen from Table II above, 196 trainees were in training for a career in Clinical Neurology during the period of training ending June 30, 1958. At present 249 individuals are in training and 61 are expected to complete training in June 1959. It is projected that eventually 80 to 90 individuals will regularly complete training each year as specialists in Clinical Neurology.

Pediatric Neurology training is as yet in its initial stages of development. This condition is due to the marked dearth of personnel in the field capable of carrying out training programs, and to the fact that only recently has a demand for such highly trained personnel evidenced itself. One of the important factors contributing to this demand is the recently established Collaborative Study of Cerebral Palsy sponsored by this Institute. During Calendar year, 1958, one new program was established bringing the total active programs to three. These programs are being supported at the level of $63,768 or an average of $21,256 per program. As is indicated in Table II, there are at present 10 individuals in training for a career in Pediatric Neurology.

2. Contribution resulting from particular awards

It is as yet too early to evaluate the contribution of the various training programs. Each institution varies in its approach. Since the product is the trained investigator, only time will reveal the caliber of a program as measured by individual successes and failures in the investigative field.

3. Major problems encountered.

Up until the present time, there has been a marked dearth of Clinical Neurologists in academic positions. However, as more and more specialists in this field complete training and steadily fill available positions in the medical schools across the country, and private practice opportunities develop, the problem of attracting individuals to investigative careers will become increasingly difficult. The answer to the problem is multifaceted and primarily consists of providing adequate support of Clinical Neurologist career investigators and keeping the training in this specialty in balance with national needs.

In the field of Pediatric Neurology, it is obvious that there is a marked dearth of highly trained personnel available to conduct training programs. When all available personnel have been recruited to the training effort, a lag period will ensue until the programs can propagate themselves. Thus, the acute lack of Pediatric Neurologists will continue for many years.

4. Changes and Improvements made.

Now that the training program in Clinical Neurology has developed to a point where it can be seen which programs are succeeding in training neurologists and which, for one reason or another, are failing, continuation support is being denied the unsuccessful and additional support awarded to proven producers. In two instances, this has involved the awarding of long terminal grants. Thus, to make long terminal grants unnecessary, review of continuation applications now takes place far enough in advance so that

decision is known at least one year before the termination of the current commitment period. Under this system the only terminal grants awarded are for the purpose of financing the program to the end of the academic year.

In an attempt to establish some general guidelines as to the training likely to produce an investigator in the field of Pediatric Neurology, an ad hoc Committee was convened on May 20, 1958, to consider the problem and offer concrete recommendations. The Committee consisted of representatives from the fields of Pediatrics, Pediatric Neurology, and Clinical Neurology. It was concluded that as prerequisite training, one year of internship and two years of Pediatric residency would be essential. Trainees meeting these qualifications are eligible for Special Traineeship awards.

In regard to the program, itself, it was the consensus that the period should be a minimum of three years, consisting of Adult Neurology, Clinical Pediatric Neurology and experience in the Neurologic Basic Sciences. It was noted that in the case of special talent, further training experience would be desirable to make it possible for a trainee to develop research proficiency in his special area of interest. These recommendations were adopted by the June 1958 Council as a guide. In light of the above recommendations, training program grants in this field do not provide trainee stipends, and the 10 individuals now in training are all supported on Special Traineeships.

Prior to calendar year 1958, three graduate training grants were awarded in Neurosurgery. In an effort to clarify the manner in which training in this field could be best supported by this Institute, an ad hoc Committee was appointed to consider the matter. This Committee met on February 22, 1958, and recommended that support of training during the residency period through the award of program grants did not represent a general need at the present time. It was the consensus that whereas there is not a recognized need for an increased number of private practitioners in this field, there is a dearth of trained investigators and that support by Research Fellowship and by Special Traineeship awards during and after the residency period would be the most effective method to aid Neurosurgery.

5. Program Objectives

It has become apparent that the evolution of the Clinical Neurology grant-in-aid training program will consist of cultivation of productive programs, the aiding of those with real potential and the cessation of support to programs which, for one reason or another, have failed to develop after a reasonable trial period. Further, it is recognized that a longer period than the usual three year training program is required to train an investigator in Clinical Neurology. Accordingly, various Program Directors are beginning to think in terms of enlarging the scope and capacity of the program in order to be able to offer training for a period of from four to six years. Individuals can be supported on Special Traineeship awards during the period beyond three years. It is expected that a core of the most productive programs will develop along these lines.

It is estimated in regard to Pediatric Neurology training that optimum growth will probably be reached when 15 to 20 programs have been established. During the next calendar year, it is projected that five new programs will evolve. As was mentioned previously, slow growth results from the marked dearth of adequately trained individuals to establish programs. Now that definite training guide lines have been established, this probably represents the greatest difficulty in this facet of the training program.

6. Volume of Applications (Clinical Neurology and Pediatric Neurology)

	Requested		Approval		% Approval	
Councils	Amount	No.	Amount	No.	Amount	No.
March 1958	$478,301	16	$195,134	7	40.8%	43.8%
June 1958	$222,352	10	$150,806	8	68.0%	80.0%
November 1958	$210,513	8	$148,609	7	70.6%	87.5%
Total	$909,167	34	$495,449	22	54.5%	64.7%

7. Staff Assignment

Lawrence A. Farber, M. D., Executive Secretary,
Neurology Graduate Training Grant Committee

1958 Annual Report

of the

National Institute of Neurological Diseases and Blindness

III. Graduate Training Grants

B. Neurologic Basic Sciences

The primary purpose of the training programs in this area is the training of postdoctoral basic science personnel for careers as teacher-investigators. Programs are being established in the basic departments such as pathology, anatomy, physiology, pharmacology, and biochemistry. Except for pathology most trainees will probably be Ph.D.'s. It is hoped that training programs will be broadly enough oriented so as to produce mature, productive investigators.

The fields included in this category are Neuropathology, Neuroanatomy, Neurophysiology, Neuropharmacology, and Neurochemistry.

Table III

	Active Grants 12/31/57	Programs Awarded Initial Support	Contin. Grants	Committed Contin.	Active Grants 12/31/58	Increase During CY 1958
Neuropathology	(4) $ 96,021	(8) $151,109	(1) $ 27,093	(3) $ 59,538	(12) $237,740	(8) $141,719
Neuroanatomy	---	(3) 82,706	---	---	(3) 82,706	(3) 82,706
Neurophysiology	(1) 24,779	(1) 104,048	---	(1) 40,503	(4) 144,551	(3) 119,772
Neuropharm.	(1) 48,650	(1) 29,576	---	(1) 30,564	(2) 60,140	(1) 11,490
Neurochemistry	(1) 17,921	(3) 134,736	---	(1) 15,585	(4) 150,321	(3) 132,400
Total	(7) $187,371	(16) $502,175	(1) $ 27,093	(6) $146,190	(25) $675,458	(18) $488,087

Figures in parentheses indicate number of programs

1. Programing Accomplishments

Prior to calendar year 1958, there were four training programs in Neuropathology, none in Neuroanatomy and one each in Neurophysiology, Neuropharmacology, and Neurochemistry, for a total of seven. This program represents an area of major programing emphasis during calendar year 1958. The type of program evolving in this basic science area is broad based, designed to not only

allow an individual to acquire techniques and knowledge in a narrow area of his own particular interest, but also to make him aware of the work being carried out in each spectrum of his field of endeavor and broaden his horizon. This is somewhat different training than that customarily received by a trainee serving as a research assistant or fellow on a research project.

Eight new program grants were awarded in Neuropathology bringing the total to twelve. These programs are supported at the level of $237,740, an average of $19,812 per program. It is the belief of our consultants that the most desirable prerequisite training is three years of residency in general pathology. Thus, most individuals are eligible for Special Traineeship support and accordingly when grants are awarded in this field, they provide at most one trainee stipend for the occasional trainee who for one reason or another is not eligible for such an award. Consequently, the average training grant in Neuropathology is somewhat smaller in amount due to the absence of large sums for trainee stipends.

Three new programs were established in Neuroanatomy, three in Neurophysiology, one in Neuropharmacology, and three in Neurochemistry, bringing the total programs in these four fields to 13. They are supported at the level of $438,718, an average of $33,748 per program. The basic science program grants in these fields are somewhat higher, due to the need for funds to provide specialized equipment and supplies as well as trainee stipends.

Since the majority of the basic science training programs were established during the latter half of calendar year 1958, the number of trainees is as yet quite low. Approximately 20 individuals are in training for careers in neuropathology. It is estimated that approximately 20 or more are in training in the remaining 13 basic science programs. Although the number of trainees is still relatively small, this program has increased by 350 per cent in number of grants and by 360 per cent in the amount of funds involved during 1958.

2. Contributions resulting from particular awards

It is as yet too early in the basic science training program to evaluate its contribution. However, since the product of this area of training will be a basic scientist, the private practice enticement does not exist. It is believed that almost all individuals trained will enter into full-time investigative careers.

3. Major problems encountered in programing

Major problems encountered in programing in these basic science areas is the marked dearth of highly trained personnel available to carry out postdoctoral training. Whereas almost all graduate institutions provide training in basic science areas for the doctorate, only a very few in the past have been interested or have developed postdoctoral programs. At present all areas potentially capable of establishing training programs are being encouraged to do so.

Recruitment of trainees to established training programs is another major problem encountered by the various Program Directors. This problem has arisen partially from the lack of knowledge that postdoctoral training opportunities exist, but primarily because of the dearth of Ph.D. personnel to undertake advanced, highly-specialized training. Through announcements in the

[illegible] gradually being conquered. Favorable results are very great in some cases, thus illustrating a definite demand for such training. However, the primary problem still exists. It is expected that the predoctoral training supported by the Division of General Medical Sciences will partially solve the situation.

4. Changes and improvements in programs

With the shift of programing emphasis to the basic science area, it became evident that the Neurology Graduate Training Grant Committee which consisted largely of clinical neurologists would have to have the benefit of advice from individuals with special basic science competence Therefore, during 1958, a Neuroanatomist and a Neurochemist were added to the Committee, and a Neurophysiologist has been nominated and is expected to be appointed in the near future.

5. Program Objectives

It is estimated that in order to meet national needs the establishment of approximately 20 training programs in each of the five basic science areas will be necessary. This would result in 25 to 30 individuals completing training in each field every year. During the next calendar year, it is expected that four or five new programs will be established in each field. It is quite apparent that since this program is as yet in its initial stages, its impact upon the research effort will be slow in developing. Undoubtedly there will be a considerable lag in growth until the established programs produce enough trained investigators to essentially propagate themselves. During the next calendar year, two aims will be highly significant. First, programing will be very active if the number of programs indicated above are to be realized. Second, existent programs will be developed and aided in all possible ways

6. Volume of applications (Basic Neurologic Sciences)

Councils	Requested		Approval		% Approval	
	Amount	No.	Amount	No.	Amount	No
March 1958	$297,423	7	$165,499	5	55.6%	71.4%
June 1958	$216,187	7	$124,347	6	57.5%	85.7%
November 1958	$334,673	9	$227,383	8	67.9%	88.9%
Total	$848,283	23	$517,229	19	60.7%	82.6%

7. Staff Assignment

Lawrence A. Farber, M. D., Executive Secretary,
Neurology Graduate Training Grant Committee

1958 Annual Report

of the

National Institute of Neurological Diseases and Blindness

Predoctoral Training Grants

Ophthalmology

1. Program accomplishments

In the pursuit of the goal of developing and maintaining postdoctoral training programs to train career teacher-investigators in ophthalmology, 1958 saw an increase of programs from 35 to 38. These programs provide a total of 296 trainees with approximately 87 individuals per year finishing residency training who are potential teacher-investigators. The three new programs initiated in 1958 were added to the clinical ophthalmology training program because of their judged potential for training personnel orientated toward research careers. There was a small amount of growth of the clinical training program during the year. Thirteen applications for a requested $292,039 were received, and of these 9 were approved for a total of $175,482. Within the same period of time financial support of three programs was discontinued.

2. Contributions resulting from particular awards

Due to the short period of time the ophthalmology training program has been in operation, it is not yet possible to single out specific programs which are outstanding in consistently producing men of high calibre as teacher-investigators. No program has yet run for a long enough period to establish a tradition of training teacher-investigators and thus attract applicants primarily interested in such careers and train them to the limit of its potentialities.

3. Major problems encountered

Two major problems have been encountered. (1) The slow evolution of programs of training from those traditionaly organized to develop entrepreneurs into those with sufficient flexibility to provide experiences, especially in the basic sciences, necessary for training teacher-investigators. (2) The second problem is inherent in the field of ophthalmology - that of recruiting individuals into academic careers. The lessening of this second problem through the passage of time is predicted as young research trained individuals become Program Directors and influence trainees in their programs.

4. Changes and improvements in program

As of the May Committee meeting, Dr. Jerome became Executive Secretary of the Ophthalmology Graduate Training Grant Committee. His appointment achieves an administrative organization which provides constant guidance of and attention to the details of Committee business.

In the month of September a small conference was held of professional personnel interested in the subject of glaucoma. Participants were Dr. Braley, Dr. Becker, and Dr. Shaffer. A recommendation was forwarded to Council that a series of five or six yearly working conferences on the subject of glaucoma be held beginning the academic year 1960-61. It was invisioned that they be international in character, include both clinicians and basic scientists, and that invitations be issued to a small number of participants which probably would not number over 30. The November Council authorized the holding of such a series.

A Program Directors Conference was held in the month of January. This was attended by approximately 100 invitees. The day was spent in clarifying the goals of the training grant program and discussing both methodology and content of clinical and basic science aspects of training teacher-investigators.

Forty-three young, potentially productive investigators in ophthalmology were granted financial help to attending the 18th International Congress of Ophthalmology held at Brussels, Belgium. The purpose of this was to provide experience and stimulation of these young investigators at a critical stage in their development as researchers.

Changes in the personnel of the training grant Committee occurring during 1958 are as follows: (1) appointment of Dr. George LeRoy, Associate Dean of the Biological Sciences, University of Chicago, to the Commitee for a four year term, (2) appointment of Dr. Bernard Becker to the chairmanship succeeding Dr. Alson Braley, and (3) retirement of Dr. Braley from the Committee as of December 31, 1958.

At its November meeting the National Advisory Neurological Diseases and Blindness Council adjusted its ceilings on trainee stipends from $3800, $4200 and $4600 plus $350 per dependent for each of three years to $4500, $5000 and $5500 plus $500 per dependent for each of the three years constituting the regular residency period.

In October at the request of the Director, Dr. Braley spent 10 days visiting institutions in England, Scandinavia, and on the continent which train ophthalmologists and have facilities for ophthalmolgic research. While the trip was made with the primary goal of evaluating opportunities for the training of

Special Trainees, information of value to the conduct of the ophthalmology training grant program also was gathered.

5. Program Objectives for 1959

The primary goal for this year will be to promote the training of greater numbers of teacher-investigators by increasing the percentage of trainees from present programs who select this type of career. This will be achieved by disseminating the goals of the program to trainees through the cooperation of present Program Directors, site visits in connection with continuation and supplemental applications, and directing attention to the organization and content of training programs which appear successful in producing men orientated toward careers as teacher-investigators rather than as entrepreneurs. The above goal in no way rules out adding programs which appear likely to turn out highly trained men of the type desired. A secondary goal of the coming year is to gather general ideas as to the types of training programs and areas of special emphasis incorporated in those which may have special significance in developing teacher- investigators

6. Staff assignment

Dr. Jerome

SUMMARY OF COUNCIL ACTIONS
Ophthalmology Applications

Calendar Year 1958

arch

	Amt. Req.	Amt. App.	No. Req.	No. App.
ew or Revised	$47777	$30000	1	1
ontinuations	20304	20304	1	1
upplements	25800	12043	2	2
otal	$93881	$62347	4	4
% Amt. App. 66			% No. App. 100	

ine

ew or Revised	$75031	$17280	2	1
ontinuations	52828	10800	2	1
upplements	4320	0	1	0
otal	$132179	$28080	5	2
% Amt. App. 21			% No. App. 40	

ovember

ew or Revised	$ 0	$ 0	0	0
ontinuations	36320	36320	1	1
upplements	29659	25659	3	2
otal	$65979	$61979	4	3
% Amt. App. 94			% No. App. 69	

AND TOTAL	$292039	$175482	13	9
% Amt. App. 60			% No. App. 69	

1958 Annual Report

of the

National Institute of Neurological Diseases and Blindness

III. Graduate Training Grants

D. Otolaryngology

1. Program Developments

The year 1958 saw an increase in number of programs from 6 to 18. While this is not the growth projected, it still is a sizeable increase. As of December 31, 1957 the 6 programs were financed in the amount of $101,976 and the new programs established increase this total to $340,710. Present number of trainees total 116 of whom 25 are in the third year, 9 in the fourth, and 1 in the fifth. At the present rate of flow of trainees, between 20 and 30 potential teacher-investigators will be available each year. For the next few years this figure can be expected to vary considerable as new programs without an even flow participate in training. Also, for the past few years many residency programs in otolaryngology were filled mostly with foreigners. As American citizens apply in increasing numbers foreigner's applications are usually rejected by Program Directors even though the total residents on a service total fewer individuals.

2. Contributions resulting from particular awards

It will be a matter of some years before outstanding programs can be identified. Stable programs with a continuity of training may now appear to be outstanding only because there is not a sufficient number of competitive programs for valid comparison.

3. Major problems encountered

There is an insufficiency of adequately trained individuals to take over positions as Program Directors and it is predicted that this situation will continue for at least 3 to 5 years. The economic lure of life as an entrepreneur is well known in otolaryngology as the need for such specialists is extensive. As yet there is apparent wide spread misunderstanding of the goals of the Otolaryngology Training Grant Program. Some of this misunderstanding is more apparent than real. Gradually, however, the point is being driven home that the purpose of these grants is not to train private practioners.

4. Changes and improvements in program

The establishment of Clinical Audiology Programs has been approached with caution. This has been necessitated because non-medically and non-research orientated predoctoral programs in speech departments are anxious to expand into the postdoctoral training field without change in orientation. Due to ground work

[illegible]

[illegible] Additional basic science personnel is needed for membership on this Committee including a Clinical Audiologist. Selection of proper personnel of this type is of prime importance and this action is to be avoided.

[illegible] Council has also recently approved a four year stipend for trainees and all residents entering training after July 1, 1960 will be required to complete a four year residency. The fourth year stipend will have a $4,000 ceiling per depending ceiling. This is an extension of the new stipend minimum established by Council in November.

At the September meeting, the Committee voted to hold a Program Directors Conference on May 1, 1959. This will be patterned after the format of the St. Louis conference in February. Invitations will be issued to Program Directors, Potential Program Directors, selected deans, and representatives from the Armed Forces. Planning for this Conference is underway.

The Chairman of the Committee and the Executive Secretary interviewed prospective applicants for training grants during the winter meetings of the American Academy of Ophthalmology (Otolaryngology). Approximately 30 people were interviewed at this time.

During the months of July and August Dr. Lindsay [illegible] training centers in England, Scotland and Ireland. As Deputy Director, NINDB, on training facilities and will make any [illegible] to the training program. Dr. Lindsay performed a similar mission on a trip to Ireland and Scandinavia.

Program Objectives for 1959

Expansion of the training program during 1959 is anticipated. Grants is envisioned. This does not include [illegible] ed by clinical audiology program. To accomplish this [illegible] present status of available Program Directors, it will be necessary to stimulate applications from institutions where competent and [illegible] personnel are available. The projected May Conference

may be depended upon to help in achieving this goal. Outstanding trainees from grant supported programs will be encouraged to take further training as Special Trainees. Only a very few applications have come from otologists for Special Traineeships and this field needs the highly trained individual which can be expected to be developed as a result of five or six years of training.

6. Staff assignment

Dr. Jerome

SUMMARY OF COUNCIL ACTIONS
Otolaryngology Applications

Calendar Year 1958

March

	Amt. Req.	Amt. App.	No. Req.	No. App.
New or Revised	$300302	$77367	8	4
Continuations	8030	8030	1	1
Supplements	0	0	0	0
Total	$208332	$85397	9	5
% Amt. App. 28			% No. App. 56	

June

New or Revised	$ 99764	$ 42660	3	2
Continuations	0	0	0	0
Supplements	18098	9400	2	1
Total	$117862	$ 52060	5	3
% Amt. App. 44			% No. App. 60	

November

New or Revised	$61616	0	2	0
Continuations	0	0	0	0
Supplements	0	0	0	0
Total	$61616	0	2	0
% Amt. App. 0			% No. App. 2	

GRAND TOTAL	$487810	$137457	16	8
% Amt. App. 28			% No. App. 50	

1958 Annual Report

of the

National Institute of Neurological Diseases and Blindness

III. Graduate Training Grants

E. Sensory Physiology

1. Program accomplishments

Early in the development of the Neurophysiology Training Grant Program it became apparent that it was not attracting the interest of certain individuals whose main areas of endeavor were the senses. In order to interest these in training, the Sensory Physiology Training Grant Program was instituted during the latter half of 1958. This is a postdoctoral program designed to train basic scientists for highly specialized research interests. The area of the special senses needs cultivating if significant research studies are to be expected. While the main goal of the Sensory Physiology Program is the training of basic science personnel, it is not the plan of this training program to exclude M.D.'s from the program.

Nine potential training institutions have been contacted to ascertain their interests toward making grant applications. These nine include interests in audition, vision, and olfaction. At present, four applications for training programs in this area in the amount of $249,475 are being readied for review by the March Council. The University of Michigan has applied for a grant to train both in audition and vision; Florida State University to train in olfaction; University of Colorado to train in olfaction; and the Childrens Hospital Society of Los Angeles to train in audition. Applications will be reviewed by either the Otolaryngology or Ophthalmology Committee or in some cases both, before Council consideration.

2. Contributions resulting from particular awards

Not applicable

3. Major problems encountered

It has been the policy to avoid any real or apparent conflict with the development of Neurophysiology Training Grants. Where overlapping interests of potential Program Directors occur, there has been hesitancy on the part of some men to apply for grants.

4. Changes and improvements in program

Not applicable

5. Program objectives for 1959

A goal of 10 programs by the end of 1959 has been set. These programs to cover the areas of audition, vision, and olfaction (including taste).

6. Staff assignment

Dr. Jerome

IV SPECIAL TRAINEESHIPS

1. Program accomplishments

Substantial advances have been made during 1958 in the development of this program which forms the cap-stone of the Institute's total training program by providing the advanced, highly specialized and diversified training indispensable to the preparation of a competent investigator.

A total of $905,750 was awarded to 125 trainees, an increase of approximately 50% over 1957. These trainees are located in 50 institutions in the United States, Canada, Europe and South America, under the direct guidance of 79 outstanding investigators in the neurosensory field.

One hundred of these were first awards to those who had not received support previously, and 25 were for the continuation of training already supported for 1 or 2 years.

These awards are being used by investigators at all stages during their careers. The postdoctoral experience of applicants for Special Traineeships this year ranged from 3 to 21 years, with an average of 7.5 years. Although, as always, the majority of the applicants were medically trained, increased general awareness of the availability of this award to basic scientists is reflected in the 10 awards made this year to persons holding the Ph.D. degree.

Anatomists, biochemists, pharmacologists, and psychologists have been the first to apply for and receive support for advancing their knowledge and skills in the neurosensory aspects of their disciplines.

In the face of an acute need for neuropathologists, neurochemists, and those trained for research in the neurological deficits of the young, the Special Traineeship program contributed materially to training in these gap areas: 16 awards in neuropathology, 7 in neurochemistry and 18 in pediatric neurology were made in 1958, which doubled the support given in 1957 in these areas.

much of this increase reflects the expansion of the Institute's Training Grant program in these areas which provides applicants.

A comprehensive report* on the development of the Special Traineeship program, from its inception to June 30, 1958, and an analysis of its procedures and activities during this initial phase was prepared this year serving to provide detailed information for our advisers and consultants on this phase of the National Institute of Neurological Diseases and Blindness extramural program, and for use in guiding future program development.

2. Contributions resulting from particular awards

More than 60% of the Special Trainees supported to the present time are still in training status, and the remainder concluded training no longer than a year ago. For this reason, information has not yet been gathered systematically from the group, as to their subsequent activities and accomplishments. However, we do know that a large proportion of those who have completed training have been selected to fill academic posts, and that most of these are undertaking independent research programs.

3. Major problems encountered

Two major problems have been encountered this year, both relating to the review and approval of applications.

The first problem resulted from shifting the Special Traineeship applications from the National Institute of Neurological Diseases and Blindness Traineeship Review Board to the 3 Training Committees for review. When our consultants were drawn from the senior scientists of the Institute's intramural staff, meetings could be arranged as the volume of applications required. It has been found that restriction of the review of applications to the 3 meetings held yearly by each

*Report on the Special Traineeship Program from its inception to June 30, 1958; E.C. Hartman, 9/15/58.

training committee results in undue delay in acting on a considerable number of requests. It became increasingly apparent that such delays would soon seriously impair the usefulness of the program. Accordingly, arrangements were made for the chairman of each training committee to appoint 3 members to serve as an ad hoc Interim Special Traineeship Committee, which would consider applications between the regular full committee meetings, as the volume of applications necessitated. In addition to expediting action on applications, the Interim Committee could also serve an important function in coordinating program policies, as they develop in the areas of special interest represented, and in promoting mutual understanding between the three Graduate Training Committees. This procedure, still regarded as an experiment, has met with some resistance from our consultants, particularly those on the Ophthalmology and Otolaryngology Committees. This resistance stems largely from the disproportionately small number of applications in these areas as compared with those in the neurological field. The experiment is being continued, since it is believed that this procedure will prove to be to the advantage of the review process as a whole.

The second problem arose because of the increasing number of applications to go to European institutions for training. A great deal of hesitancy was felt by our consultants in recommending approval for training at institutions where facilities and conditions of training were largely unknown to them. This problem was met by arranging for the chairmen of the 3 training committees (Drs. Bordley, Braley and Sahs) and the Chief of the Extramural Programs, Dr. Seger, to visit a considerable number of European institutions where training is most frequently requested. The observations of this group are being made available to the committees, and should result in more effective review of these applications. An added dividend of these visits is an increased understanding of our program by the sponsors who were visited.

4. Changes and improvements in program

The only major change in the program this year has been an increase in the amount of funds earmarked for this program, from $1,000,000 to $1,500,000, which was deemed necessary in order to meet needs stimulated by the expansion of Institute programs in

basic science and hearing and speech fields. In addition, this increase has allowed us to meet the needs of an increasing number of trainees desiring renewal of awards to complete a program of training on which they have already embarked.

Other changes have been small ones, as required in adapting our procedures to the major changes made last year, i.e. enlarging the program to include basic scientists, and shifting of the responsibility for application review to the Training Committees.

5. Program objectives for 1959

This program will continue to be expanded as necessary and as fund availability allows to provide the highly trained investigators needed. A continuous survey of former trainees will be initiated. Questionnaires will be sent to each individual during the second, fifth, and tenth year following conclusion of traineeship support. Analysis at regular intervals of information derived from the questionnaires will serve as the basis for evaluation of the progress made by the Special Traineeship program in reaching its objectives.

6. Volume of applications

Applications reviewed:	175	$1,371,500
Applications approved:	125	905,750

Average award - $7,247

7. Staff assignments

Mrs. Hartman - Training Analyst, in immediate charge of the program, with the advice of:
Dr. Farber - Executive Secretary, Neurology Training Grant Committee for applicants in the neurological field.
Dr. Jerome - Executive Secretary, Otolaryngology and Ophthalmology Training Grant Committees for applicants in the otological and ophthalmological field.

1958 Annual Report
of the
National Institute of Neurological Diseases and Blindness

V 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 level in 1958 as in 1957.

One hundred ninety one awards for a total of $443,654 were made in 1958. Slightly more than half of the funds went to 32 Predoctoral Research Fellows, whose training was directed toward the Ph.D. degree, and 36 Postdoctoral Research Fellows for research training support during the years immediately following receipt of the Ph.D. or M.D. degree. The remaining funds were distributed on an institutional basis, and were used to support 94 students for part-time training, and 24 medical students electing to interrupt medical courses to secure a year of specialized basic science training. The individuals to be supported are selected by the institution. As the Institute's contribution to the establishment of the Foreign Fellowship program, a small portion of the fellowship funds was made available to the National Institutes of Health and used to support 5 European scientists selected in their respective countries to take a year of training in the United States.

2. Contributions resulting from particular awards

Detailed information about the activities and contributions of research fellows subsequent to completion of their support is unavailable to us at the present time.

3. Major problems encountered

The major problems encountered in administering the Research Fellowship program stemmed this year, as in the past, from the fact that control of the program operation and responsibility for it has been placed almost exclusively outside the National Institute of Neurological Diseases and Blindness. The resulting lack of Institute participation in and intimate knowledge of the Research Fellowships supported by its funds has led to difficulty in adapting the program to meet Institute program needs. In addition, there was frequently protracted delay in acting on applications.

It is hoped that certain changes initiated as of July 1, 1958 may serve as an initial step in improving this undesirable situation.

4. Changes and improvements in program

A. As of July 1, 1958 funds available for this program were increased from $525,000 to $536,000. This increase is somewhat larger than the figures indicate, since the total amount will be available for Predoctoral and Postdoctoral Fellowships; fellowships in the post-sophomore, part-time and foreign categories are being supported by the Division of General Medical Sciences recently created.

B. Since July 1, 1958, Research Fellowships have undergone a completely revised procedure for review and award, modelled in a general way on the method of Research Grant review. Applications are received by the Research Fellowship Review Branch, and assigned to the appropriate discipline panel (composed of intramural scientists) for evaluation, and to the appropriate Institute for support.

The recommendations of the Review Panels, and the priorities assigned the applications are made available to the Institute, which then advises the Research Fellowship Review Branch which are to be paid. At the present time, the three National Institute of Neurological Diseases and Blindness Training Committees (Neurology, Ophthalmology and Otolaryngology), are acting as our consultants on Research Fellowships as well as Special Traineeships.

All procedures relating to the duplication of applications and supporting credentials, preparation of applications for review, preparation of routine letters and encumbrance lists is the responsibility of the Research Fellowship Review Branch. Encumbrance lists are signed by the Chief, Extramural Programs Branch and the Director of the Institute. Letters of award and disapproval are signed by the Chief, Extramural Programs Branch.

The above procedure is still frankly experimental and subject to further modification as the needs arise.

Already some increase in direct communication between the Fellows and this Institute has occurred, as a result of the signature on the letters of award. It is inevitable that this will lead to improvement in our relations with the Fellows supported by the National Institute of Neurological Diseases and Blindness, and increased understanding on our part of the kind of people and the nature of the training the Institute is supporting.

5. Program objectives for 1959

During the coming year, the gains made during 19[illegible] in Institute participation in this program will be exploited insofar as possible. It is realized that applicability to the Institute's central mission should be interpreted in its broadest sense, and that the majority of the awards in the predoctoral area will be made for training that is not strictly categorical. However, an effort will be made in the Postdoctoral field to employ a certain number of the Research Fellowships to train those who need special skills for research in the neurosensory diseases.

6. Volume of applications.

Applications reviewed: Unknown
Applications awarded:

Regular Predoctoral and Postdoctoral Research Fellowships	68	$[illegible]
Postsophomore Fellowships	24	38,270
Part-time Fellowships	94	82,208
Foreign Fellowships	5	[illegible]
Total	191	[illegible]

7. Staff assignments

Mrs. Hartman - Training Analyst, in immediate charge of the program, with the advice of:
Dr. Farber - Executive Secretary, Neurology Training Grant Committee for applicants in the neurological field
Dr. Jerome - Executive Secretary, Otolaryngology and Ophthalmology Training Grant Committees for applicants in the otological and ophthalmological field.

ANNUAL REPORT
Calendar Year 1958
Extramural Programs Branch
National Institute of Neurological
Diseases and Blindness
National Institutes of Health

VI REVIEW AND APPROVAL OF GRANTS

1. PROJECT DESCRIPTION

This activity provides for the support of the activities of the National Advisory Council on Neurological Diseases and Blindness, the advisory training committees in neurology, ophthalmology and otology, the advisory committees reviewing applications for field investigations and pilot projects, and the professional and clerical staff engaged in the processing, analyzing and management of the grants and awards. Included within this activity are funds for the Institute's proportionate share of costs for services performed centrally by the Division of Research Grants.

The progress and problems in the management of the extramural programs are discussed in individual progress reports for I Research Grants; II Field Investigations and Pilot Projects; III Graduate Training Grants; IV Traineeships; and V Research Fellowships.

Sixth Annual Report of Clinical Investigations of the National Institute of Neurological Diseases and Blindness

1958

The Clinical Director's Report

The Sixth Annual Report of the Clinical Investigations Unit of the National Institute of Neurological Diseases and Blindness includes 94 projects, and 73 publications either published or in press. Some of such publications obviously reflect results obtained from previous years. Six-hundred and six patients were admitted, for a total of 19,278 patient days. This is an increase of total patients of 81, but a decrease in patient days of approximately 2,000. This reflects, in part, the tumor program utilizing radioactive isotopes, in which patients were admitted but for 48 hours.

Two investigators were lost through death during the past year: Dr. J. Godwin Greenfield died of a heart attack on March 2, 1958, during his third stay at the National Institute of Neurological Diseases and Blindness, and Dr. Francis Enomoto met an accidental death, also during the first part of the year. The Institute also lost two of its senior investigators: Dr. Kenneth Hall accepted a post as Associate Professor of Anesthesiology at Duke University, in charge of research, and Dr. Jose del Castillo, Chief of the Section of Clinical Neurophysiology, will undertake his new position as Associate Professor of Neuropharmacology at the University of Puerto Rico. Clinical Investigations gained one senior investigator in Dr. Herbert Lansdell, who has taken over the Section of Psychology.

As always, the Unit has benefited by visiting scientists and guest workers from Abroad. From England were two senior investigators -- Dr. Greenfield, and now Dr. Tansley from the Institute of Ophthalmology in London; from Japan, Dr. Kyoji Tasaki and Dr. Enomoto; from India Dr. Lele; from Austria, another senior scientist Dr. Dodt; from Sweden, a senior scientist Dr. Widen; from Australia Dr. Strang; from Canada, Dr. Wherrett; from Spain, Dr. Miquel; from France, Dr. Gerin; and from Mexico, Dr. Ortiz. Drs. Tansley, Lele, Dodt, and Tasaki were or are attached to the Branch of Ophthalmology; Drs. Ortiz and Miquel, to the Branch of Neurosurgery; Drs. Widen, Gerin and Enomoto, to the Branch of Electroencephalography and Neurophysiology; and Drs. Greenfield and Wherrett, to the Branch of Medical Neurology. These are visiting scientists

in the true name, and they are with the Institute for a period of six months to two years, and will be returning to their native countries. 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 Sections of Electroencephalography and Clinical Neurophysiology, report the following projects: Under the combined Branch of Electroencephalography and Clinical Neurophysiology:

Dr. Enomoto, before his untimely death, was working with Dr. Ajmone-Marsan on the epileptic activation of unitary elements of the cat's cerebral cortex, and their relationship to the EEG discharge. In this particular study, epileptic foci were produced experimentally on the suprasylvian gyrus of the cat by means of local application of different convulsive drugs. The development of the slow EEG discharges was monitored from a routine surface electrode and upon their appearance a survey was then undertaken on the various units within the different layers of the nearby cortex by means of tungsten microelectrodes. The investigators report several thousand observations on such units analyzed in 29 experiments carried out on cat. They noted that such unit behavior occurring in coincidence with an EEG discharge was characterized by a paroxysmal appearance of high frequency bursts, plus a marked tendency towards synchronization of different units. This property, however, was not absolute in that a given unit may be characterized by rhythmical high frequency firing in "resting" condition, in which case it tends to become inactivated in coincidence with the EEG discharge. They feel that, in the latter such case, the unit recorded is very likely injured. Dr. Enomoto and Dr. Ajmone-Marsan concluded, from these observations, that probably the number of units activated, in a certain instant; their firing pattern, their location, and temporal inter-relationship are at least closely related to, if not even responsible for, the final shape, amplitude, and polarity of the slow EEG event.

Still using the tungsten microelectrode, Dr. Ajmone-Marsan and Dr. Widen have recently embarked upon a study of the relationship of the cortical unitary elements to slow surface responses evoked in the visual cortex, after stimulation of the lateral geniculate nucleus. This problem has just been initiated and as yet these investigators do not report definitive results.

Dr. Gordon Long, in the same laboratory, has finished a complete study on the modification of sensory mechanisms by subcortical structures, in which he has attempted to elucidate the effects which the brain stem reticular formation, the non-specific thalamic system, and other subcortical structures (basal ganglia, thalamic associative nuclei, rhinencephalic formations) may have upon cortically evoked potentials from peripheral sensory stimulation. Dr. Long reports his experiments on some 55 cats. The subcortical structures were localized stereotaxically and histologically controlled. Varying parameters of conditioning stimuli were used. Dr. Long found that in the unanesthetized preparation he could more easily modify somatic and visual potentials, and that this effect was more prolonged when the conditioning stimuli were applied in the reticular formation of the brain stem. In decreasing order, the same was found to be true of non-specific thalamic system, the amygdala, the putamen, the globus pallidus, and the lateral half of the head of the caudate nucleus. Stimulation of the pulvinar-lateralis posterior complex of the thalamus produced some modification of visual response only. The changes recorded in the evoked potentials were more marked at the cortical level than they were at the specific thalamic relay nuclei. Thus, Dr. Long confirmed previous impressions that high frequency stimulation of the reticular formation will depress the amplitude of evoked somatic and visual responses, and that this depression was more consistent and of longer duration than the somato-sensory system. In addition, however, it was shown that lower frequencies of stimulation to the reticular formation and its projections will augment the amplitude of the evoked visual and somatic responses. Both modifications of the sensory potentials were abolished or diminished by barbiturate anesthesia. Dr. Long suggests an analogy between the reciprocal effect of augmentation and depression of afferent conduction, and the facilitation and inhibition of motor responses by the reticular formation.

Dr. Abraham and Dr. Richards, in Dr. Ajmone-Marsan's laboratory, after noting certain characteristics in the EEG of patients receiving steroids, undertook a study of the EEG changes induced with photic stimulation in patients treated with ACTH and adrenal corticoids. Eighty such patients were reviewed; 9 of these 80 showed an unusually marked response to photic stimulation. This places the occurrence of such responses in the group study at 11.2 percent; however, if one takes a population of non-selected subjects, such an instance is only 1.3 percent. These investigators concluded that ACTH and adrenal corticoids contribute to the lowering of the convulsive threshold, probably acting at the brain stem level.

upon probably already abnormal structures, and the abnormal reaction to intermittent photic stimulation is, in fact, a manifestation of such a convulsive tendency. They also indicate that such a convulsive tendency may be in fact dependent upon the disease state, in that a majority of their patients had either lupus erythematosus or lymphatic leukemia, either of which may have, at some time during their course, central nervous system lesions.

Dr. Ajmone-Marsan, Dr. Abraham and Dr. Van Buren have continued their studies of depth electrography in seizure patients, and have reported their first findings in a temporal lobe epilepsy monograph by C. C. Thomas. As noted in the 1957 report, these investigators found the metrazol activation of extreme importance in such indwelling electrodes, and found many cases in which epileptic discharges may be present at a cortical level but would not be recorded with the routine EEG. On the other hand, a certain number of discharges will, instead, be recorded from the routine EEG, and their amplitude appears to be directly proportional to that of the cortical discharges. But another group demonstrated amplitude which is definitely not related to that of the original discharge, and may vary from as high as 58 to 1, to as low as 2 to 1. Such amplitude and extent of area covered by the discharge, however, were not the only factors responsible for the presence or absence of spread to the scalp, and spikes do not appear to project more easily or more constantly than the paroxysmal waves. A complete analyses of this report may be seen in the reference listed above.

Dr. Ajmone-Marsan and Dr. Baldwin are continuing their observations on temporal lobe epilepsy and they have reported the electrocorticographic findings in a large series, again in the monograph by C. C. Thomas, during the past year. The procedure and ultimate course of this project follows the lines reported in the 1957 annual report.

Dr. Ajmone-Marsan is also still continuing, with the cooperation of Dr. Abraham, the atlas of seizure patterns accompanying Metrazol activation, and the correlation between such movements and behavior with simultaneous electroencephalograms. The publication of this atlas has been undertaken by the Journal of EEG and Clinical Neurophysiology. For the latter Journal, Dr. Ajmone-Marsan and Dr. Henry have also undertaken a bibliography covering the last ten years, from 1948 to 1958.

In addition to this productive research, the Unit has carried on a large service function in which a total of 1,502 electroencephalograms were completed. Many of these were from other Institutes - thus, the National Cancer Institute accounted for 306; 23 electrocorticographic studies were performed on neurosurgical patients in the operating room, and extensive EEG studies were carried out on many patients with indwelling cortical electrodes.

The Section of Neurophysiology has been closely aligned with the Branch of Biophysics of the Basic Unit (NIMDB), with Dr. Kenneth Cole. One of Dr. del Castillo's largest projects was in the study of excitation in medullated nerve, in that the results previously obtained in the giant axons cannot be applied indiscriminately to the medullated fibers of vertebrates or man. The extremely small surface area of the nodes of Ranvier makes, in many ways, myelinic fibers particularly appropriate for exploration of certain aspects of nerve excitation, which could not be resolved when the whole axon is studied. Counteracting this, however, is the difficulty of isolated single nodes of Ranvier. The study was a combination of an improved technique to perform voltage clamp experiments in the membranes of the nodes of Ranvier, and to combine this with the electronic resistance multiplier method of Frankenhauser. These investigators found original difficulties due to the high longitudinal impedence of the inner nodes through which the controlling ionic currents are injected. These were overcome eventually by the resistance multiplier method, which was also adapted in these experiments to minimize the external leaks of the controlling current projected into the interior of the clamped node. Dr. del Castillo and Mr. Moore found that depolarization of a mammalian nodal membrane conforms to pattern similar to those found, and thoroughly analyzed, by previous investigators in invertebrate material. They made an important incidental observation, however, that when a medullated nerve fiber is sectioned, the cut end of the myelin tube tends to close in such a way that the leak of axoplasm is minimal and a high electrical resistance is maintained. Thus, one has a basis for providing an artificial single node of Ranvier. Dr. del Castillo and Moore have reported in a paper the technical methods utilized in this procedure, entitled "An Electronic Electrode", which is to be presented at the 1959 National Convention of Institute of Radio Engineers. In the future, these investigators wish to study the mechanism by which certain organic cations, such as hydrazinium ions may replace sodium ions in the excitable mechanism of the membrane.

Dr. del Castillo's other project pertains to the mechanism of transmitter liberation at presynaptic nerve endings, in which intracellular capillary microelectrodes will be used to record potential changes at the endplate membrane of nerve to muscle, or of synaptic potentials. Ionophoretic methods will also be utilized to apply substances to the localized spots of the nerve ending. In their initial studies, these investigators found that the depolarization of the post-synaptic membrane produced by externally applied acetyl-choline is markedly influenced by the pH of the extracellular solution. This project will, in essence, place at intracellular level some of the findings reported by Dr. Irwin in the Section of Neuropharmacology.

In the Section of Neurological Disorders, a new investigation as to the medical treatment of seizures has been undertaken, which is dependent upon the findings of Brody and his colleagues in the National Heart Institute, of new monoamine oxidase inhibitors. Monoamine oxidase is the primary enzyme necessary for the break-down of 5-hydroxy-tryptamine to the 5-hydroxy-indoles, the most important of which is 5-hydroxy-indoleacetic acid. The formation of the sulphate ester of this group in the urine has already been associated with a neurological disorder characterized by cerebellar symptamotology, dermitis, and mental retardation, under the name of Jepson's disease. Brody and his colleagues have found that the utilization of monoamine oxidase inhibitors in animals markedly reduces the epileptogenic threshold. Thus a double blind procedure has been instituted in which patients with centrencephalic seizures, having as many as 50 or more attacks per day, have been admitted, and a double blind procedure initiated, using the new monoamine oxidase inhibitor JB-516. As this is a double blind procedure, the results will not be known for approximately six months' time. At the present time, ten patients have entered into this project. This project is being carried out by Dr. Bushnell Smith and Dr. Darwin Prockop.

It has been noted that cases of orthrostatic hypotension have been noted to have many neurological disorders, in particular loss of sweating, loss of external sphincter controls, impotence, mental dulling, and, in some cases, a Parkinsonism-like syndrome, with or without ciliary atrophy. Three such patients have now been studied and one such patient has come to post mortem. To date no thorough anatomical study has ever been accomplished on a patient dying from orthrostatic hypotension. The importance of this single case, hence, is not to be underestimated. It was the decision that serial sections

should be accomplished through the hypothalamus, sympathetic ganglia, the intermediate cell columns, and the cranial nerve nuclei of III, V, VII, IX, and X, as well as the basal ganglia, anterior horn cells, and cortex. This necessitates literally thousands of sections, and the strict correlation of anatomy and pathology. It is anticipated that the thorough study of this one post mortem case will need, in time, investigative use of a neuroanatomist for at least six months. This is being undertaken, at the present time, by Drs. Drager and Shy, and to date important findings have already been found in intermediolateral cell columns, the ventral cell columns, in Clarke's column, the dorsal nucleus of the vagus, the ventricular gray, and in the inferior olives. Degenerative changes in the cerebellum were also found with many torpedos. There were marked degenerative changes in the substantia nigra and in the mesencephalic nucleus of the trigeminal, as well as in the larger cells of the corpus striatum and the pyramidal cells of the cortex.

Similar to this is the study of a new syndrome recently described with rapid central nervous system deterioration, central blindness, myoclonus, and death in approximately three to four months. Here again, a long-term anatomical and pathological correlative study is being undertaken, with serial sections. This case will be studied extensively by Drs. Drager and Bushnell Smith.

As in past years, many of the patients admitted to the Branch of Medical Neurology are suffering from diseases of the motor unit. Recent advances in isotopic procedures and muscle pathology have changed radically this program from the past year. In combination with the Association of Research in Nervous and Mental Diseases, this Institute undertook, during the past year, a review of the effects of metabolic and endocrine abnormalities upon diseases of striated muscle. This was an over-all survey, employing chemical studies of muscle biopsies, combined with various metabolic tests with gonadotrophin, corticoids, ketosteroids, TSH, etc. Of particular interest were two disorders: Familial Periodic Paralysis and so-called McArdle's glycogen disease of muscle. In the former disorder, aldosterone levels were determined by the double isotope derivative methods. Intracellular cations on muscle removed both before and during attacks were also studied, as was pathology before and during attacks. And finally, microelectrode recordings of single muscle fibers in vivo before and during attacks. Potassium turn-overs were also studied in this disease. The pathol

of this disorder was quite striking in that large accumulations of fluid appeared intracellularly in approximately one-third of the fibers. Chemical determinations showed that, in spite of this accumulation of fluid, the cationic concentration of the cell remained approximately within normal limits. This was confirmed by microelectrode recordings, which showed a resting potential of 71.2 ± 11.3, which is what might be anticipated if intracellular potassium were at normal levels. Studies on aldosterone on these particular patients revealed that there was no increase preceding the attack, as previously reported by Conn. There was also a decrease in potassium in the urine preceding the attack, which indirectly confirms the latter observation done on double isotope derivative methods, in that if there had been aldosterone excretion there should have been a potassium diuresis.

Twenty-three cases of infantile neuromuscular disorders associated with hypotonia were also studied in reference to pathology, electromyography, and clinical course. From this, five different types of disorders were found in the disease state, which have been recently grouped into but one disorder. These findings have been reported by Drs. Greenfield, Cornman, and Shy, in the December issue of Brain.

The recent findings that DNA is probably inert in non-proliferating cells is now leading to the utilization of tritium labelled thymidine. This will be a powerful tool in the study of regeneration and growth of muscle, and this, combined with electron microscopy, will be undertaken by the Section of Biophysics.

Dr. Haase has, in addition, undertaken a long-range study of the pathological findings of intramuscular motor and sensory nerve endings in normal and in neuromuscular disease states, using the Coers technique of intravital methylene blue staining. He has confirmed axonal regeneration in neurogenic diseases, but the other abnormalities described by Coers and Wolfe have, as yet not been verified.

The Section of Biophysics has completed its investigations on the localization of cerebral neoplasia by collimating techniques, utilizing various isotopes. Over 200 such patients now have been studied, with a confirmed accuracy of 86.2 percent. The final techniques and instrumentation utilized in this study, as well as the statistical evaluation, have been reported in monograph form by E. & S. Livingstone. This monograph was also utilized at the International Conference for

Peaceful Use of the Atom. Similar procedures have now been initiated by the Institute for the Johns Hopkins University, the National Navy Medical Center, Oak Ridge National Laboratories, and now at Los Alamos.

The studies of microelectrode recording in single muscle fibers has been utilized in familial periodic paralysis and myasthenia gravis. Due to the scarcity of the first disorder, this was done by cut-down method. In the myasthenic patients, continuing attempts are made to record single muscle fibers through the intact epidermis. The Bak Unity Gain Amplifier has been utilized as optimal with a constant current sent back into the grid of the cathode follower. This latter allows constant sampling of the condition of the probing electrode. To date, endomysium and perimysial connective tissue has been the chief stumbling-block, in that the electrodes intermittently plug or break. Of the literally hundreds of recordings which have been attempted to date, only 5 successful intracellular penetrations have been made through the intact epidermis. The continuity of this project will depend upon the ability to overcome the difficult techniques listed above.

In the Clinical Director's Report each year, an attempt has been made to select areas of outstanding contribution. This year the studies conducted in the laboratory of clinically applied pharmacology, under Dr. Richard Irwin, has accomplished much which will show considerable insight as to the interrelationship of blood and tissue cholinesterase systems, their substrata, other enzyme systems working upon such substrata, and basic fundamental knowledge as to the differentiation between depolarizing and competitive blocks, as well as insight as to where in the muscle fiber the blockading compound has its maximal effect. Thus, Dr. Irwin and his colleagues have demonstrated that competitive blocking compounds, such as d-tubocurarine and depolarizing blocking compounds such as decamethonium, may be differentiated in their action by inhibition or excitation of muscle cholinesterase; thus, the competitive block of d-tubocurarine is reduced or prevented by inhibition of muscle cholinesterase. On the other hand, the block of depolarizing drugs is prolonged by the inhibition of plasma cholinesterase or muscle cholinesterase. In the case of decamethonium, this cannot be due to destruction by cholinesterase, per se, as decamethonium has no ester group and hence could not be destroyed by cholinesterase. Succinylcholine, on the other hand, has an ester group, and thus could be destroyed by cholinesterase. It is of interest,

however, that the prolongation of the blockade by inhibition of plasma cholinesterase is identical to the two substances, thus showing that this inhibition prolongation is not of necessity due to destruction, or the depolarizing compound. Thus one can assume, I believe correctly, as Dr. Irwin and his colleagues have assumed, that muscle cholinesterase has but a minor role in relation to the total block. If this substance, however, is not metabolized by plasma cholinesterase, then inhibition of muscle cholinesterase has a marked effect on the blocking activity, and the non-depolarizing substances upon such inhibition of muscle choline demonstrate a decrease in their blocking power, whereas the depolarizing substances demonstrate an increase in their blocking power.

Dr. Irwin and his group have continued their studies on the action of directly stimulated innervated and denervated muscle. In this they have been aided by a device, created by Mr. Wells, of an optical-isotonic lever system, recorded through a cathode ray oscilloscope. With this mechanism, they have been able to demonstrate that the block is not due to increased muscle compliance, as added compliance in series does not give contractile responses similar to those obtained with succinylcholine or decamethonium. If this isotonic system is observed closely, one may see there is less shortening of the fiber and reduced velocity of shortening, again showing that this is not an increased compliance of the muscle fiber. The isotonic-optical system allows this, in fact that it reduces the elastic component of muscle. This system, however, does demonstrate a prolonged latency from the onset of the stimulus to the time of contraction after administration of depolarizing compounds. These investigators feel there is a spatial distribution of the depolarizing blockade over the muscle membrane, indicating either multiple end plates upon the muscle membrane, or a temporal spread from a single membrane, i.e. one end plate. These investigators point out that muscle cholinesterase is low in quantity and is not uniform in various species and/or organs, and hence has a species and organ specificity. It is thus dependent upon the substrate and enzyme activity. Thus, muscle cholinesterase studied as to substrate specificity and well-known inhibitors would give considerable information as to the chemical interchange between the substrate and the enzyme.

The cholinesterase of muscle homogenates, in which the blood was removed so the plasma cholinesterase was not present, was studied. Such homogenates hydrolyzed acetylcholine more rapidly than benzoylcholine, or butrylcholine. An excess of the substrate, however, would inhibit

such hydrolyzes, the optimal level being 5×10^{-3}. The optimum level of concentration for substrates other than acetylcholine are higher. Thus, muscle cholinesterase is highly specific. However, since benzoyl- and butrylcholine are hydrolyzed at measurable rates, small amounts of non-specific enzyme must also be present. It is of interest that neostigmine depolarizes the membrane at 10^{-3}, whereas pyridostigmine (mestinon) will not. This becomes of double interest in that both drugs are highly useful in the treatment of myasthenia gravis. Galanthamine, which has been isolated from an alkaloid in the United Soviet Socialist Republic, and utilized in the treatment of myasthenia gravis, was also studied by these investigators. Galanthamine is a phenanthrene derivative and not a carbamine ester. Dr. Irwin and his group found a 50 percent inhibition at 6×10^{-6}. The value for the inhibition of plasma cholinesterase was the same. Neostigmine and physostigmine inhibit at lower concentrations as far as cholinesterase in the muscle is concerned, but in vitro inhibit more rapidly than with galanthamine.

Finally these investigators are studying the possibility of choline esters other than acetylcholine occurring as natural constituents of biological systems; the object being to determine to what extent the choline esters are found in such biological systems and related compounds, and how they depolarize tissue membrane. Secondly, to relate the depolarizing properties of these compounds to their stimulation or blocking activity of synapses, and finally to study the metabolism of these compounds by tissue enzymes. To study this, the travelling fluid electrode technique is used to measure depolarization of the isolated frog sartorius muscles, and microelectrodes will be utilized to determine the resting membrane potentials, presumably through the Bak Unity Gain Cathode Follower. These investigators have found, in high concentrations, i.e. 10^{-3} molar, that butrylcholine, benzoylcholine, and imidazoleacrylcholine, all resemble acetylcholine in their depolarizing properties. Methacholine, however, does not depolarize muscle membrane. These investigators have also found the plasma from myasthenic patients have been observed to metabolize imidazoleacrylcholine at the same rate as plasma from non-myasthenic patients. And finally, these investigators are attempting to find to what extent depolarization of the muscle membrane may effect the efflux of enzymes from inside the muscle fiber, in particular aldolase. This latter project is projected into the coming year.

The Section of Neuroradiology suffered in having its chief investigator, Dr. Giovanni Di Chiro, undergo surgery for a major illness. In spite of this setback,

however, Dr. Di Chiro was able, upon his return to duty, in addition to his heavy service responsibilities, to carry out, in combination with Dr. Martin Rubin of Georgetown University, a research project which culminated in a paper concerning the metal chelates as possible contrast media for myelography. These chelating compounds were tested against commonly used iodinated contrast media. Different concentrations of the various chelating compounds were tested in order to determine the concentration for optimal opacity. Once such opacity was determined in vitro, it was tested in vivo on dogs and rabbits. Chelating agents used are listed in Dr. Di Chiro's report, with primary interest on lead ethylenediaminetetraacetic acid. This substance was administered at the dose level of 10 milligrams per kilo, and appeared in the urine to the extent of 85-89 percent of the injected dose within two days. Of that retained in the animal, i.e. 10-15 percent, 50 percent was found in the liver and some 20 percent in the bone marrow. This demonstrated that, despite the large amount of excretion, the amount retained is not to be discounted. The experiments in vivo show that studies of good diagnostic quality may be obtained as far as X-ray contrast and detail are concerned, with radiopaque metal chelates. However, the acute toxicity of the metal chelates in myelography, as well as in most of the other X-ray examinations carried out, proved to be too high. Accordingly, Drs. Di Chiro and Rubin are going on to undertake studies in other metal chelates with high atomic number, in hope that in this screening one agent of local toxicity would be found which was so low as to suggest it could be used in clinical myelography.

The Section of Neurochemistry continued its efforts in the major fields listed in the 1957 report. Dr. Horvath continued his studies in the distribution of actin and tropomyosin in normal and diseased muscle, his comparative biochemistry studies of smooth muscle and striated muscle, and alterations of actomyosin tensile strength and muscle proteins in neuromuscular diseases.

Dr. Tower and his colleagues have continued their studies on the metabolism of γ-aminobutyric acid in neural tissue, with the aid of Dr. McKhann and Dr. Wherrett. Studies on the relation of pyridoxine to certain seizure states, in particular in those cases known as pyridoxine dependency continued. Dr. Tower Continued his elaborate studies on amino acid metabolism in normal and epileptogenic cerebral cortex in vitro, and in electrolyte energy metabolism in normal and epileptogenic cerebral cortex. The unit as a whole continued its clinical evaluation of amino acids and related compounds in control of seizures in man.

Dr. Curtis continued in the realm, predominantly of surface-chemistry, and in other physico-chemical methods in determining constituents of human spinal fluid, ocular fluid, etc.

Dr. Tower's studies specifically now revolve around C^{14} and N^{15} labelled compounds. Two-deoxyglucose was utilized as a competitor for glucose utilization, by inhibiting the hexokinase step primarily due to depletion of available ATP required for this step. Dr. Tower found it was possible to overcome the 2-deoxyglucose block in glucose utilization by adding either ATP or glucose-6-phosphate to the slices in anaerobic conditions. No effect of these additions, however, was obtained in aerobic metabolism, presumably due to their failure to penetrate the slices. Dr. Tower felt that 2-deoxyglucose inhibition did not result in any activation of the glucose-6-phosphate dehydrogenase or in any oxidative shunt pathway. These findings were checked by incubating the control and inhibited slices with glucose-1-C^{14} and glucose-6-C^{14} phosphate, determining the utilization of $C^{14}O_2$ and C^{14}-lactic acid production. Since the ratios of the C^{14} lactate from the C-6 compared to C-1 samples were 1.0 in both cases, whereas C-6/C-1 would be less than 1.0 if the shunt pathway were utilized, this would indicate that this inhibition was not due to an oxidative shunt pathway. This was indirectly confirmed by the finding of low level brain TPN by other investigators in that TPN is the necessary coenzyme for the shunt pathway. Dr. Tower found also that 2-deoxyglucose inhibition not only resulted in marked decrease in glycolysis, but also in oxidative metabolism. Thus, with glucose-U-C^{14}, less $C^{14}O_2$, less labelling of the free amino acid pool, and less C^{14} lactic-acid were all obtained. From these studies with C^{14}-labelled glucose, the distribution of glucose utilized by normal slices to various intermediary steps could be estimated, thus glycolysis to lactate, 70 percent; amino acids measured by glutamate, 22 percent; respiratory CO_2, 7 percent, and other intermediaries, such as lipid and protein, 1 percent. If one calculates the oxygen uptake as 85μM./g/hr., it is clear that if 30 percent of the latter is accounted for as amino acid and respiratory CO_2, this almost exactly balances the oxygen uptake, assuming 6 moles of the latter per mole of glucose oxidized. This is consistent with studies of other laboratories, and this laboratory, that non-glucose substrates, such as amino acids, normally support oxidative metabolism by the brain and they are repleted subsequently by part of the glucose utilized. Studies with

2-deoxyglucose clearly demonstrate, according to Dr. Tower, that glucose is necessary to make repletion of non-glucose intermediates possible, and energy production rapidly falls in its absence, and that this is not only by depletion of ATP and creatine phosphate, but also by deleterious effects on glutamic acid and electrolytes in the inhibited slices. As in his 1957 report, Dr. Tower points out that such inhibited slices fail to extrude excess sodium and reconcentrate potassium in normal manner. This is similar also to defects seen in slices which have been removed from epileptogenic patients, and Dr. Tower has also found this in cortical slices from cats with seizures enduced by 3-methyl-3-ethylglutarimide, and by methionine sulfoximine. Utilizing the Cotlove apparatus, Dr. Tower and his colleagues find that the swelling of normal and epileptogenic slices during incubation is confined to the chloride space, and that calculations of electrolyte concentration per litre of non-chloride space water at the end of slice incubation demonstrates again a loss of potassium and a gain of sodium.

Dr. Tower has continued his studies on incubating slices of cat cerebral cortex with L-glutamic acid labelled with C^{14}; L-glutamine labelled with C^{14}; γ-aminobutyric acid labelled with C^{14}; L-aspartic acid labelled with C^{14}; D-L-asparagine labelled with 2,3-C^{14}; D-glucose labelled with C^{14}; Sodium Pyruvate-3-C^{14}, and 2-pyrrolidinone-2-C^{14}. Using these compounds, Dr. Tower was able to determine the order of labelling in amino acids, and was able to show this had considerable significance since the aspartic acid could prime the Krebs cycle by providing both oxalacetate and acetyl-Coenzyme A (from pyruvate) in the absence of the latter from glycolysis. Dr. Tower concludes that these studies indicate how active the components of the glutamate-aspartate amino acid group are in metabolic participation in the Krebs cycle, and feels that the release of CO_2 measured by C^{14} liberated during these experiments confirmed this conclusion.

In the second part of his experiment, Dr. Tower analyzed the liberation and formation of glutamic acid, glutamine, γ-aminobutyric acid and free ammonia metabolism in incubated slices from non-cortical areas of the cat brain; these were the sub-cortical white matter, the thalamus, the caudate nucleus, and the cerebellar cortex. He found the levels and metabolic behavior in all gray, i.e. neuronal areas, were similar to that previously observed in the cortex, but that the white matter

exhibited extremely low levels and little change on incubation for glutamic and γ-aminobutyric acids, while the white matter glutamine was not greatly different from the cerebral cortex. In studies on the levels of these substances, Dr. Tower felt, using the calculations of Elliott and Heller, that at least 85 percent of cortical glutamic and γ-aminobutyric acid content was associated with neurons, while only about 5 percent of the glutamine appeared to be neuronal in location. The cerebral cortex was fractionated by the Brody and Bain technique, and Dr. Tower found the majority of glutamic and γ-aminobutyric acids were associated with fraction R_3 or the mitochondrial fraction, whereas glutamine was distributed almost equally between that fraction and the combined R_1 + R_2 fractions which contained cell debris, axon fragments, nuclei, etc. No content of any of the three amino acids was found in the microsomal fraction. The finding of these substances in the mitochondrial fraction is compatible with their close association with the Krebs cycle.

Dr. Tower's studies also indicated that the inhibition of glutamine synthesis by methionine sulfoximine is primarily an interference with ammonia moiety, possibly by the imine group of the toxic compound, and that by adding only ammonium chloride such a block could not be overcome unless adequate amounts of glutamic acid are available to amidate to glutamine. Studies with similar epileptic agents, such as Megimide showed that the glutamic acid metabolism was blocked to include γ-aminobutyric acid, in that the latter compound was significantly lower than normal. The same was true when inactivators of pyridoxal phosphate were used. If malonate however, was used, the amount of glutamic acid and γ-aminobutyric acid in the slices rose to double the normal values. The action of malonate is to inhibit succinic dehydrogenase. Since this was accompanied by reduction of oxygen uptake, it was previously not clear why such slices did not also show succinate accumulation. Dr. Tower's data suggest that in the whole cell preparation it is glutamate and γ-aminobutyrate rather than succinate which accumulates and requires a study of the relationships among these three components of Krebs cycle. Dr. Tower plans to continue these interesting experiments, using the microanalytical method of Dr. O. H. Lowry.

Clinical evaluation of various amino acids and related compounds in the control of seizures in vivo in man has been continued by Dr. Tower and Dr. McKhann, and the Branch of Electroencephalography. Patients on γ-aminobutyric acid have continued to do well in Dr. Tower's estimation, one patient being seizure-free after three

three months on the compound, compared to multiple daily seizures previously. On stopping the compound, the seizures returned and have again been abolished by starting γ-aminobutyric acid. Several other patients are getting more benefit from γ-aminobutyric acid than from l-asparagine. Gamma-aminobutyric acid has been given intravenously to levels of 4 mM/kg. body weight, with no untoward effects, in dogs. However, when 1/200 of this dose is administered to man, there is immediate agitation, flushing, hyperpnea, and a drop in diastolic blood pressure. Recovery occurred within 5-10 minutes. Dr. Tower rightly points out, despite the reports by Elliott that such occurrences can be ignored, it would seem that this potentially is a dangerous drug given intravenously. Another case of pyridoxine dependency has been worked up by Dr. McKhann and Dr. Tower. These patients were also studied by the Krypton^{85} technique for measuring cerebral metabolism developed by Sokoloff. The original case of Hunt was restudied, and the patient now 7 years old is still dependent, regularly developing seizures within 72 hours of omission of her regular daily dose of 10 mg. of pyridoxine. Typical EEG abnormalities could be abolished in 30-60 seconds by intravenous pyridoxine-HCl (15 mg.). During a typical period of depletion, cerebral metabolism was measured by Krypton^{85} technique, and the decreased oxygen consumption during the depleted state in this case was similar to the situation reported by Sokoloff for hypoglycemia subjects. Thus, the interpretation tentatively put upon the data obtained in this case is that during pyridoxine depletion a deficiency of the substrate for cerebral oxidative metabolism exists which is promptly corrected by pyridoxine administration. Since pyridoxine deficiency affects γ-aminobutyric acid metabolism primarily, and since that compound appears to be a significant substrate, Dr. McKhann and Dr. Tower rationalize that this case may actually represent an example of γ-aminobutyric acid deficiency, with a consequent reduction in oxidative metabolism. Drs. McKhann and Tower have continued their studies of the metabolism of γ-aminobutyric acid in neural tissue by using the Fluorimetric method, as described in the 1957 report. They appear to have demonstrated that the shunt pathway, i.e. glutamate to γ-aminobutyrate appears to be active and important in cerebral oxidative metabolism, and is significantly involved in certain dysfunctions of the brain, such as seizures. They plan to undertake further studies to see how such a pathway may exert a regulatory control on oxidative metabolism and hence on energy production in terms of normal function and of seizure states.

Dr. Curtis is continuing his studies on physico-chemical methodology in an attempt to obtain quantitative data from fluids which contain extremely small amounts of organic metabolites. He is working particularly on the surface tension of urine, and in particular optical measurements by polarized light and its reflection off of surfaces utilizing the elliptical polarization as an indication of the thickness of the surface interface. The apparatus has been built in combination with the Naval Research Laboratories and exploration of this approach is now being orientated towards the use of photomultipliers, so that the square function may be utilized, and monochromatic light. Parallel with this he is continuing his studies of adsorption on solid surfaces, such as column resins, in foams and interfaces in urine and water-immiscible liquids. Dr. Curtis has now found that there is so much gross interference in the acetylcholine-boron-flavonaol reaction to biological materials as to make this procedure unsuccessful, in the determination of microchemical amounts of acetylcholine. His studies on guinea pig serum asparaginase, detailed in 1957, have now been completed, except for some electrophoretic and ultracentrifuge data now in progress. He finds that the purified enzyme preparation contains two macromolecular contaminants which have defied attempts at separation by electrophoresis or ultracentrifugal means; that enzyme can be quantitatively adsorbed on a modified cellulose and in carbon dioxide foam, and purification by these means is currently being attempted.

Dr. Horvath is continuing his work on proteins of muscle in normal and diseased states, and has calculated total solids, total protein, non-protein solids (i.e. fat), non-collagenous proteins, collagen, water-soluble proteins, myosin, alkali-soluble proteins, non-protein nitrogen, electrolytes, and tissue water. He finds differences in the normal and dystrophic muscle analyses are reflected by connective tissue and fat, and by an increase in sodium and chloride in dystrophic specimens. He finds there is a relative increase of myosin and decrease in alkali-soluble proteins in most cases. These changes seem to be independent of the remaining muscle mass. The water-soluble proteins appear to be increased relative to other proteins in most dystrophic samples and an inverse relationship is indicated between the remaining muscle mass and the percentage of water-soluble proteins in the muscle on the other hand. He concludes that samples of dystrophic muscle not only contain less muscle and more connective tissue and fat than normal muscle, but that the protein composition of the remaining muscle is different from the normal.

In the study of actin and tropomyosin in normal and diseased muscle, Dr. Klatzo and Dr. Horvath are turning to immunological properties of functionally important muscle proteins. They find that rabbits immunized against serum tropomyosin A, clam tropomyosin A, mammalian myosin, and antisera to human and cat myosin precipitate clam tropomyosin A. No such cross-reaction was found between antisera to chick tropomyosin B on the one hand and clam tropomyosin A or mammalian myosins on the other. Using antibodies to myosin conjugated with fluorescein, myosin in sections of normal human muscle was clearly and distinctly demonstrated under the fluorescent microscope. Preliminary sections of dystrophic muscle similarly treated showed myosin in residual islands of muscle and a suggestion that in areas of active degeneration myosin-reactive material was present in macrophages. Thus the immunological findings are consistent with the present concepts of the myosin molecule consisting of subunits - tropomyosin A, B and actin, the latter can be prepared in a higher state of purity than myosin itself, so that it is more suitable for investigational purposes. Since these proteins are also iso-antigenic, the immunological response 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 circulation of the body. Dr. Horvath is continuing the same studies in muscle protein and electrolytes in dystrophic mice obtained from Bar Harbor.

Dr. Korengold and Dr. Hampp have concluded their studies, which were an attempt to confirm the findings of spirochetes in the cerebral spinal fluid, with patients suffering from multiple sclerosis. Identical material to that used by Ichelson was utilized, and a trip was made to Dr. Ichelson's laboratory to be certain that there were no differences. Twenty-two patients were studied in the outpatient area, at which time spinal fluid was removed. No positive cultures were obtained, and it was felt desirable to terminate the project, after this number of studies.

The Branch of Ophthalmology has, over the past year, continued in its investigations directed towards further understanding of the metabolism and growth of the lens of the eye in relation to cataract; basic studies and clinical studies in the function of retinal elements; studies directed towards further knowledge of the formation and outflow of the aqueous of the eye and its relation to glaucoma, and studies of primary tumors of the eye, and infections of the uveal system. Although orientated in such given areas, a multi-disciplined approach is used so that widely dispersed laboratories may be engaged on different aspects of a given problem. This coordinated research is possible, largely, through the able direction of the Branch Chief.

Specifically, basic studies of the retina from a unicellular approach have continued by Dr. Fuortes, Dr. Gouras, and Dr. Tasaki, in an attempt to study the features of the activity of the visual nerve cells in the eye, as well as the more general problem of the transducer action of sense organs, whereby external energy is transferred into a change capable of stimulating nerve cells. Dr. Fuortes has found the frequency of impulses discharged in response to light stimulation is approximately a linear function of the logarithm of light intensity in the single cell. The frequency of discharge of the same cells in response to depolarizing electric current is, however, a simple linear function of current intensity. It would appear, therefore, that the logarithmic transformation which is typical of light perception may be exerted in this case by the photochemical processes inherent in the perceptual cells. If light of supraliminal intensity is utilized there is a sustained depolarization upon which may be seen superimposed impulses. If a subliminal intensity is used in the natural stimulus, only a sustained depolarization is recorded. It is apparent, therefore, that light evokes the firing of the nerve cells by depolarization of the membrane. Dr. Fuortes's analysis of the interaction between light and electrical currents in a single cell of the limulus indicates that the depolarization evoked by light is the result of change of conductance of the nerve cell's membrane, and that impedence measurements show directly that a change of the membrane conductance occurs during illumination. In contrast, no conductance change occurs during electrical stimulation. It is of interest that it has been reported in other laboratories that the eyes (of certain fish at least), respond with a depolarizing change to lights of one wavelength and a hyperpolarizing change to other wavelengths. Dr. Tasaki plans to work in this particular area. Dr. Gouras, before leaving to join Rushton, at Cambridge, brought to a conclusion the work on relations between slow electrical waves and impulse activity produced by illumination in amphibian retina. His results suggest that both the ganglion cells and receptor cells produce electrical potentials during illumination and both contribute to the electroretinogram. During this observation, Dr. Gouras also described a phenomenon comparable to "cortical spreading depression", occurring in the excised amphibian retina. This process is spontaneously reversible, and recovery occurs in 5-15 minutes.

Just recently Dr. Katharine Tansley, from the Institute of Ophthalmology, has joined the Ophthalmology Branch as Visiting Scientist, for a year. She plans to continue ERG work in pure-cone mammalian retinae. In her study she will use, nearly as possible, a monochromatic

light source. Both flickering and single flash stimuli will be used to study the responses and dark adaptation curves. This study is important in that one of the great difficulties in studying the human ERG is the separation of the photopic (cone) response from the scotopic (rod) response. Many members of the squirrel family, on the American Continent, possess pure double-layered cone retinae. It is hoped that further study of the electroretinogram in these animals will lead to further understanding in the ERG in man.

Studies in man have continued with Dr. Copenhaver and Dr. Gunkel, in which they combine electroretinography with adaptometry; the latter is a dark adaptation plot to determine the paramacular retinal area on the Goldmann adaptometer. In the past the ERG, in the hands of Dr. Bornnshein, Dr. Dodt, and others, has yielded information of significant value in the diagnosis of many retinal color-defective subjects, comprising 8 percent of the male population. The defects responsible for the typical color abnormalities are demonstrated to be retinal in location rather than in the optic pathways or the cerebral cortex. These findings are of some importance in that Le Gros Clark postulated that color reception was done at geniculate level. The electroretinographic method allows the determination of the type of defect and to some extent the degree of deficiency. Thus the peak absorption due to the red-sensitive pigment erythrolabe, which was found by Rushton, was found to be absent in the retina of this type of color defective. The sensitivity loss in deuteranopes agrees well with the green-sensitive pigment present in normals and also in deuteranopes, and hence suggests an interruption of the electrical impulses from the green-sensitive cones at a retinal level rather than a loss of pigment.

Further ERG studies were undertaken by these investigators with Dr. Dodt, a Visiting Scientist to the Ophthalmology Branch. These studies were directed towards spectral sensitivity curves on deeply pigmented and albinotic human eyes. These investigators found that the relative spectral sensitivities for wavelengths longer than 583mμ were high in albinos and low in negroes, while the dark caucasians and subjects with "blond" fundi showed intermediate sensitivities. The maximum sensitivity in the albinos occurred at 610mμ as compared with a peak sensitivity of 558mμ for caucasians and negroes. These investigators felt the difference in spectral sensitivity in the albino and negro is due to the reflection of light by blood in the former. By trans-scleral illumination, these investigators found that only selective absorption of light in the tissue coats of the eye was due to blood. It was also determined that the blood volume in the

sclera and choroid cannot be ascertained with this method. Hence, this work demonstrates the important effect which the density of the pigment epithelium has on the electroretinal spectral sensitivity.

Other studies on such pigments were carried on by the Section of Cytology and Histopathology, of the Ophthalmology Branch, by Dr. Wolf, Dr. Aronson, and Mr. Caravaggio. Tissue cultures of choroid pigment epithelial cells and ciliary body pigment epithelial cells were raised in the Paul Chamber in the presence of staining concentrations of Acridine Orange, and the fluorescent image observed at regular intervals through the lifetime of the cultures. Although Acridine Orange is toxic in tissue culture, at concentrations of 1:100,000, it permits growth at concentrations of 1:1,000,000; it has a photodynamic effect in stained cultures, and makes them more susceptible to light injury than unstained controls. Healthy cells will fluoresce green predominantly in the nucleus and nucleolus. With continuing illumination, the cells become brighter, and red granules appear in the cytoplasm. At this stage the light injury is still reversible. However, if illumination is continued, the nucleolus and the entire cytoplasm acquire nonspecific fluorescence and at this stage the cell is irreversibly injured. Thus the study of staining of living cells may provide important information about the chemical state of the components of living cells. For example, the metachromatic granules in irreversibly injured cells probably are not ribonucleic acid, because the cells observed do not contain granular aggregations of ribonucleic acid large enough to produce the image observed. These are some of the major studies concerning the retina and the choroid.

The Branch has, in addition, continued its studies on experimental cataracts, and growth of lens tissues. Dr. von Sallmann has continued his studies on diet and drug induced experimental cataract by directly applying Mimosine, or to initiate Mimosine cataracts by studying the effects of high pyridoxine and niacin levels upon the toxicity of Mimosine. Such lens were subjected to electron microscopic examination as well as to histochemical examination; the first by Dr. Wanko, and the latter by Dr. Kuhlman. Tryptophan deficiency cataracts were also used in this study, and the eyes were studied biomicroscopically, as well as histologically, for six, eight, and fourteen weeks after the five-day-old animals were put on the diet. The Mimosine cataract has a unique histologic picture in that, initially, there is selective

damage of the cells of the germinative zone in the earlier stages, and proliferation of these cells in a circumscribed area, described previously in Dr. von Sallmann's report. The combination of such lens changes with conjunctival, corneal and anterior uveal changes, suggested the local use of the compound in the form of frequent instillation of 1/2 percent solution in the conjunctival sac. However, such treatment completely fails to produce any of the surface changes or signs of lens damage as they are seen in Mimosine-fed animals. The chemical structure of Mimosine is much like that of the vitamin pyridoxine (see Dr. Tower's studies), and to a less extent to that of niacin. Therefore, to examine the possibility that in this case cataract formation is connected with an antivitamin effect of the toxic compound, high levels of vitamins were administered in an effort to protect the animals from such effects of Mimosine. However, such treatment does not in any way alter the ocular syatemic Mimosine effects. Electron microscopic examination of such cataracts by Dr. Wanko will show a conspicuous development of endoplasmic reticulum in the equatorial cells, and distention of cystic space between the membranes of the reticulum. There is a dispersion of the RNA granules and accumulations of abnormal, fine granular material in the cell nucleus. Thus the abnormality implicates both the nucleus and cytoplasm. The specificity of such changes to lens cell structure, however, is to be doubted in view of the findings in muscle disease by Drs. Wanko and Shy, in disorders of muscle. Dr. Kuhlman's investigations of enzymes, particularly dehydrogenase, have not led to conclusive results in these Mimosine cataracts. In tryptophan deficiency cataract, the equatorial zone remains unaffected, and the structure of this area is now preserved, while the lens cortex and lens nucleus are destroyed. Here the first changes are seen in the peri-nuclear zone around the anterior pole of the lens nucleus. There is a progressive decomposition of fibers which spreads later to the surface of the lens along the sutures. While the epithelium does not undergo such changes as described above initially, in advanced stages it proliferates to form multilayered plaques or knobs. These changes resemble those seen in galactose and alloxan cataract. The D-isomer form of tryptophan was fed to such animals on a tryptophan deficiency diet. Clinical examination did not reveal any differences in the utilization of the D-isomer by itself or when fed simultaneously with the L form. Electron microscopic studies of the epithelium, the capsule and the fibers of the lens, and on the epithelium of the ciliary bodies and optic nerve, were made by Dr. Wanko and Dr. von Sallmann.

in an attempt to investigate the normal characteristics of lens tissues as seen in the electron microscope; to investigate the ultrastructure of the lens epithelium and the lens fibers after cataractogenic agents had been administered; and to study the morphology of the ciliary epithelium with the electron microscope. In addition to the 1957 studies reported last year, the cortical layers of lens fibers were studied in rat, rabbit, monkey and calf. Such fibers represented elongated, prismatically shaped cells, outlined by dense membranes and separated from each other by small intercellular spaces. The nuclei, the mitochondria, the endoplasmic reticulum, the Golgi complex, and low density elements in the normal lens were all described by these investigators. Experimental cataracts were induced by 1500 rad X-rays, and the lens studied in the electron microscope. Structural changes in the mitochondria were noted and the nucleoplasm appeared in dense masses inside a lighter matrix. In the cytoplasm, profiles of endoplasmic reticulum appeared larger than normal and there was a considerable increase in RNA granules. In Myleran cataracts a great quantity of low density filaments in the cytoplasm of the lens epithelium was noted, as well as a deposition of a dense amorphous substance in areas beneath the epithelium. The findings in the Mimosine cataracts were described above. Investigations on the ciliary body has been initiated, and as-yet no definitive findings have been reported by the investigators.

Dr. von Sallmann has, in addition, described the submicroscopic structure of the lens tissue by phase contrast microscopy, in tissue culture of lens epithelium. Cultures in the Paul Chamber have been successfully maintained for periods up to one month, while cultures on roller tubes have been maintained up to two months. Hanks' medium with horse serum has been used as the culture medium. These investigators, feel in their preliminary stages that there is some evidence to indicate that elongated forms of cells are more constantly produced in cultures containing chick embryo extract, while cells grown without this media grow in sheets more analogous to the in vivo condition. They feel that the use of phase microscopy and tissue culture techniques enable the direct observation of the effects of cataractogenic agents on living cells, and that artifacts of the histological method are eliminated and the cell responses that escape detection are recorded by time-lapse cinematography for further study.

Investigation of the enzymatic systems present in the lens, cornea, and aqueous humor, has been undertaken by Dr. Kuhlman and Dr. Resnik, with particular reference to lactic dehydrogenase in the corneal epithelium, in glucose metabolism of the cornea, using tracer experiments and similar studies upon the lens and cornea after administration of 1000 R of X-rays. Three species were investigated: the rat, the rabbit and the cat. The rabbit cornea

epithelium had the highest general level of enzymatic activity. Two enzymes of citric acid cycle, namely malic and isocitric dehydrogenase were found in all three groups to be present at levels equivalent to those present in cellular areas of the brain and retina. While all species also contained glucose-6-phosphate dehydrogenase at a level equivalent to brain and retina, aldolase and hexokinase were lower. The rabbit corneal epithelium was unusual in that it had a very high lactic dehydrogenase activity, being 20 times higher than that in the cat to rat. These investigators found that the whole cornea oxidizes glucose at a rate of approximately one-half that of liver or diaphragm, and the presence of a direct oxidative shunt in corneal metabolism is confirmed. In addition, they found that the cornea is able to oxidize lactate, and may do so even in the presence of glucose. The removal of the epithelium from the cornea reduces this ability to oxidize glucose by a factor of 80 to 97 percent, whereas lactate oxidation is reduced only 27 percent. After irradiation, although there may be morphological changes, there was no alteration in the lens content of hexokinase or glutathione reductase. Preliminary investigations of such enzymes in the aqueous humor are now started.

Dr. Resnik has continued his studies concerning the primary proteins of the lens. In his additional studies, he finds that the value for the sedimentation coefficient of alpha crystallin is 17.0×10^{-13}. This value is slightly lower than that reported previously, but is based upon additional data. He feels the molecular weight of alpha crystallin is now 900,000 to 950,000. In collaboration with Dr. Wanko, isolated preparations of this protein, and low density elements seen in sectioned lens fibers, were also carried out. At the present time these investigators cannot state whether the elongated structures in preparations of the low density elements are alpha crystallin. These studies do indicate, however, that such low density structures are proteins and that the soluble lens proteins alpha, beta, and gamma crystallin are present in these structures. Thus again a coordinated program is in force in the understanding of the normal and abnormal lens, in relation to growth, degeneration, and cataract formation.

Studies in normal and abnormal control of intraocular pressure are also continuing. Dr. von Sallmann and Miss Grimes report on the anatomy of the posterior ciliary nerves in cat and monkey, and the preparations from these species have in common the fact that nerves close to the globe are generally mixed nerves, and they contain fifth and third cranial nerve fibers. Isolated

"long" ciliary nerves may be found in the cat, however, which are not observed within the orbit, and which do not fuse with any of the post-ganglionic branches of the ciliary ganglia. In the monkey, the ciliary ganglion receives three or four communicating nerves from the fifth cranial nerve. Nerves arising in the ciliary ganglion going directly to the eye without joining branches of the fifth cranial nerve have not been observed in either species, but the segregation of fibers within mixed nerves and the subsequent branching, might, in the opinion of these investigators, give rise to a few nerves which enter the scleral coat and which are purely parasympathetic. Covering the long ciliary arteries are nerves which supposedly contain only fifth cranial nerve and sympathetic fibers. These investigators feel, however, that these are mixed nerves which also carry post-ganglionic parasympathetic fibers.

A study of afferent electric impulses induced by intraocular changes has been undertaken to see if such impulses may terminate in diencephalic centers as originally postulated by von Sallmann, et al. This study is being undertaken by Dr. Lele in animals in which external ocular muscles and connective tissue and the ciliary nerves are all disected from the globe, and connective tissue excised. Pressure within the globe is maintained by 22 guage hypodermic needles, connected by short lengths of saline columns to a pressure transducer. The nervous activity was examined by placing recording electrodes on each of the dissected nerves, and recorded from one channel of a dual-beam cathode-ray-oscillograph. The transducer, in turn, is recorded into the second channel of the same scope. Dr. Lele finds that branches of the ophthalmic division of the fifth cranial nerve did not show any spontaneous electrical activity, but in every instance, afferent impulses were evoked when the cornea or conjunctiva were mechanically stimulated. Neither spontaneous nor evoked activity, however, were recorded from the short ciliary nerve originating from the ciliary ganglion and entering the globe. Responses obtained from ciliary nerves of mixed origin were essentially similar to those of the long ciliary nerves. All the long and mixed ciliary nerves tested showed a response to increased intraocular pressure. In each instance there is a sharp but transient increase in the frequency of the impulses, lasting as long as the increased ocular pressure rises. The maximum frequency was proportional to the rate and the height of the rise of increased ocular pressure. In approximately 60 percent of the preparation, such activity was sustained while the pressure was sustained. Every eye tested showed this type of sustained response in one or more of the nerves.

Dr. Macri, Dr. von Sallmann, and Miss Grimes have continued their studies on the effects of drugs on intraocular pressure. Dr. Macri reports that muscle relaxants such as decamethonium (see Dr. Irwin's studies), and succinylcholine probably have their effects on spasms of extraocular muscles (during 1957). To further clarify this an attempt was made to record intraocular pressure upon stimulation of the third nerve intracranially, and after rigor mortis. The procedures were essentially those described in the 1957 report. These investigators find that spasm of the extraocular striate muscles induced by third nerve stimulation and/or rigor mortis produced changes in the elasticity of the eye similar to those obtained after the administration of these two drugs. This effect could be abolished by resection of the extraocular striate muscles or enucleation. These investigators feel that since three different methods inducing muscle spasm produced similar changes in the elasticity of the eye, it appeared very unlikely that factors other than muscles could be involved. If the eye was also placed in a chamber filled with saline, and the pressure of the chamber raised to various levels and the elasticity determined, the effects on such elasticity of the eye, under these conditions, were almost identical to those obtained by muscle spasm. Utilizing the method reported last year for the determination of aqueous outflow, devised in their laboratory, these investigators find that now there is a second biphasic outflow pattern which is not proportional to the internal ocular pressure throughout the pressure range examined. The biphasic curve was characterized by a very fast outflow at lower intraocular pressures, which then inflected at pressure levels between 35 and 50 mm. of mercury. Such an outflow pattern could be induced by parenteral administration of Diamox.

Since it has been reported that the pressure in the veins to which the aqueous humor flows is essentially constant and independent of internal ocular pressures, then the outflow pressure should be the difference of pressure values between two ends of the channels, i.e. the intraocular and the venous. These investigators felt it was, therefore, important to study the venous pressures on the surface of the eye. Three veins, the anterior ciliary, the long posterior ciliary, and the vortex,can be cannulated. A cast material was injected into the anterior chamber under continuous pressure until many of the episcleral vessels were seen to be filled. The material was allowed to harden, and the tissue was digested away. Thus the aqueous outflow channels were demonstrated in their course from the trabecular area to the Circle of Hovius. Pressure readings in the anterior ciliary and

vortex veins, and those of intraocular pressure, appeared almost identical under resting conditions. However, if the intraocular pressure was either raised or lowered, the venous pressure fell. Thus they felt that they had shown that changes in internal ocular pressure can alter venous pressure. They summarize this by four points:

1) The biphasic outflow patterns become more pronounced when the outflow pressure is calculated as internal ocular pressure against venous pressure.

2) Acetylcholine, Arterenol, histamine, sympathetic and parasympathetic nerve stimulation all produced changes in the venous pressure which paralleled the changes in the internal ocular pressure.

3) Trauma of the eye induced identical elevations of internal ocular pressure and venous pressure.

4) Diamox lowers both the internal ocular pressure and venous pressure.

Thus, for example, Diamox may have a double action in that it may reduce the aqueous inflow, but it may also reduce the venous pressure in the eye. Such studies are important in the further understanding of glaucoma.

Glaucoma studies at clinical level are being continued by Dr. Paton and Dr. von Sallmann, in an attempt to determine the most valuable diagnostic tests and the prognosis and adequacy of glaucoma therapy. With present day techniques, measurement of intraocular tension, visual fields, and the individual response to test situations, a diagnosis of glaucoma is often uncertain. This study is concerned with information gained from tonography in borderline cases of glaucoma. It is also concerned with distinct subdivisions of glaucoma noted as "low tension", "hypersecretion", "pigmentary narrow angle", or "inflammatory" forms of glaucoma. Patients are accepted to this study by admission for a minimum of several days in order that an extensive glaucoma workup may be performed under rather constant environmental conditions, and at all times of the day or night. Tests include tonometry with day curve determinations, applanation tonometry, measurement of depth of anterior chamber, biomicroscopy, gonioscopy, visual fields, and photography of the optic discs. At the present time, cases of borderline glaucoma have not been followed for sufficient time to judge the value of the data obtained. In addition it is apparent that more normal control subjects must be studied. These then are the primary studies concerned with increased intraocular pressure.

A study of the inflammatory disorders of the eye is continuing in two separate areas: The first one by Dr. Kaufman on toxoplasmosis and its therapy, and the second one by Dr. O'Rourke and his colleagues in determining the effects of endocrine glands upon exascerbations of inflammatory disorders of the uveal tract. In the first study Dr. Kaufman is maintaining strains of the organism in the chick embryo, and from the chorioallantoic membrane, as reported in the 1957 report. These were then kept alive in tissue culture roller tubes. The time of attachment of the micro organism to the cells could be determined by washing out the inoculum at desired intervals. The organisms were allowed to multiply, but the culture could be fixed and stained before cell destruction had occurred. The effect of Daraprim on toxoplasmosis in vitro was investigated. Testing of patient material was continued as in previous years. It was found that slow growing organisms were much more resistant to Daraprim than the rapidly growing organisms. These studies also showed that appreciable time is required for the organism to be in contact with the cell before an invasion of the cell takes place, and that chronic infection of the tissue culture can be produced with slow growing strains. In such chronic states, the organism and culture seem to be in symbiosis, and the damage to the culture is not apparent. When the organisms are incubated with serum containing a high titer of dye test antibodies, these organisms were killed by this serum, suggesting that the dye test antibodies may, in fact, be toxoplasmocidal.

As noted in the 1957 report, Dr. O'Rourke, in an attempt to explain the multiple remissions and exascerbations of uveal infections, has turned his energies to endocrine studies, in particular thyroid hormone turnover, and he has now completed radiothyroxine turnover studies in 30 uveitis patients, and 5 normal controls. His data suggest that in the main uveitis patients show retarded rate of utilization of circulating thyroid hormone as compared to the normal. The major difference lies in the daily rate of degradation of I^{131} thyroxine. Treatment with thyroid hormone has, in a few patients, in Dr. O'Rourke's opinion, resulted in correction of these abnormal results.

Finally, Dr. van Alphen is studying immunological relations in ocular tissues, in an attempt to determine the possible antigenicity of lens capsule, and to produce cataracts immunologically by immunization with lens capsule and lens proteins; also to see if the various tissues

of the eye are immunologically related. Dr. Van Alphen finds that the sera of guinea pigs immunized with capsule and guinea pigs' lens proteins or with calf lens capsules and calf lens proteins, show cross-reactions with corneal epithelium and vitreous, but do not react with donor blood, -iris, -retina, or -aqueous. Anti-calf vitreous sera show strong cross-reactions with calf blood and none with other calf antigens. Anti-calf corneal sera react with corneal epithelium only, and not with calf blood or ocular calf antigens. In none of the animals immunized with lens capsules and lens proteins did cataracts appear, although repeated paracenteses were carried out and some lenses were traumatized. These studies then represent the collected research directed towards infection and immunization in ocular disease.

Dr. O'Rourke is also continuing his studies on the detection of ocular tumors by isotope tracer methods, using radiophosphorus, and trans-scleral counting done as a surgical procedure. He finds evidence in four patients most recently studied, the trans-scleral counting results were correctly positive, although results of the trans-conjunctival method were negative or equivocal. The former method seems to be the present one of choice, as might be anticipated by the low energy range of the beta particles of radioactive phosphorus. Thus it would appear that surgical procedure must still accompany a diagnostic method of determination of intraocular neoplasia.

The Ophthalmology Branch has initiated a new project this year, studying the basic factors in refraction anomalies. This is an effort to complete a statistical analysis of the interrelations of the five optical elements in the human eye, and to test in part a theory which assumes the tension in the choroid by reducing the pressure on the sclera as a factor in determining the size of the globe. It is clinically known that wherever the choroid is absent, the sclera becomes ectatic. A quantitative confirmation could be obtained by measuring the pressure in the subarachnoid space, comparing this to the intraocular pressure. The subscleral pressure in 12 eyes appeared to be lower than the intraocular pressure. The differences amount to 2 to 6 mm. of mercury. Parasympathetic stimulation of the ciliary ganglion leads to decreased pressure, and sympathetic stimulation of the cervical sympathetic leads frequently (but not always) to increased pressure. Several investigators have considered the choroid as too fragile to stand pull and pressure. Dr. van Alphen, however, by trephining scleral windows in the posterior pole, may make the choroid bulge out and cause

it to retract on parasympathetic stimulation. If a large scleral window is cut, there is a large herniation of the choroid, but even when overstretched the choroid is able to stand 90 mm of intraocular pressure before rupturing.

Dr. Gunkel is continuing his program of design and construction of optical instruments, and has, during the past year, in correlation with Dr. Copenhaver and others, established retinal profiles using new color filters and smaller test spots. Dark adaptation curves were obtained with the modified instrument which have been found to be quite satisfactory. This and other tentative data with other eye diseases indicate the potential usefulness of such testing procedures for a variety of disease entities.

Dr. Kaufman, Dr. van Alphen, and Dr. von Sallmann have reported upon the highly interesting findings in the vitreous in primary familial amyloidosis. The muscle of such cases were also examined in the Medical Neurology Branch, and there is no doubt this almost pathognomonic appearance in the vitreous confirms the diagnosis of unsuspected amyloidosis. In none of the five cases seen by the Clinical Director has one presented with the classical findings of amyloid. Biopsy of gum, muscle, skin, nerve and vitreous paracentesis, however, have confirmed the accuracy of this finding, and indicates that a close examination of the vitreous is important if this disease is to be considered.

Dr. Bruce Cohan has undertaken a study in intraocular angiography, using radiopaque dyes after replacement by such dyes of the aqueous humor. He utilizes laminagraphic techniques, using a 0.3 mm. focal spot tube. The anterior ciliary vein of the cat's eye is also cannulated, and radiographs are taken during hand injection of radiopaque dyes into the venous system, with and without paraorbital tissues. The study has resulted in the successful demonstration of the X-ray anatomy of the intraocular venous systems in the essentially intact cat eye which will allow a more detailed study of the anatomy of the intrascleral venous plexus, and the dynamics of intraocular vasculature.

Finally, Dr. Wanko is continuing with his observations of normal and abnormal striated muscle, as examined by electron microscopy. Four normal specimens have been examined, four cases of myotonic dystrophy, and one case of Werdnig-Hoffman's disease have been studied. In the myotonic dystrophies, there appears to be an increase in the RNA granules, and a rarefication of the

electron microscope confirming the findings in the light microscope. In Werdnig-Hoffman's disease, peculiar shaped mitochondria have been observed. The total series of all such cases is too small to make definite conclusions at this time.

The Branch of Surgical Neurology reports intensive investigation of 119 patients with cerebral seizures, the majority being temporal in location. The pathology, the physiology (at the operating room), the autonomic concomitance of temporal lobe epilepsy, the language characteristics, and the psychological abnormalities are described in detail. Study of microelectrode techniques in tissue culture in neurological and muscle elements have continued, as well as studies in cortical neurones, and the effects of hallucinogenic agents upon higher primates after removal of specific areas of brain. The anatomical effects of temporal lobectomy have continued, and a new stereotaxic device has been developed which will be directed to the treatment of involuntary movements. In combination with the Cancer Institute, studies in hypophysectomy have continued. Further developments of the underlying factors in cerebral palsy have been reported, and attempts to correlate this with the embryology of the central nervous system. Tissue culture studies have continued, as well as the effects of hypothermia upon the central nervous system and cerebral edema. The pathological characteristics of a rapidly degenerating disease found in New Guinea are described. New anesthetic agents, and their effect upon cerebral circulation, have been studied. Specifically, the following investigators have reported their projects as follows:

Dr. Baldwin reports 119 cases of cerebral seizures, the majority of which are afflicted with temporal lobe abnormalities. From his most recent studies he feels that a cryptic angioma is a significant cause of this form of seizure, and that this vascular abnormality is found more commonly in the mesial temporal structures close to the junction of the circulation of the middle cerebral and anterior choroidal artery. Dr. Baldwin suggests that the peri-insular tissue, through its epileptogenic characteristics, may initiate a perceptual process in the opposite intact temporal lobe. Dr. Baldwin reports a series of patients admitted as probable temporal lobe seizures, which after further study, appeared to arise from the cingulate area. These patients had a clinical seizure pattern characterized by epigastric aurae, altered affect, altered awareness, posturing and adversive movements, as well as autonomic changes. Dr. Baldwin is studying these cases in conjunction with a similar series at the Mayo Clinic under Dr. David Daly, and he hopes that this series will provide a means for differentiating those seizures arising from the cingulate gyrus. Dr. Baldwin has continued his

of the motor phenomena of the temporal lobe seizures; he now feels that there are certain movement patterns of the hands and upper extremity which are characteristic of epileptic activity in one or both temporal lobes. He feels that such movements have a lateralizing significance, and that they occur on the side opposite to the most active temporal lobe. The movements of the head and neck in a temporal lobe seizure are usually such that there is a turning to one or the other side; this movement is a slow postural movement, and is thus different from the adversive movement, which is pathognomonic of the supplementary motor area, and the chin points downward. He notes that during epileptic automatism, the fine digital movements are lost, and the hand is used apparently en bloc. He has now photographed 1,721 such phenomena.

Dr. Van Buren has continued his studies on the same series of patients with temporal lobe seizures, by use of polygraphic measurements of autonomic concomitants of such temporal lobe seizures. He has noted a hypertension, tachycardia, respiratory apnea, a fall in skin resistance and skin temperature, as well as swallowing movements and inhibition of gastric motility. There is no strict correlation of such autonomic activity to the electrographic tracings. Perceptual aberrations do not always coincide with clinically recognized seizures. They may occur without other stigmata of temporal lobe seizures. The perceptual disorders of space and color perception are most frequent. Dr. Baldwin feels such perceptual aberrations are never separate from differences in affect. In fact, in disturbances of the temporal lobe by epileptic processes, the most frequent combination is that of fear and perceptual aberration. Dr. Baldwin feels the physical basis of fear may be one of the most significant sources of the clinical characteristics in temporal lobe seizures. This has prompted him to turn his research in the direction of searching for catechol amines or other adrenaline-like substances, which may increase in amount as a result of mesial temporal discharge. Dr. Baldwin feels, in addition, that during a seizure, the patient does not have the usual appreciation of body image. The "memory difficulty" which has been noted so frequently in patients with temporal lobe seizures has also been studied. It is the impression of these investigators this difficulty is not so much in memory as in relating space and time. In the laboratory, Dr. Baldwin has continued his seizure project utilizing penicillin-induced seizures. Penicillin lesions within one or both temporal lobes will usually project first to the

cortex of the hemisphere in the paraga[illegible] on the side opposite to the involved temporal lobe. As such a seizure discharge spreads across the cortex it is preceded by discernible vascular change, and if massive may be followed by severe and occasionally critical edema.

In the operating room, Dr. Baldwin has continued in the electrical stimulation of human and higher primate temporal lobes. In the human operating room the interest has been focused on the so-called psychical responses. The majority of such responses have come from depth stimulation, but may also be found from surface stimulation. Approximately 200 positive responses have been obtained from the chimpanzee cortex, which were motor in nature, and Dr. Baldwin feels there may be a centralateral inhibitory motor area. The study of ablation preparations continues, and chimpanzees have been studied now up to four years after bilateral temporal lobectomy. Dr. Baldwin finds the animal now adjusting more socially, and remains more placid than his contemporaries. After four years, in the case of the bilateral frontal lobectomy, however, a similar animal does not regain his place in the social hierarchy and his individual and social habits remain abnormal. Dr. Baldwin notes the mesial temporal lesions affect communication in the chimpanzee for approximately four weeks after their creation. He also notes that hallucinogenic substances which are contained in the Mexican mushroom do not affect the chimpanzees whose temporal lobes have been removed, yet such substances affect the normal chimpanzee as to make him tame, relatively unaware of his surroundings, and somewhat ataxic. Dr. Van Buren, and Dr. Paul Yakovlev from Harvard, have been studying the anatomical pathology following temporal lobectomy. With an anterior temporal lesion, nuclear degeneration appeared in the inferior and lateral portion of the pulvinar, and the posterior portion of the medial geniculate body, and the lateral part of the lateral geniculate body. In posterior temporal lesion the degeneration appeared in the middle and posterior part of the pulvinar, the anterior portion of the medial geniculate body, and the medial part of the lateral geniculate body. They note also a loss of cells in the posterior part of the nucleus medialis dorsalis. These investigators feel that the stria terminalis in man appears to arise from the cortical and medial accessory basal nuclei of the amygdala since it remains intact when the lateral portions of the amygdala are destroyed by surgery. The

anterior commissure, however, was nearly entirely degenerated from such lateral lesion of the amygdala, suggesting that the retained medial portions of the amygdala and region of the uncus received very little projection from the anterior commissure. A descending pathway from the amygdala to the brainstem in the lateral part of the cerebral peduncle is also found. This could be followed as low as the lower pons.

Dr. Van Buren reports in some detail his experience in hypophysectomy of graded nature in man. Thirteen cases formed the basis of this study. Serial sections of the sella were obtained insofar as the size of the retained pituitary fragment, and differential cell counts were made in this fragment. Such findings were correlated in each case with the patient's clinical course and the response of the thyroid and adrenal function, and the level of gonadatrophins, and the presence or absence of diabetes insipidus. The amount of pituitary remaining after surgery varied from 0.3 cubic mm. to 160 cubic mm. Immediately after such surgery there was profound depression of thyroid and adrenal activity, and the gonadatrophin levels fell to negligible figures. It was furing this time that remission of a primary tumor might occur, and this was seen in approximately 50 percent of the cases. The most striking feature noted by Dr. Van Buren was that there was no correlation between the amount of pituitary tissue left and the amount of hypopituitarism, or tumor remission present in the patient. In a patient having 160 mm., the thyroid and adrenal function returned to normal, but the patient's 16 month post-operative gonadatrophin levels remained near negligible figures. Thus, there was strong suggestion that depression of individual trophic pituitary hormones are not the same for all trophic hormones. The only feature common to all cases was surgical section of the pituitary stalk and this may indeed be the essential feature, according to Dr. Van Buren. The amount of diabetes insipidus present could also not be correlated in any way with the amount of pituitary tissue remaining. An initial rise of cholesterol was noted in 5 cases that were seen to fall to normal in one to three months following surgery. The initial rise nor the ultimate fall again did not correlate with the amount of pituitary tissue left. Histologically there appeared to be a lack or decrease of specific granules of the chromophile cells, presumably to meet the increased demand of pituitary hormones. These same cases also provided Dr. Van Buren with valuable post mortem material for study of the visual system.

In the visual system studies, which were a continuation of the 1957, 38(c) studies, Dr. Van Buren studied human and primate retina; the effects of lesions of the optic pathways upon the retina; the effects of lesions of optic pathways and the lateral geniculate body; and the visual field defects following temporal lobectomy. Dr. Van Buren and Dr. Baldwin reported these findings partially in the 1957 report, and a paper now has been published, in Brain, 1958. That portion of the study having to do with the visual field following temporal lobe defects terminated with this report. Eight additional retinal studies and four lateral geniculate studies are still in progress.

Dr. Van Buren has now tested his new stereotaxic instrument on cadaver material at NIH. Obtaining of such material has been difficult, and the first such cadaver was undertaken in May, 1958. Since this time only five other cadavers have been available. Dr. Van Buren feels, however, on the whole the results have been encouraging in that they show the principle of the arcuate electrode carrier is a sound one under practical operating circumstances, and that the apparatus is mechanically accurate. Simultaneous ventricular and cisternal punctures were necessary for good pneumography in such cadavers. Dr. Van Buren is continuing in the preparation of a brain atlas for the utilization with this stereotaxic instrument. The utilization of this stereotaxic instrument will be exceedingly important in the future approach to minute lesions in the treatment of involuntary movements. Measurements of such involuntary movements again is difficult and the surgical unit is attempting the preparation of accelerimeters as a means of simple graphic recording of such movements.

In 1957 Dr. Van Buren reported that his findings suggested that patients complaining of pain which appeared more functional than organic in origin, had unusually unstable autonomic responses. He has continued these examinations during the present year in an attempt to correlate the degree of the autonomic responsiveness with other features of the patient's clinical picture. He feels, however, that his results have been practically, in this case, of no value. Autonomic responses to apparently the same pain stimulus varied from examination to examination, on the whole tending to decrease as the patient became more used to the examiner and the testing

situation. He feels, at this time, that autonomic recording does not seem a probable lead for accurate measurements of pain in an objective fashion.

Dr. Li has continued his studies of intracellular recordings in the cerebral cortex and in tissue cultured nerve and muscle cells, as well as studies of neurotransmission in hypothermia. Dr. Li has found five separate types of intracellular potentials recorded from the cortex, the first type being a steady potential unresponsive to afferent stimulation and/or local application of strychnine. He feels that these potentials originate probably from glia elements. Secondly, large slow potentials originate, probably from glia elements. Secondly, large slow potentials, probably originating from dendrites; third, small potentials presumably originating from synaptic regions; fourth, brief spikes with an inflexion in the rising phase, presumably recorded from cell bodies; and finally, simple brief spikes from axons The miniature potentials, arising from presumably synaptic regions, show a marked similarity to that recorded previously by Fatt and Katz, in England, in neuromuscular junctions. He further feels that the mechanism of such synaptic transmission in the central nervous system hence indicates the importance of dendrites in the production of electrical activities of the cerebral cortex may be over-emphasized.

He has continued to measure the activity of nerve cells in the motor cortex with micropipettes while electric stimulation was applied to various subcortical structures and peripheral sensory nerves. The cells which are intimately related to motor function, and which have descending axons to medullary pyramid were identified by their responses to antidromic stimulation, and those cells in the motor cortex which do not have descending axons were identified as internuncial cells. Dr. Li verified his previous report to the effect that the nucleus ventralis lateralis of the thalamus activates the cells with descending axons but suppresses the activity of the internuncial cells, and he suggests that this thalamic nucleus may have some control over the motor activity of the experimental animal. He also found that what he identifies as internuncial cells in the motor cortex could be influenced by a sensory volley from the peripheral cells. Such a sensory volley, however, was also capable of exciting a motor neurone in the cerebrum. The study demonstrated, to Dr. Li's satisfaction, that the refractory periods of the pyramidal fibers varied from 1.5 to 2.5 milliseconds, and that the conduction velocity was 8 meters to 95 meters per second.

The synchronous activity of nerve cells in the cerebral cortex was the subject of a further study by Dr. Li, in which he found only a very few nerve cells in a sphere of 1 mm. in the cerebral cortex would discharge precisely at the same instant; secondly, that a synchronous volley evoked discharges of nerve cells with time discrepancies varying from 2 milliseconds to 20 milliseconds; third, that the application of strychnine activates about 85 percent, but not all of the nerve cells; and fourth, that there is a time relationship between neuronal activity in the "aroused" cortex. Since it is generally pictured (see the reports of Dr. Ajmone-Marsan, et al), that neurons in the epileptogenic cortex tend to fire in unison, the present study suggests that this is a generalization with a certain degree of truth, from the strychnine experiment, but that in the normal cortex in an alert subject cells are firing randomly.

In his studies in hypothermia, Dr. Li has concluded with publications on the effect of cooling on the neuromuscular transmission in the rat. This study indicated that there was a critical body temperature in mammals below which the transmission of impulses across the neuromuscular junction could not occur, and if the body temperature would be further lowered to 4°C, transmission is completely blocked. Similar studies on conduction of impulses in cranial and peripheral nerves were carried out by Dr. Li and Dr. Ortiz, in which small segments of the optic nerve and sciatic nerve were subjected to -150°C for 30 seconds. The animals were then kept alive for 1 day - 4 months, and impulse conduction was tested at various intervals. This study is in its initial stage, and is designed to see if extremely low temperatures locally applied to tissue has ablation experiments by surgical procedures.

Finally, in coordination with Dr. Baldwin's projects listed above, Dr. Li and Dr. Ortiz have been studying the effects of the hallucinogenic activity of the Mexican mushroom in both cats and monkeys. Multiple electrodes were used which were capable of injecting minute quantities of the testing chemical agents, and inserted into various depth structures of the brain. Recording of the electrical activity and responses to stimulation from these structures and from the cortical surface were made. At the present time such studies are still inadequate for conclusive statements to be made.

In the Section of Clinical Neuropathology, Dr. Klatzo reports his initial investigations in pinocytosis

of labelled proteins in tissue culture. This consists of labelling proteins with a fluorescent component and feeding cultures with these labelled proteins. The differences between individual cell types could be demonstrated in this aspect as cellular protein metabolism may be studied by altering pH, temperature, and chemical substrate. Newborn kitten and rat cerebellum were grown in vitro. Cat serum albumin and rabbit serum globulin were labelled with fluorescein isothiocyanate. The cultures were "starved" for a period of three hours, and consequently fed with the labelled substance. The preliminary findings indicated that it was possible to demonstrate protein uptake by living cells grown in vitro. A significant difference in metabolism of proteins by various cellular elements was observed. Cultures washed for a brief period of time after feeding showed abundant labelled proteins in the macrophages and only a few fluorescent droplets in the glial elements. Cultures washed for several hours in balanced salt revealed abundant green fluorescent droplets in glial cells, whereas the macrophages showed mostly autofluorescence of various lipid substances. This study is also important in relation to collimation techniques for detection of brain tumors with radioactive serum albumin labelled with I^{131}. This would seem to indicate that the uptake of such substance was not only due to break in the blood-brain barrier, but to actual ingestion of the labelled protein by the tumor cell.

Dr. Klatzo, Dr. Horvath, and Dr. Emmart, are continuing their studies of the localization of myosin in human striated muscle by fluorescent antibody, using the Coons' fluorescent antibody technique. They find in normal muscle, the specific stain for myosin was observed in the A band, and that the I and H band appeared unstained with the Z band showing an occasional non-specific autofluorescence. In studies of the various pathological processes in human muscle, there was a striking persistence of antigenic reactivity of myosin in fibers with far advanced degeneration. Regenerating fibers observed in cases of muscle injury and polymyositis showed similar features to those muscle fibers grown from chick embryo. An occasional macrophage also contained green-fluorescent inclusions in their cytoplasm. This observation may be of importance for interpretation of possible mechanism of hypersensitivity due to release of muscle proteins. Attempts at the present are now being made to induce allergic myopathy in laboratory animals.

Dr. Klatzo and Dr. Gajdusek have now completed their findings in Kuru disease, the main pathological findings being a widespread neuronal degeneration; myelin degeneration affecting predominantly cortico-spinal and spino-cerebellar tracts; intense and widespread astroglial and microglial proliferation; perivascular cuffings with mononuclear elements; and the presence of peculiar plaque-like bodies in half the cases studied. In many ways this disease resembles that described by Jakob-Creutzfield. Together with Dr. Ortiz-Galvan, and Dr. Laskowski, Dr. Klatzo reports some studies on regeneration in the central nervous system, after the application of cold, and then injection intra-cysternally with prednisolone. The progress of the regeneration will be followed by photic stimulation recordings from various parts of the central nervous system.

Dr. W. K. Engel, Dr. Li, and Dr. Klatzo report on the histochemical and electrophysiological observations of muscle fibers grown in vitro. The muscle tissue is obtained from 14-day-old chick embryo or newborn rat, and studies in the RNA (ribonucleic acid) content was demonstrated with gallocyanin and Toluidine blue methods. The first appearance and localization of myosin in myofibrills has been followed with specific fluorescent antibody. Data on the electrical activity has been obtained from cultures several weeks old. This study has demonstrated that spontaneous activity may occur in such fibers. In earlier days of culture, spontaneous pulsation of muscle fibers may also be seen. Dr. Engel is testing specific blocking agents to such tissue culture after administering electronic stimuli to the muscle fibers.

Dr. Miquel and Dr. Horvath, and Dr. Klatzo, are utilizing a new quantitative method for estimating precipitin reaction, by applications of antigen-antibody mixtures to chromatographic paper. By using fluorescent antibody instead of serum in the tests, the ratio between the amount of antibody to antigen in the precipitate may be quantitatively measured. This method has been applied to the precipitin reaction between antigens of contractile muscle proteins and their respective antibodies. The quantitative data obtained by this method is in agreement with the much more complicated and cumbersome Kjehldal nitrogen determinations. The sensitivity of the method was estimated to be as low as 1 gamma of nitrogen.

Dr. Laskowski and Dr. Klatzo are continuing their studies on the relationship between edema, blood-brain-barrier and tissue elements in experimental brain injury. Sodium fluorescein was used for this study of the blood-brain-barrier. These findings have now been published in the Journal Neuropathology and Experimental Neurology, in which the development of edema was observed within 6 hours in the white matter underlying the site of cold application. This edema exhibited strong PAS-positive staining of astrocytes and less intense PAS staining of interstitial spaces. The histochemical analysis of PAS positive staining in the edematous white matter suggested a glycoprotein nature of the substances involved. Electrophoretic studies performed at the time of maximal intensity of the edema and break-down of blood-brain-barrier indicated an appreciable increase in total proteins with a striking elevation of albumins in the area of edema. This fluorescence in the superficial layers of the cortex persisted one month after injury, and was associated with presence of small astrocytes, while that of the deeper layers, i.e. white matter, disappeared in this period of time.

In the Section of Developmental Neurology, Dr. Dekaban is continuing his studies concerning the site, type, and extent of lesions involving the central nervous system in cerebral palsy, and allied conditions. During 1958 Dr. Dekaban reports 56 patients studied in great detail as inpatients, and 28 as outpatients. Analysis of his results shows that in 62 percent of the cases the site of the lesion was determined; in 27 percent such an abnormality was of diffuse character, and in 11 percent the localization was not possible. In 29 percent of such cases the lesion was compatible with a destructive process, in 12 percent with a congenital malformation, and in 21 percent it was of diffuse character, and in the remainder of 38 the lesion could not be estimated with confidence. An etiological diagnosis was made in 43 percent of all patients by elimination of antibodies, lipid contents in cerebrospinal fluid, special retinal studies, and estimation of amino acids in urine, phenylalanine in blood, and a genetic assay. This brings to a total 141 patients that Dr. Dekaban has studied since the beginning of this project.

Dr. Dekaban's extensive survey of 4,480 products of pregnancy at the National Navy Medical Center and Walter Reed Hospital, between March 1, 1956, and March 1, 1957, is continuing. Over 80 percent of such products have now been evaluated, and the final statistics of

injury to the central nervous system in such a large group of patients taken at random is of extreme importance in estimating the incidence in cause of cerebral palsy.

Dr. Dekaban has also continued his study of the pathological lesions in patients coming to post mortem who have central nervous system lesions occurring during prenatal, intranatal and early postnatal life. Twenty brains have now been obtained, which are being processed and studied. Analysis of the pathological findings reveal that in 9 children the abnormality of the central nervous system was of prenatal origin; in five it was compatible with birth injury, and in only four was it the result of intracranial infection. In one cerebral neoplasm was present, and no central nervous system abnormality was detected.

Dr. Dekaban and Dr. Baird are continuing their studies of the products of diabetic mothers, in which they note that the total for all wastage of pregnancies in diabetic mothers was 43.4 percent as compared to 17.6 percent in the normal control. Of the surviving offspring born to the diabetic mothers, 6.7 percent showed congenital abnormalities or various neurological areas; this compares with only 0.48 percent of abnormal children in the non-diabetic control group. The tables of their findings may be seen in their detailed presentation.

Dr. Dekaban has also continued his measurements of external and internal orbital distance in males and females from birth to adulthood, and has now accomplished these measurements on approximately 600 normal children. This material is being currently validated and subsequently will be subjected to statistical analysis.

His study of the normal development of the mouse brain is continuing and has now resulted in an atlas of the normal mouse brain. Careful dissection of the brain and brainstem in 11 age horizons have been performed. Production of malformations by means of x-radiation has also been attempted. Approximately 10 percent of the litters of x-radiated mice have major abnormalities; about 25 percent minor abnormalities, and the remaining are free of detectable pathology.

Dr. Lansdell, in the Section of Clinical Psychology, has undertaken studies to the effect of "fear-provoking" stimuli on visual discrimination in primates, and an intensive study on the psychological evaluation of temporal lobe disease. He and Mrs. Weissbach and Miss Blevins, have reported a tendency for patients with left temporal lobe removal to be "poor communicators". Since Dr. Lansdell has recently joined this Clinical Investigative Unit, much of his program is projected into the future.

As noted by Dr. Baldwin, the Clinical Unit has been injured by the loss of Dr. Kenneth Hall, who has joined the staff of Duke University as Associate Professor in Anesthesiology, in charge of research. Before leaving, Dr. Hall terminated his fluothane studies, showing that fluothane has proven to be a potent non-combustible, non-toxic anaesthetic agent, and that Fluether was found to be non-combustible and a relatively stable agent by various chemico-physical criteria. The physiological effects of the latter drug in acute dog experiments generally paralleled those of fluothane, the latter being the more potent of the two. Dr. Hall and Dr. Norris have reported their findings in dog in Anesthesiology, in May, 1958, and September, 1958. Dr. Hall continued also his work in hypothermia in neuro-anesthesiology, and in the use of succinyl choline in the awake craniotomy. This latter project has been developed to the degree of proficiency that it represents an important adjunct to the surgery of epilepsy, and renders more successful the electrocortical studies performed in human patients.

Finally, Dr. Pritchard and Dr. Edgar have been studying the effects of hypertonic urea solution in reducing intracranial pressure in patients undergoing surgery with suspected brain tumor, and with ward patients with clinical evidence of increased intracranial pressure. They find that the brain volume and intracranial pressure may be reduced by the application of urea. Thus far no serious side effects have been noted, but not enough cases have been reported for full documentation at this time.

In closing, Clinical Investigations Unit also again acknowledges its debt to Miss Hulburt and her staff, particularly through a period of difficult transition during which each of the major branches transferred the majority of its admissions into given areas.

Much of the data reported herein is done with the cooperation of the Clinical X-ray Department, Clinical

Pathology, and the Instrument Section of the Central Services. Finally, we would like to acknowledge the cooperation and aid of the Atomic Energy Commission, Central Intelligence Agency, National Naval Medical Center, School of Aviation Medicine, and Walter Reed Army Hospital, as well as other Institutes of the National Institutes of Health, with whom many of the projects were undertaken.

At the request of the Director, National Institutes of Health, the projected program of the Clinical Investigations Unit to the year 1970 has been submitted. The suggestions of the Board of Scientific Councilors have been incorporated into this report.

G. Milton Shy, M.D.

SERVICES GIVEN BY THE CLINICAL INVESTIGATIONS UNIT OF THE NATIONAL INSTITUTE OF NEUROLOGICAL DISEASES AND BLINDNESS

Services given by the Clinical Investigations Unit to the Clinical Center of the National Institutes of Health are as follows:

One thousand four hundred and eighty-five (1,485) consults were rendered in either the in-patient or out-patient areas of other Institutes. Of these, one thousand and thirty-three (1,033) were Ophthalmology, ninety-six (96) Neurosurgery, and three hundred and fifty-six (356) Medical Neurology. Electroencephalographic Laboratory carried out one thousand five hundred and two (1,502) examinations. Of these, five hundred and forty-six (546) were patients referred by other Institutes. These were distributed as follows:

NCI	306
NHI	76
NIAMD	81
NMI	56
NIMH	27

Twenty-three electrocorticograms were performed on seizure patients in central surgery. Indwelling electrodes were implanted on thirteen subjects.

These figures show that the consultive services of the Institute have now levelled off with complete activation of all beds of the Clinical Center and reflect almost the exact figures given in the previous year.

Services by the Neurosurgical Unit were continued in which major intracranial or intraspinal operations were done on patients who were admitted to other Institutes. The collaborative project with the National Cancer Institute on hypophysectomy has continued and will be detailed in the report by Dr. Van Buren.

Eighty-seven (87) radioactive scan techniques were done on patients as a consultive service during the last year.

The Neuropathology Laboratory processed one hundred and twenty-four (124) surgical specimens, eighty-five (85) of these being muscle biopsies and eleven (11) of the latter were from

outside sources. One hundred and ninety-one (191) autopsy cases were processed and these included thirteen (13) Kuru cases and seven (7) cases from the Belgian Congo.

Collaborative work with the Physics Division of the Atomic Energy Commission has continued, as well as with the Central Intelligence Agency, National Naval Medical Center, Walter Reed Army Medical Center, and Johns Hopkins University.

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Collaborative work with the Physics Division of the Atomic Energy Commission has continued, as well as with the Central Intelligence Agency, National Naval Medical Center, Walter Reed Army Medical Center, and Johns Hopkins University.

SUMMARY

As in the previous years, the Branch activity has consisted of routine diagnostic service (for the entire Clinical Center) and research, the latter chiefly utilizing part of the patient population of NINDB, but also of other Institutes, as well as animal material for experimentation.

Since the last report up to the date this report is being prepared (November 30, 1958) a total of 1,502 electroencephalographic examinations has been carried out, patients from the various Institutes being distributed as follows:

NCI	306
NHI	76
NIAMD	81
NMI	56
NIMH	27
NINDB	956
Total	1,502

In collaboration with the Branch of Neurological Surgery 23 electrocorticographic studies were performed on occasions of cortical exposure during the surgical treatment of epileptic patients. In a few epileptic subjects in which electrodes were chronically implanted within subcortical structures for diagnostic localization purposes, extensive EEG studies were carried out under different conditions.

As in the past, a considerable number of EEG examinations were performed as part of research projects outside of our Branch and as a requested adjunct to research projects of Institutes other than NINDB, and this service has taken up a relatively large portion of the total activity of the Branch. Fortunately the active and proficient help of some of the staff members and of Dr. K. Abraham in particular, as well as skillful technicians and secretaries has made this collaborative service possible and, it is hoped, of some practical usefulness.

From the Branch of Electroencephalography a total of eleven research projects are in progress or have been completed within 1958. Of these eight are from the Section of EEG, five are new (4C, 5C, 6C, 7C, 8C), three are continuations of long range projects previously outlined in the 1956 and/or 1957 reports. Three are from the Section of Clinical Neurophysiology.

Projects 1C, 2C and 3C are all related to clinico-electrographic problems in the field of the epilepsies and their description has already been given in detail in the 1957 report.

Some data pertaining to projects 2C and 3C (79C-1957 and 84C-1957, respectively) have appeared in printed form in three papers during 1958 calendar year.

Project 1C (continuation of 78C-1957) is progressing very satisfactorily. The investigators are now processing and preparing the wealth of material accumulated in the last 30 months in form suitable for printing. Arrangements have already been made with the editor for the publication of a monograph - Atlas illustrating in detail the multiform patterns of the epileptic fit as well as their electrographic correlates in a large series of cases.

Among the new projects of the EEG Section, one is considered completed 6C, some of the data of two other projects are ready for publication or are actually in press 4C and 7C and the remaining two 5C, 8C are still under way and will be carried out through the coming calendar year.

Project 6C was carried out and completed by one of the research associates and the final paper has been accepted by the J. of Neurophysiol. It deals with the "control" exerted by a number of subcortical structures upon the thalamic and, chiefly, cortical potentials which can be evoked by peripheral nerve stimulation and which are considered as one of the electrographic manifestations of the arrival of centripetal sensory messages. This project had the honor of being officially commended by the Chairman of the Editorial Committee of the NINDB.

Project 4C is an experimental approach to the problem of epilepsy and specifically deals with the investigation on the nature of those EEG discharges considered as the characteristic and typical electrographic signs of epileptic lesions. The first part of this project was completed, the results presented at a National meeting and a paper is now in press. The study of other facets of the same general problem is now in progress.

Project 5C was initiated recently and no results are yet available for this presentation. It deals with the general problem of the relationship between relatively slow EEG changes in the cat's visual cortex and the behavior of local unitary elements. Most of the technical details involved in this experiment have been solved and the first results appear quite promising and of interest. It is hoped that some definite answers to the problem under investigation can be obtained within the first half of the coming calendar year.

Project 7C [illegible] investigation [illegible] has been completed. Much control data are still collected before the paper is ready for publication. The project deals with the unusual (and abnormal) EEG reaction which can be elicited in certain patients on steroid treatment when submitted to intermittent photic stimulation. In view of the observation that only patients with certain types of systemic disorders present this photic activation, a synergistic mechanism was tentatively suggested between the existing CNS pathology and the steroid effect. The high incidence of seizures in the same group of patients confirms the relationship between convulsive tendency and the observed type of photic activation and, indirectly, cautions against the (ab)use of steroid therapy in those cases in which such an activation is present.

Project 8C does not really deserve such a qualification and it is mentioned in this report only for the sake of offering a complete picture of the activity of the Branch. Notwithstanding the non-research character of this "project", however, it is felt that the little time spent in its actuation is far from being useless. At the present rate of scientific publications the availability of complete and specialized bibliographic references becomes almost indispensable and, unfortunately, the task can only be successfully undertaken by people having great familiarity with the field.

From the Section of Neurophysiology three projects were undertaken and partial results are now ready for publication.

Project 9C deals with the investigation of excitation and conduction of the nervous impulse in myelinated fibers and, in particular, with experiments on mammalian nodes of Ranvier. Preliminary observations suggest a similarity of patterns of ionic currents in mammalian and invertebrate nervous tissue. Some of the results chiefly relating to technical details are to be presented in early 1959 at the National convention of the Institute of Radio Engineers.

Project 10C is a study of the mechanisms of synaptic transmission. Besides a few interesting observations on the effects of the pH of the extracellular medium on the artificial depolarization of the post-synaptic membrane, most of the work is still concentrated on technical-methodological aspects of the problem.

A further project of the Section of Neurophysiology is only briefly mentioned here because it appears in full in the report of the Laboratory of Biophysics of NINDB. It was carried out in collaboration at the Marine Biological Laboratory at Woods Hole and deals with the interpretation of nerve function in terms of the fast transports of ions across the membrane of the squid giant axon. Preliminary results have been obtained and a paper (J. del Castillo and J. W. Moore: "On increasing the velocity of a nerve impulse") has been submitted for publication to the J. Physiol.

projects which have been completed in previous annual reports have appeared in their final form in this calendar year:

1. Ralston, B. L.: The mechanism of transition of interictal spiking [illegible] into ictal seizure discharges. EEG Clin. Neurophysiol. 1958, 10: 217-227

2. Ajmone Marsan, C.: Recruiting response in cortical and subcortical structures. Arch. ital. Biol. 1958, 96: 1-16.

A considerable contribution to the routine activity and research productivity of the Branch was provided by the numerous scientists who selected the Branch itself for either training or active cooperation in original investigative work. Visiting scientists (2), clinical associates (2), research associates (2) and guest workers (1), have taken active part in several research projects and in the diagnostic service. Some were already with the Branch in the last calendar year, while others plan to continue their work through 1959. Their enthusiasm and eagerness are only matched by their high sense of adaptability to the precarious space situation which, as already mentioned in the 1957 report, represents the only facet in the Branch physical organization which could stand some improvement.

It is with great sorrow that we wish to mention the untimely and sudden death of Doctor T. F. Enomoto who was one of our most promising and efficient research associates.

In closing this report, the Chief of the Branch wishes to express his sincere appreciation to the Clinical Director for his help, guidance and constructive cooperation.

National Institute of Neurological

Diseases and Blindness

Clinical Research

Electroencephalography Branch

Serial Numbers of Projects:

NINDB-1(c), NINDB-2(c), NINDB-3(c), NINDB-4(c), NINDB-5(c), NINDB-6(c), NINDB-7(c), NINDB-8(c), NINDB-9(c), and NINDB-10(c).

Estimated Obligations for FY 1959

Total: $96,500

Direct: $84,000

Reimbursement: $12,500

ORP-2

Serial No. NINDB-1(C)
1. Electroencephalography and Clin. Neurophysiol.
2. EEG
3. Bethesda, Maryland
4. Continuation of 80C, 1956; 78C, 1957

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Analytical study of focal cerebral seizures

Principal Investigator: Cosimo Ajmone Marsan

Other Investigators: Kristof Abraham

Cooperating Units: None

Man Years (calendar year 1958):
Total: .10
Professional: .10
Other: .10

Project Description:

Objectives: Outlined in the title; described in previous reports and in the Method.

Methods employed: Described in detail in the 1957 report (78C). Briefly it consists of a special photographic technique by which one obtains a series of closely spaced single frames synchronized with the EEG tracing. This provides a continuous and permanent recording of all the electrographic-clinical events taking place throughout the development of an epileptic seizure, and, chiefly, it permits a very analytical study of all the details which would likely escape the simple visual observation. It furthermore enables one to closely correlate motor-EEG phenomena thus obtaining results of unquestionable physiopathogenetic interest.

Part B included Yes ☐ No ☒

ORP-2(a)

Major findings: Over 150 seizures of different types have been elicited and recorded with the new method. These are routinely used in the weekly conference in the discussion of the patient's case. About 60 cases, representing the most interesting, unusual or demonstrative examples are now being selected for display and analysis with the respective portions of the EEG record. It is of interest to note obvious discrepancies between clinical and electrographic behavior, changes in the former showing no correlate with changes in the latter or vice versa. On the other hand, certain motor phenomena appear more often accompanied by EEG modifications than others, etc.

Significance to the program of the Institute: This project is part of a vast research program related to diagnostic, etiopathogenetic and therapeutical aspects of focal epilepsy and of temporal lobe epilepsy in particular, which is one of the main projects carried out by the Branch of Neurosurgery.

Proposed course of the project: Continue the routine collection of seizures for discussion of each patient's case. Complete the selection and analysis of the demonstrative examples and publication of an Atlas of such analyses to illustrate clinical-electrographic correlates as well as the details and the variability of patterns in focal cerebral seizures. The Atlas should be ready in early 1959.

ORP-2

Serial No. NINDB-1(C)
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4. Continuation of 80C, 1956
78C, 1957

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Part B included Yes [ ] No [x]

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ORP-2

Serial No. NINDB-2(C)
1. Electroencephalography and Clin. Neurophysiology
2. EEG
3. Bethesda, Maryland
4. Continuation of 81C, 1956, 79C, 1957.

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Depth electrography in epileptic patients

Principal Investigator: Cosimo Ajmone Marsan

Other Investigator: Kristof Abraham and John Van Buren

Cooperating Units: None

Man Years (calendar year 1958):

Total:	.10
Professional:	.10
Other:	.10

Project Description:

Objectives: See Project 79C, 1957

Methods employed: (See Project 81C-1956, 79C-1957). Since the last report only three new cases were studied in which electrodes were implanted in the depth of both temporal lobes and also on the cortical (or dural) surface of temporal and frontal lobes of patients affected with various types of epilepsy. The electrodes were kept in place for about 10 days during which time daily EEG tracings in various conditions could be obtained.

Major findings: The findings described in projects 81C-1956 and 79C-1957 have now appeared in published form (see Part B of this project). The data from the more recently collected cases are still in the process of being elaborated, particularly in regard to the relationship between deep and cortical electrographic changes. Of special interest is the study of the electrographic modifications, as recordable by means of implanted electrodes, during metrazol-induced (or spontaneous) seizures. The latter have been analyzed and will be presented together with the large series of seizure studies described in the previous project.

Part B included Yes [x] No [ ]

Significance to the program of the Institute: Same as in previous project.

Proposed course of the project: Patients suitable for this study have to be carefully selected in view of the possible risks involved in the technique and for this reason their number has been necessarily limited. Collection of further cases is contemplated and the proposed course of this project remains fundamentally unchanged.

Serial No. NINDB 2(C)

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Abraham, K and Ajmone Marsan, C.: Patterns of cortical discharges and their relation to routine scalp electroencephalography. EEG. Clin. Neurophysiol., 1958, 10: 447-461.

2. Ajmone Marsan, C. and Van Buren, J.: Epileptiform Activity in Cortical and Subcortical Structures in the Temporal Lobe of Man - in TEMPORAL LOBE EPILEPSY, C. C. Thomas, Springfield, Ill., 1958, 78-108.

Honors and Awards relating to this project.

NONE

ORP-2

Serial No. NINDB-3(c)
1. Electroencephalography and Clin. Neurophysiology
2. EEG
3. Bethesda, Maryland
4. Continuation of 79C-1956, 84C-1957

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Electrocorticographic studies in temporal lobe epilepsy and in focal cerebral seizures.

Principal Investigator: Cosimo Ajmone Marsan

Other Investigators: Maitland Baldwin

Cooperating Units: None

Man Years (calendar year 1958):
Total: .10
Professional: .10
Other: .10

Project Description: See 84C - 1957

Part B included Yes [x] No [ ]

Serial No. NINDB-3(C)

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Ajmone Marsan, C. and Baldwin, M.: Electrocorticography in TEMPORAL LOBE EPILEPSY, C. C. Thomas, Springfield, Ill. 1958: 368-395

Honors and Awards relating to this project:

None

ORF-2

Serial No. NINDB-3(C)
1. Electroencephalography and Clin. Neurophysiology
2. EEG
3. Bethesda, Maryland
4. New

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Epileptic activation of unitary elements of the cat cerebral cortex and their relationship with EEG discharges.

Principal Investigator: Takayuki F. Enomoto

Other Investigators: Cosimo Ajmone Marsan - Paul Gerin

Cooperating Units: None

Man Years (calendar year 1958):

Total:	1
Professional:	1
Other:	0

Project Description:

Objectives: To investigate the intimate nature of the EEG epileptiform paroxysmal patterns commonly referred to as "sharp waves" or "spikes".

Methods employed: Epileptic foci were produced experimentally on the gyrus suprasylvian of cat by means of local applications of different convulsant drugs (strychnine, penicillin, curare, etc.). The development of the relatively slow EEG discharges was monitored with a routine surface electrode and, upon their appearance, a systematic survey of the behavior of the various units within the different layers of the nearby cortex was carried out by means of Tungsten microelectrodes made according to Hubel's description. A similar method was applied in a few experiments in which paroxysmal discharges were elicited following intravenous administration of different drugs.

Several thousands of units were recorded and analyzed in 29 experiments carried out on cats.

Part B included Yes [x] No [ ]

Major findings: Observations were made on a) the general pattern of unitary activity, b) the general and detailed relationship between unit activity and slow EEG paroxysmal discharges, c) topographical distribution of the various activated unitary elements, d) analogies among the effects of the various convulsant drugs, and e) interaction between unitary elements.

It was found, among other things, that the two most characteristic features of unit behavior in coincidence with an EEG discharge are the paroxysmal appearance of high frequency bursts and a marked tendency towards synchronization of a very large number of different units. This hypersynchrony is not absolute because when a given element is characterized by rhythmical, high frequency firing in "resting" conditions, the common pattern in coincidence with the EEG discharge is an arrest either temporary or permanent, of the firing itself. This characteristic behavior was analyzed and discussed.

Unit activation may take place in correspondence with any phase of the EEG event, however, for a given unit the time course of its firing and the pattern of relationship with the whole of the slow event or a given phase of it, tend to remain quite constant. From these and other observations it is concluded that the number of units activated at a certain instant, their firing pattern, location and temporal interrelationship are closely related to - but not responsible for - the final shape, amplitude and polarity of the slow EEG event.

Significance to the program of the Institute: A better understanding of the essence of those EEG changes, which are considered almost pathognomonic in human epilepsy, is greatly needed. The significance of the above-mentioned data, obtained experimentally but closely pertinent to such a problem is evident if one considers that one of the main clinical projects at NINDB is the study of focal epilepsy.

Proposed course of the project: Part of this project is completed, the results presented at the June meeting of the American EEG Society and a paper submitted and accepted for publication. Further studies on the same line are now under way with the purpose of a) comparing local ("original") discharges with distant ("projected") ones in regard to unitary behavior; b) investigating the unit activity during fully developed seizures (the above study was limited to the inter-ictal discharges); c) extend the analysis of cortical unitary patterns following systemic administration of convulsant drugs.

Serial No. NINDB-4(C)

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Enomoto, T. F. and Ajmone Marsan, C.: Epileptic activation of single cortical neurons and their relationship with EEG discharges. EEG Clin. Neurophysiol. 1959, in press.

Honors and Awards relating to this project:

None

CRP-2

Serial No. NINDB-5(C)
1. Electroencephalography and Clin. Neurophysiology
2. EEG
3. Bethesda, Maryland
4. New

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Unit analysis of the responses elicitable in the visual cortex

Principal Investigator: Cosimo Ajmone Marsan and Lennart Widen

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1958):
Total: .10
Professional: .10
Other: .10

Project Description:

Objectives: As implied in the title, the purpose of this research is to analyze the various components of the complex potential evoked in the visual cortex (following stimulation of the lateral geniculate nucleus) with particular regard to the activation of the cortical unitary elements and their relationship with the slow surface response.

Methods employed: Acute experiments in cats either nembutalized or only curarized after a brief period of pentothal anesthesia for the craniotomy. Subcortical structures localized stereotaxically for stimulation. Recording from g. lateralis with silver macroelectrode and with tungsten microelectrode. Systematic survey with the latter through depth of cortex and underlying white matter.

Part B included Yes [ ] No [x]

Major findings: This series of experiments was started only recently and at the moment of the present report the various data have not yet been elaborated. From the first experiments however, there appears to be a wealth of interesting findings.

Significance to the program of the Institute: The study of the behavior of unitary elements in various cortical areas has been carried out quite extensively by a number of other investigators. In this project the emphasis is placed on the relationship between slow cortical event(s) and single cell activity. Its significance rests on the information one can obtain thereby for a better knowledge of the intimate essence of the EEG phenomena.

Proposed course of the project: Carry out this recently started project.

ORP-2

Serial No. NINDB-6(C)
1. Electroencephalography and Clin. Neurophysiology
2. EEG
3. Bethesda, Maryland
4. New

PHS NIH
Individual Project Report
Part A. Calendar Year 1958

Project Title: The modification of sensory mechanisms by subcortical structures.

Principal Investigator: R. Gordon Long

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1958)
Total: 1
Professional: 1
Other: 0

Project Description:

Objectives: To further elucidate the effects which the brain stem reticular formation, the non-specific thalamic system and other subcortical structures (basal ganglia, thalamic associative nuclei, rhinencephalic formations) may exert on peripherally evoked sensory potentials at thalamic and cortical levels.

Methods employed: Experiments carried out on 55 cats induced with ether, curarized and maintained on artificial respiration (some studies also performed on nembutalized animals). Bipolar stimulation and bipolar and monopolar recording technique was used. Subcortical structures for recording and stimulation were located stereotaxically and histologically controlled. Frequency for the test stimuli (to contralateral peripheral nerves) at 0.5/sec (rarely at 5-10/sec). Conditioning stimuli delivered at these approximate frequencies: 0.5/sec; 5-10/sec; 250/sec. Cortical recording from primary receiving areas (mostly somatosensory and visual) by means of Tektronix amplifiers and Dumont CRO.

Part B included Yes [x] No [ ]

Major findings: In the unanesthetized preparations the modification of somatic and visual potentials was obtained most easily and the effect was most prolonged when the conditioning stimuli were applied to the reticular formation of the brainstem. Conditioning stimuli to the non-specific thalamic system, amygdala, putamen, globus pallidus and lateral aspect of the head of the caudate nucleus were, in this order, decreasingly effective in modifying sensory impulses; stimulation of the pulvinar-lateralis posterior complex produced some modification of visual responses only. The changes recorded in the evoked responses were more marked at the cortical level than at the level of the specific thalamic relay nuclei. In general the modifications were of equal degree and duration in the primary and secondary cortical sensory areas.

The observation made by previous investigators, that high-frequency stimulation of the reticular formation will depress the amplitude of evoked somatic and visual responses, has been confirmed; this depression of amplitude was obtained more consistently and was of longer duration in the somatosensory system. In addition, it has been shown in this study that lower frequencies of stimulation to the reticular formation and its projections will augment the amplitude of evoked visual and somatic responses. The phenomenon of "rebound" increase in amplitude of evoked potentials after an initial depression has been observed and described. These modifications at sensory potentials were abolished or markedly diminished by barbiturate anesthesia.

Certain mechanisms by which these modifications may be produced are mentioned and discussed. It is suggested that the reciprocal effect of augmentation and depression of afferent conduction is analogous in certain respects to the facilitation and inhibition of motor responses by the reticular system, and that the augmentation of afferent signals may represent a mechanism which permits limited focusing of awareness or attention.

Significance to the program of the Institute: To quote from the comments of the Chairman of the NINDB Editorial Committee, "there are very few subjects of investigation which could have such important implications, not only to neurology and neurosurgery but to the psychological and epistemological branch of phylosophy as well".

Proposed course of the project: This project is completed. Findings and conclusions were written up and a paper submitted and accepted for publication in the J. Neurophysiol.

Serial No. NINDB-6(C)

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Long, R. Gordon: The modification of sensory mechanisms by subcortical structures. J. Neurophysiol, 1959, in press.

Honors and Awards relating to this project:

None

ORP-2

Serial No. NINDB-7(C)
1. Electroencephalography and Clin. Neurophysiology
2. EEG
3. Bethesda, Maryland
4. New

PHS-NIH
Individual Project Report
Part A. Calendar Year 1958

Project Title: EEG changes induced with photic stimulation in patients treated with ACTH and adrenal corticoids.

Principal Investigator: Kristof Abraham

Other Investigators: Nelson G. Richards

Cooperating Units: None

Man Years (calendar year 1958)
Total: .10
Professional: .10
Other: .10

Project Description:

Objectives: To describe the type of EEG changes obtained with photic stimulation in patients undergoing steroid treatment, review their clinical diagnoses and investigate possible physiopathogenetic mechanisms.

Methods employed: The record file of the last four years of the EEG Branch was reviewed. Out of 80 patients who had been on steroid treatment at the approximate time of their EEGs, 9 were found who showed an unusually marked response to photic stimulation. All of their charts were reviewed and their several EEGs including follow-ups, were re-analyzed in detail. The clinical diagnosis of the 9 patients was: lupus erythematosus (4), lymphatic leukemia (3), rheumatoid arthritis (1) and progressive ossificans myositis (1). Six of these patients developed seizures during corticoid or ACTH medication.

Part B included Yes ☐ No ☒

Major findings: Eight of these patients showed a "recruiting" type of response and one presented a "hypersynchronous" response to intermittent photic stimulation. Although the occurrence of such responses in the group studied is relatively low (11.2%), one must consider the fact that in a population of non-selected, non-epileptic subjects it has an incidence of only 1.3%. It is concluded that ACTH and adrenal corticoids contribute to lowering the convulsive threshold, acting at the brain stem level, upon probably already abnormal structures and the abnormal reaction to intermittent photic stimulation is, in fact, a manifestation of such a convulsive tendency.

Significance to the program of the Institute: In view of the fact that the occurrence of seizures in those patients who show an abnormal photic response is significantly higher than among those who did not present any particular activation to the same stimulation, practical considerations of prognostic value may be inferred. The abnormal photic response could actually be an early sign of impending convulsive disorder with manifest clinical fits and, therefore, caution should be exerted in such cases toward the continuation of a given steroid treatment.

Proposed course of the project: The first data have been collected in one paper almost ready for publication. Further cases and control studies are now gathered to better determine the role of the systemic disease or/and of the corticosteroids in the pathogenesis of the abnormal photic response and seizures.

ORP-2

Serial No. NINDB-8(C)
1. Electroencephalography and Clin. Neurophysiology
2. EEG
3. Bethesda, Maryland
4. New

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Bibliography on Electroencephalography and Clinical Neurophysiology, 1948-1958.

Principal Investigators: Cosimo Ajmone Marsan and Charles Henry

Other Investigators: None

Cooperating Units: Institute of Living, Hartford, Connecticut.

Man Years (calendar year 1958)
Total: .01
Professional: .01
Other: .18

Project Description: This is a minor project which the two investigators (both in the editorial board of the Journal of Electroencephalography and Clinical Neurophysiology) have been asked to undertake as a continuation of a similar work done by M.A.B. Brazier in 1948. The project title is self-explanatory. Most of the work is routine secretarial and is performed, as much as possible, without interfering with active clinical and experimental research projects. In view of the tremendous number of publications appearing in the last 10 years in the various scientific journals, both American and foreign, there is a need for a complete list of pertinent papers in this field, properly divided into subgroups and indexed in a practical and rational way. The organization of a cumulative 10-year index of the EEG Journal is also part of this project.

Part B included Yes [ ] No [x]

ORG-2

Serial No. NINDB-9(C)
1. Electroencephalography and Clin. Neurophysiology
2. Clinical Neurophysiology
3. Bethesda, Maryland
4. New

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Excitation in medullated nerve.

Principal Investigator: Jose del Castillo, M. D.

Other Investigators: J. W. Moore, Ph.D.

Cooperating Units: Laboratory of Biophysics, NINDB

Man Years (Calendar year 1958):

Total:	.50
Professional:	.50
Other:	.50

Project Description:

Objectives: The study of the basic mechanisms of nerve excitation and conduction of impulses has been mainly carried out in amyelinic fibers of invertebrates, which are extremely suitable, because of their large size, for the application of intracellular recording techniques. The results obtained in giant axons cannot, however, be applied indiscriminately to the medullated fibers of vertebrates and of man. The first objective of this project is, therefore, to repeat in vertebrate nerve some of the experimental work done in giant invertebrate fibers and obtain enough quantitative information to allow the computation of the equations governing the behavior of vertebrate excitable membrane. On the other hand, the extremely small surface area of the membrane exposed at the nodes of Ranvier makes the myelinic fibers particularly appropriate for the exploration of certain aspects of the excitation process which cannot be resolved when dealing with larger areas such as those offered by non-medullated axons, muscle fibers, or neuron bodies.

Part B included Yes [X] No [ ]

Methods employed: The experiments on medullated fibers will be carried out to begin with, in isolated motor axons of the frog (R. pipiens) and toad (B. marinus) and eventually in mammalian (mouse) fibers. The basic preparation will consist of a single node of Ranvier separated from the adjacent nodes by seals which create high external resistances. The potentials generated across the nodal membrane under study will be amplified and recorded by means of a circuit provided with a negative capacity input stage. One of the adjacent nodes will be used as a recording probe into the inside of the central node while the other will serve to inject the electric currents needed to stimulate, polarize and "clamp" its membrane.

Major findings: Since October 1957 until the end of April, 1958, full time was devoted to the development of an improved technique to perform "voltage clamp" experiments in the membrane of nodes of Ranvier of vertebrate medullated nerve fibres. Isolated motor axons of the frog (R.pipiens) and, in some experiments, mammalian nerve fibres have been used.

The immediate objective of this work was to combine the electronic resistance multiplier method of Frankenhaeuser with the special instrumentation for voltage clamp technique developed by Dr. K. S. Cole and his collaborators at the Biophysics Laboratory, NINDB. Many difficulties were at first encountered due to the extremely high longitudinal impedance of the internode through which the controlling currents are injected. Eventually, these difficulties were overcome and a method for the study of the permeability changes and ionic currents underlying excitation processes in vertebrate nerve is now available. The resistance multiplier method was also adapted in these experiments to minimize the external leak of the controlling current injected into the interior of the clamped node.

Preliminary experiments performed, for the first time, in mammalian nodes of Ranvier gave results that while pure qualitative in nature are of great interest from the viewpoint of the comparative physiology of excitation, as they have shown that the ionic currents elicited by controlled

depolarization of the mammalian nodal membrane conform to patterns similar to those found, and thoroughly analyzed, by previous investigators in invertebrate material. Their results can now be applied with confidence to mammalian nervous tissue. One of the original objectives of this research project may be considered as acomplished.

An incidental observation, worth mentioning, made during these experiments is the fact that when a medullated fibre is sectioned the cut end of the myelin tube tends to close in such a way that the leak of axoplasm is minimal and a high electrical resistance is maintained. This observation is interesting as it might explain why the resting potential of neurons in slices of nervous tissue is higher than one would expect on the basis of the short length of the cut nerve fibres. It might also have some technical significance as it provides the basis for a "single node of Ranvier" preparation which might have useful applications.

A paper dealing with technical details (J. del Castillo & J. W. Moore, "An Electronic Electrode"), will be presented in the Technical Program of the 1959 National Convention of the Institute of Radio Engineers.

Significance to the program of the Institute: The basic mechanisms of nervous activity, both in the CNS and the periphery, is the excitation process in which nerve impulses are generated. As pointed out above, practically all the information we possess on those processes derives at the present moment from studies in organisms widely different from the human. A reinvestigation of these mechanisms in vertebrates and, eventually, in mammalian nerve fibers is considered to be of importance for a more complete understanding of the physiology of the human nervous system, both health and disease. Furthermore, such knowledge is necessary for the elucidation of the mechanism of action of several types of drugs, mainly of local anesthetics, whose selective blocking action on the excitation process of nerve and muscle membrane is still in need of clarification

Proposed course of the project: Apart from experiments designed to obtain quantitative information on the behavior of the nodal membrane in conditions of controlled membrane polarization a number of subjects can be investigated with the technique developed. One of the most interesting being the study of the mechanism by which certain organic cations, such as the hydrazinium ions may replace Na ions in the excitable mechanism of the membrane.

Serial No. NIMH-00[illegible]
1. Electroencephalography and Clin. Neurophysiology
2. Clinical Neurophysiology
3. Bethesda, Maryland
4. New

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Study of Mechanisms of Transmitter Liberation at Presynaptic Nerve Endings.

Principal Investigator: Jose del Castillo, M.D

Other Investigators: None

Cooperating Units: None

Man Years (Calendar year 1958):
Total: .50
Professional: .50
Other: .50

Project Description:

Objectives: The studies of the mechanisms of synaptic transmission, both at the nerve-muscle junction and at the motoneurons of the spinal cord, have so far not thrown much light on the physiology of the presynaptic nerve endings, i.e. on the liberation of the chemical transmitters. This is due both to the small size of these nerve terminals and to the fact that, although a number of drugs acting on the post-synaptic membrane are available, very few agents capable of influencing the activity of the presynaptic nerve endings have so far been discovered. An attempt will be made, therefore, to find substances influencing the liberation of chemical transmitter, and to develop new methods for the investigation of the mechanism of action of those so far known.

Methods employed: The frog (R. pipiens) nerve muscle junction is the synapsis to be used because of its easy access, large size of the

Part B included Yes ☐ No ☒

post-synaptic cell and the possibility of direct microscopic observation of the motor nerve endings. Intracellular capillary microelectrodes will be used to record potential changes at the end-plate membrane. New optical methods will be employed to obtain a better view of the presynaptic nerve endings and to position external recording microelectrodes in its immediate vicinity. Ionophoretic methods will also be employed to apply substances to localized spots of the nerve endings.

Major findings: Experiments have been performed in order to study some of the ionic factors that influence the liberation of chemical transmitter and its combination with the post-synaptic receptors. In the course of this investigation it was discovered that the depolarization of the post-synaptic membrane produced by externally applied acetyl-choline is markedly influenced by the pH of the extracellular solution. This phenomena may help to elucidate the nature of the acetyl-choline receptor reaction.

Significance to the program of the Institute: The physiology and pharmacology of the presynaptic nerve endings are still in its very beginning; however, its importance for our understanding of nervous function does not need to be emphasized, as well as the interest of its pharmacological and therapeutic implications. Although we possess today a number of pharmacological agents which produce post-synaptic blocking on potentiating actions (ranging from d-tubocurarine to prostigmine) the potentialities of presynaptic influences on nervous junctions have not been explored so far.

Proposed course of the project: a) Setting up the necessary experimental apparatus for the electrical study of nerve-muscle junction. b) Exploration of new methods for direct microscopic observation of presynaptic motor endings, c) Future course of research to be determined in view of the technical improvements achieved.

Annual Report
Medical Neurology Branch
National Institute of Neurological
Diseases and Blindness

December, 1958

SUMMARY

The Branch of Medical Neurology admitted during the period covered by this report 215 patients for 5,974 total patient days. Thus each patient stay was 27.8 days. One hundred and fifty-six out-patients were seen.

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

In the Section of Neurological Disorders, a new investigation as to the medical treatment of seizures has been undertaken, which is dependent upon the findings of Brody and his colleagues in the National Heart Institute, of new monoamine oxidase inhibitors. Monoamine oxidase is the primary enzyme necessary for the breakdown of 5-hydroxy-tryptamine to the 5-hydroxy-indoles, the most important of which is 5-hydroxy-indol-acetic acid. The formation of the sulphate ester of this group in the urine has already been associated with a neurological disorder characterized by cerebellar symptomatology, dermitis, and mental retardation, under the name of Jepson's disease. Brody and his colleagues have found that the utilization of monoamine oxidase inhibitors in animals markedly reduces the epileptogenic threshold. Thus a double blind procedure has been instituted in which patients with centrencephalic seizures, having as many as 50 or more attacks per day, have been admitted, and a double blind procedure initiated, using the new monoamine oxidase inhibitor JB-516. As this is a double blind procedure, the results will not be known for approximately six months' time. At the present time, ten patients have entered into this project. This project is being carried out by Dr. Bushnell Smith and Dr. Darwin Prockop.

It has been noted that cases of orthrostatic hypotension have been noted to have many neurological disorders, in particular loss of sweating, loss of external sphincter controls, impotence, mental dulling, and, in some cases, a Parkinsonism-like syndrome, with or without ciliary atrophy. Three such patients have now been studied, and one such patient has come to post-mortem. To date no thorough anatomical study has ever been accomplished on a patient dying from orthrostatic hypotension. The importance of this single case, hence, is not to be underestimated. It was the decision that serial sections should be accomplished through the hypothalamus, sympathetic ganglia, the intermediate cell columns, and the cranial nerve nuclei of III, V, VII, IX, and X, as well as the basal ganglia, anterior horn cells, and cortex. This

necessitates literally thousands of sections, and the strict correlation of anatomy and pathology. It is anticipated that the thorough study of this one post-mortem case will need, in time, investigative use of a neuroanatomist for at least six months. This is being undertaken, at the present time, by Drs. Drager and Shy, and to date important findings have already been found in intermediolateral cell columns, the ventral cell columns, in Clarke's column, the dorsal nucleus of the vagus, the ventricular gray, and in the inferior olives. Degenerative changes in the cerebellum were also found with many torpedos. There were marked degenerative changes in the substantia nigra and in the mesencephalic nucleus of the trigeminal, as well as in the larger cells of the corpus striatum and the pyramidal cells of the cortex.

Similar to this is the study of a new syndrome recently described with rapid central nervous system deterioration, central blindness, nyclonus, and death in approximately three to four months. Here again, a long-term anatomical and pathological correlative study is being undertaken, with serial sections. This case will be studied extensively by Drs. Drager and Bushnell Smith.

As in past years, many of the patients admitted to the Branch of Medical Neurology are suffering from diseases of the motor unit. Recent advances in isotopic procedures and muscle pathology have changed radically this program from the past year. In combination with the Association of Research in Nervous and Mental Diseases, this Institute undertook, during the past year, a review of the effects of metabolic and endocrine abnormalities upon diseases of striated muscle. This was an over-all survey, employing chemical studies of muscle biopsies, combined with various metabolic tests with particular reference to hormonal levels of aldosterone, gonadotrophin, corticoids, ketosteroids, TSH, etc. Of particular interest were two disorders: Familial Periodic Paralysis and so-called McArdle's glycogen disease of muscle. In the former disorder, aldosterone levels were determined by the double isotope derivative methods. Intracellular cations on muscle removed both before and during attacks were also studied, as was pathology before and during attacks. And finally, microelectrode recordings of single muscle fibers in vivo before and during attacks. Potassium^{42} turnovers were also studied in this disease. The pathology of this disorder was quite striking in that large accumulations of fluid appeared intracellularly in approximately one-third of the fibers. Chemical determinations showed that in spite of this accumulation of fluid, that the cationic concentration of the cell remained approximately within normal limits. This was confirmed by microelectrode recordings, which showed resting potential of 71.2 ± 11.3, which is what might be anticipated if intracellular potassium were at normal levels.

Studies on aldosterone on these particular patients revealed that there was no increase preceding the attack, as previously reported by Conn. There was also a decrease in potassium in the urine preceding the attack, which indirectly confirms the latter observation done on double isotope derivative methods, in that if there had been aldosterone excretion there should have been a potassium diuresis.

Twenty-three cases of infantile neuromuscular disorders associated with hypotonia were also studied in reference to pathology, electromyography, and clinical course. From this, five different types of disorders were found in the disease state, which have been recently grouped into but one disorder. These findings have been reported by Drs. Greenfield, Corrman, and Shy, in the December issue of Brain.

The recent findings that DNA is probably inert in non-proliferating cells is now leading to the utilization of tritium labelled thymidine. This will be a powerful tool in the study of regeneration and growth of muscle, and this, combined with electronmicroscopy, will be undertaken by the Section of Biophysics.

Dr. Haase has, in addition, undertaken a long-range study of the pathological findings of intramuscular motor and sensory nerve endings in normal and in neuromuscular disease states, using the Coers technique of intravital methylene blue staining. He has confirmed axonal regeneration in neurogenic diseases, but the other abnormalities described by Coers and Wolfe has, as yet, not been verified.

The Section on Biophysics has completed its investigations on the localization of cerebral neoplasia by collimating techniques, utilizing various isotopes. Over 200 such patients now have been studied, with a confirmed accuracy of 86.2 percent. The final techniques and instrumentation utilized in this study, as well as the statistical evaluation, have been reported in monograph form by E. & S. Livingstone. This monograph was also utilized at the International Conference for Peaceful Use of the Atom. Similar procedures have now been initiated by the Institute for Johns Hopkins University, the National Naval Medical Center, Oak Ridge National Laboratories, and now at Los Alamos.

The studies of microelectrode recording in single muscle fibers have been utilized in familial periodic paralysis and myasthenia gravis. Due to the scarcity of the first disorder, this was done by cut-down method. In the myasthenic patients, continuing attempts are made to record single muscle fibers through the intact epidermis. The Bak Unity Gain Amplifier has been utilized as optimal with a constant current sent back into the grid of the cathode follower. This latter allows constant

sampling of the condition of the probing electrode. To date, endomysium and perimysial connective tissue has been the chief stumbling-block, in that the electrodes intermittently plug or break. Of the literally hundreds of recordings which have been attempted to date, only five successful intracellular penetrations have been made through the intact epidermis. The continuity of this project will depend upon the ability to overcome the difficult techniques listed above.

In the Clinical Director's Report each year, an attempt has been made to select areas of outstanding contribution. This year the studies conducted in the laboratory of clinically applied pharmacology, under Dr. Richard Irwin, have accomplished much which will show considerable insight as to the interrelationship of blood and tissue cholinesterase systems, their substrata, other enzyme systems working upon such substrata, and basic fundamental knowledge as to the differentiation between depolarizing and competitive blocks, as well as insight as to where in the muscle fiber the blockading compound has its maximal effect. Thus, Dr. Irwin and his colleagues have demonstrated that competitive blocking compounds, such as d-tubocurarine and depolarizing blocking compounds such as decamethonium, may be differentiated in their action by inhibition or excitation of muscle cholinesterase; thus the competitive block of d-tubocurarine is reduced or prevented by inhibition of muscle cholinesterase. On the other hand, the block of depolarizing drugs is prolonged by the inhibition of plasma cholinesterase or muscle cholinesterase. In the case of decamethonium, this cannot be due to destruction by cholinesterase, per se, as decamethonium has no ester group and hence could not be destroyed by cholinesterase. Succinylcholine, on the other hand, has an ester group, and thus could be destroyed by cholinesterase. It is of interest, however, that the prolongation of the blockade by inhibition of plasma cholinesterase is identical to the two substances, thus showing that this inhibition prolongation is not of necessity due to destruction, or the depolarizing compound. Thus one can assume, I believe correctly, as Dr. Irwin and his colleagues have assumed, that muscle cholinesterase has but a minor role in relation to the total block. If this substance, however, is not metabolized by plasma cholinesterase, then inhibition of muscle cholinesterase has a marked effect on the blocking activity, and the non-depolarizing substances upon such inhibition of muscle choline demonstrate a decrease in their blocking power, whereas the depolarizing substances demonstrate an increase in their blocking power.

Dr. Irwin and his group have continued their studies on the action of directly stimulated innervated and denervated muscle. In this they have been aided by a device, created by Mr. Wells, of an optical-isotonic lever system, recorded through a cathode ray oscilloscope. With this mechanism, they have been able to

demonstrate that the block is not due to increased muscle compliance, as added compliance in series does not give contractile responses similar to those obtained with succinylcholine or decamethonium. If this isotonic system is observed closely, one may see there is less shortening of the fiber and reduced velocity of shortening, again showing that this is not an increased compliance of the muscle fiber. The isotonic-optical system allows this, in fact, that it reduces the elastic component of muscle. This system, however, does demonstrate a prolonged latency from the onset of the stimulus to the time of contraction after administration of depolarizing compounds. These investigators feel there is a spatial distribution of the depolarizing blockade over the muscle membrane, indicating either multiple end plates upon the muscle membrane, or a temporal spread from a single membrane, i.e. one end plate. These investigators point out that muscle cholinesterase is low in quantity and is not uniform in various species and/or organs, and hence has a species and organ specificity. It is thus dependent upon the substrate and enzyme activity. Thus, muscle cholinesterase studied as to substrate specificity and well-known inhibitors would give considerable information as to the chemical interchange between the substrate and the enzyme.

The cholinesterase of muscle homogenates, in which the blood was removed so the plasma cholinesterase was not present, was studied. Such homogenates hydrolyzed acetylcholine more rapidly than benzoylcholine, or butrylcholine. An excess of the substrate, however, would inhibit such hydrolyzes, the optimal level being 5×10^{-3}. The optimum level of concentration for substrates other than acetylcholine are higher. Thus, muscle cholinesterase is highly specific. However, since benzoyl- and butrylcholine are hydrolyzed at measurable rates, small amounts of non-specific enzyme must also be present. It is of interest that neostigmine depolarizes the membrane at 10^{-3}, whereas pyridostigmine (mestinon) will not. This becomes of double interest in that both drugs are highly useful in the treatment of myasthenia gravis. Galanthamine, which has been isolated from an alkaloid in the United Soviet Socialist Republic, and utilized in the treatment of myasthenia gravis, was also studied by these investigators. Galanthamine is a phenanthrene derivative and not a carbamine ester. Dr. Irwin and his group found a 50 percent inhibition at 6×10^{-6}. The value for the inhibition of plasma cholinesterase was the same. Neostigmine and physostigmine inhibit at lower concentrations as far as cholinesterase in the muscle is concerned, but in vitro inhibit more rapidly than with galanthamine.

Finally, these investigators are studying the possibility of choline esters other than acetylcholine occurring as natural constituents of biological systems; the object being to determine

to what extent the choline esters are found in such biological systems and related compounds, and how they depolarize tissue membrane. Secondly, to relate the depolarizing properties of these compounds to their stimulation or blocking activity of synapses, and finally to study the metabolism of these compounds by tissue enzymes. To study this, the travelling fluid electrode technique is used to measure depolarization of the isolated frog sartorius muscles, and microelectrodes will be utilized to determine the resting membrane potentials, presumably through the Bak Unity Gain Cathode Follower. These investigators have found, in high concentrations, i.e. 10^{-3} molar, that butrylcholine, benzoylcholine, and imidazoleacrylcholine, all resemble acetylcholine in their depolarizing properties. Methacholine, however, does not depolarize muscle membrane. These investigators have also found the plasma from myasthenic patients have been observed to metabolize imidazoleacrylcholine at the same rate as plasma from non-myasthenic patients. And finally, these investigators are attempting to find to what extent depolarization of the muscle membrane may effect the efflux of enzymes from inside the muscle fiber, in particular aldolase. This latter project is projected into the coming year.

The Section of Neuroradiology suffered in having its chief investigator, Dr. Giovanni Di Chiro, undergo surgery for a major illness. In spite of this setback, however, Dr. DiChiro was able, upon his return to duty, in addition to his heavy service responsibilities, to carry out in combination with Dr. Martin Rubin, of Georgetown University, a research project which culminated in a paper concerning the metal chelates as possible contrast media for myelography. These chelating compounds were tested against commonly used iodinated contrast media. Different concentrations of the various chelating compounds were tested in order to determine the concentration for optimal opacity. Once such opacity was determined in vitro, it was tested in vivo on dogs and rabbits. Chelating agents used are listed in Dr. Di Chiro's report, with primary interest on lead ethylenediaminetetraacetic acid. This substance was administered at the dose level of 10 milligrams per kilo, and appeared in the urine to the extent of 85-89 percent of the injected dose within two days. Of that retained in the animal, i.e. 10-15 percent, 50 percent was found in the liver and some 20 percent in the bone marrow. This demonstrated that, despite the large amount of excretion, the amount retained is not to be discounted. The experiments in vivo show that studies of good diagnostic quality may be obtained as far as x-ray contrast and detail are concerned, with radiopaque metal chelates. However, the acute toxicity of the metal chelates in myelography, as well as in most of the other x-ray examinations carried out, proved to be too high. Accordingly, Drs. DiChiro and Rubin are going on to undertake studies in other metal chelates with high atomic number, in hope that in this screening one agent

of local toxicity would be found which was so low as to suggest it could be used in clinical myelography.

The Section of Neurochemistry continued its efforts in the major fields listed in the 1957 report. Dr. Horvath continued his studies in the distribution of actin and tropomyosin in normal and diseased muscle, his comparative biochemistry studies of smooth muscle and striated muscle, and alterations of actomyosin tensile strength and muscle proteins in neuromuscular diseases.

Dr. Tower and his colleagues have continued their studies on the metabolism of γ-aminobutyric acid in neural tissue, with the aid of Dr. McKhann and Dr. Wherrett. Studies on the relation of pyridoxine to certain seizure states, in particular in those cases known as pyridoxine dependency, continued. Dr. Tower continued his elaborate studies on amino acid metabolism in normal and epileptogenic cerebral cortex in vitro, and in electrolyte energy metabolism in normal and epileptogenic cerebral cortex. The unit as a whole continued its clinical evaluation of amino acids and related compounds in control of seizures in man.

Dr. Curtis continued in the realm, predominantly, of surface-chemistry, and in other physico-chemical methods in determining constituents of human spinal fluid, ocular fluid, etc.

Dr. Tower's studies specifically now revolve around C^{14} and N^{15} labelled compounds. Two-deoxyglucose was utilized as a competitor for glucose utilization, by inhibiting the hexokinase step primarily due to depletion of available ATP required for this step. Dr. Tower found it was possible to overcome the 2-deoxyglucose block in glucose utilization by adding either ATP or glucose-6-phosphate to the slices in anaerobic conditions. No effect of these additions, however, was obtained in aerobic metabolism, presumably due to their failure to penetrate the slices. Dr. Tower felt that 2-deoxyglucose inhibition did not result in any activation of the glucose-6-phosphate dehydrogenase or in any oxidative shunt pathway. These findings were checked by incubating the control and inhibited slices with glucose-1-C^{14} and glucose-6-C^{14} phosphate, determining the utilization of $C^{14}O_2$ and C^{14}-lactic acid production. Since the ratios of the C^{14} lactate from the C-6 compared to C-1 samples were 1.0 in both cases, whereas C-6/C-1 would be less than 1.0 if the shunt pathway were utilized, this would indicate that this inhibition was not due to an oxidative shunt pathway. This was indirectly confirmed by the finding of low level brain TPN by other investigators in that TPN is the necessary coenzyme for the shunt pathway. Dr. Tower found also that 2-deoxyglucose inhibition not only resulted in marked decrease in glycolysis, but also in oxidative metabolism. Thus, with glucose-U-C^{14}, less $C^{14}O_2$, less labelling of the free amino

acid pool, and less C^{14} lactic-acid were all obtained. From these studies with C^{14}-labelled glucose, the distribution of glucose utilized by normal slices to various intermediary steps could be estimated, thus glycolysis to lactate, 70%; amino acids measured by glutamate, 22%; respiratory CO_2, 7%, and other intermediaries, such as lipid and protein, 1%. If one calculates the oxygen uptake as 85μM./g/hr., it is clear that if 30% of the latter is accounted for as amino acid and respiratory CO_2 that this almost exactly balances the oxygen uptake, assuming 6 moles of the latter per mole of glucose oxidized. This is consistent with studies of other laboratories and this laboratory, that non-glucose substrates, such as amino acids, normally support oxidative metabolism by the brain and they are repleted subsequently by part of the glucose utilized. Studies with 2-deoxyglucose clearly demonstrate, according to Dr. Tower, that glucose is necessary to make repletion of non-glucose intermediates possible, and energy production rapidly falls in its absence, and that this is not only by depletion of ATP and creatine phosphate but also by deleterious effects on glutamic acid and electrolytes in the inhibited slices. As in his 1957 report, Dr. Tower points out that such inhibited slices fail to extrude excess sodium and reconcentrate potassium in normal manner. This is similar also to defects seen in slices which have been removed from epileptogenic patients, and Dr. Tower has also found this in cortical slices from cats with seizures enduced by 3-methyl-e-ethylglutarimide, and by methionine sulfoximine. Utilizing the Cotlove apparatus, Dr. Tower and his colleagues find that the swelling of normal and epileptogenic slices during incubation is confined to the chloride space, and that calculations of electrolyte concentration per litre of non-chloride space water at the end of slice incubation demonstrates again a loss of potassium and a gain of sodium.

Dr. Tower has continued his studies on incubating slices of cat cerebral cortex with L-glutamic acid labelled with C^{14}; L-glutamine labelled with C^{14}; γ-aminobutyric acid labelled with C^{14}; L-aspartic acid labelled with C^{14}; D-L-asparagine labelled with 2,3-C^{14}; D-glucose labelled with C^{14}; sodium pyruvate-3-C^{14}; and 2-pyrrolidinone-2-C^{14}. Using these compounds, Dr. Tower was able to determine the order of labelling in amino acids, and was able to show this had considerable significance since the aspartic acid could prime the Krebs cycle by providing both oxalacetate and acetyl-Coenzyme A (from pyruvate) in the absence of the latter from glycolysis. Dr. Tower concludes that these studies indicate how active the components of the glutamate-aspartate amino acid group are in metabolic participation in the Krebs cycle, and feels that the release of CO_2 measured by C^{14} liberated during these experiments confirmed this conclusion.

In the second part of his experiment, Dr. Tower analyzed

the liberation and formation of glutamic acid, glutamine, γ-aminobutyric acid and free ammonia metabolism in incubated slices from non-cortical areas of the cat brain; these were the subcortical white matter, the thalamus, the caudate nucleus, and the cerebellar cortex. He found the levels and metabolic behavior in all gray, i.e. neuronal areas, were similar to that previously observed in the cortex, but that the white matter exhibited extremely low levels and little change on incubation for glutamic and γ-aminobutyric acids, while the white matter glutamine was not greatly different from the cerebral cortex. In studies on the levels of these substances, Dr. Tower felt, using the calculations of Elliott and Heller, that at least 85 percent of cortical glutamic and γ-aminobutyric acid content was associated with neurons, while only about five percent of the glutamine appeared to be neuronal in location. The cerebral cortex was fractionated by the Brody and Bain technique, and Dr. Tower found the majority of glutamic and γ-aminobutyric acids were associated with fraction R_3 or the mitochondrial fraction, whereas glutamine was distributed almost equally between that fraction and the combined $R_1 + R_2$ fractions which contained cell debris, axon fragments, nuclei, etc. No content of any of the three amino acids was found in the microsomal fraction. The finding of these substances in the mitochondrial fraction is compatible with their close association with the Krebs cycle.

Dr. Tower's studies also indicated that the inhibition of glutamine synthesis by methionine sulfoximine is primarily an interference with ammonia moiety, possibly by the imine group of the toxic compound, and that by adding only ammonium chloride such a block could not be overcome unless adequate amounts of glutamic acid are available to amidate to glutamine. Studies with similar epileptic agents, such as Megimide showed that the glutamic acid metabolism was blocked to include γ-aminobutyric acid, in that the latter compound was significantly lower than normal. The same was true when inactivators of pyridoxal phosphate were used. If malonate, however, was used, the amount of glutamic acid and γ-aminobutyric acid in the slices rose to double the normal values. The action of malonate is to inhibit succinic dehydrogenase. Since this was accompanied by reduction of oxygen uptake, it was previously not clear why such slices did not also show succinate accumulation. Dr. Tower's data suggest that in the whole cell preparation it is glutamate and γ-aminobutyrate rather than succinate which accumulates and requires a study of the relationships among these three components of Krebs cycle. Dr. Tower plans to continue these interesting experiments, using the microanalytical method of Dr. O. H. Lowry.

Clinical evaluation of various amino acids and related compounds in the control of seizures in vivo in man has been

continued by Dr. Tower and Dr. McKhann, and the Branch of Electroencephalography. Patients on γ-aminobutyric acid have continued to do well in Dr. Tower's estimation, one patient being seizure-free after three months on the compound, compared to multiple daily seizures previously. On stopping the compound, the seizures returned and have again been abolished by starting γ-aminobutyric acid. Several other patients are getting more benefit from γ-aminobutyric acid than from l-asparagine. Gamma-aminobutyric acid has been given intravenously to levels of 4mM/kg. body weight, with no untoward effects, in dogs. However, when 1/200 of this dose is administered to man, there is immediate agitation, flushing, hyperpnea, and a drop in diastolic blood pressure. Recovery occurred within 5-10 minutes. Dr. Tower rightly points out, despite the reports by Elliott that such occurrences can be ignored, it would seem that this potentially a dangerous drug given intravenously. Another case of pyridoxine dependency has been worked up by Dr. McKhann and Dr. Tower. These patients were also studied by the Krypton^{85} technique for measuring cerebral metabolism developed by Sokoloff. The original case of Hunt was restudied, and the patient, now seven years old, is still dependent, regularly developing seizures within 72 hours of omission of her regular daily dose of 10 mg. of pyridoxine. Typical EEG abnormalities could be abolished in 30-60 seconds by intravenous pyridoxine-HCl (15 mg.). During a typical period of depletion, cerebral metabolism was measured by Krypton^{85} technique, and the decreased oxygen consumption during the depleted state in this case was similar to the situation reported by Sokoloff for hypoglycemia subjects. Thus, the interpretation tentatively put upon the data obtained in this case is that during pyridoxine depletion a deficiency of the substrate for cerebral oxidative metabolism exists which is promptly corrected by pyridoxine administration. Since pyridoxine deficiency affects γ-aminobutyric acid metabolism primarily, and since that compound appears to be a significant substrate, Dr. McKhann and Dr. Tower rationalize that this case may actually represent an example of -aminobutyric acid deficiency, with a consequent reduction in oxidative metabolism. Drs. McKhann and Tower have continued their studies of the metabolism of γ-aminobutyric acid in neural tissue by using the Fluorimetric method, as described in the 1957 report. They appear to have demonstrated that the shunt pathway, i.e. glutamate to γ-amino-butyrate appears to be active and important in cerebral oxidative metabolism, and is significantly involved in certain dysfunctions of the brain, such as seizures. They plan to undertake further studies to see how such a pathway may exert a regulatory control on oxidative metabolism and hence on energy production in terms of normal function and of seizure states.

Dr. Curtis is continuing his studies on physico-chemical methodology in an attempt to obtain quantitative data from fluids which contain extremely small amounts of organic metabolites. He

is working particularly on the surface tension of urine, and in particular optical measurements by polarized light and its reflection off of surfaces utilizing the elliptical polarization as an indication of the thickness of the surface interface. The apparatus has been built in combination with the Naval Research Laboratories and exploration of this approach is now being orientated towards the use of photomultipliers, so that the square function may be utilized, and monochromatic light. Parallel with this he is continuing his studies of adsorption on solid surfaces, such as column resins, in foams and interfaces in urine and water-immiscible liquids. Dr. Curtis has now found that there is so much gross interference in the acethylcholine-boron-flavonaol reaction to biological materials as to make this procedure unsuccessful, in the determination of microchemical amounts of acetylcholine. His studies on guinea pig serum asparaginase detailed in 1957, have now been completed, except for some electrophoretic and ultracentrifuge data now in progress. He finds that the purified enzyme preparation contains two macromolecular contaminants which have defied attempts at separation by electrophoresis or ultracentrifugal means; that enzyme can be quantitatively adsorbed on a modified cellulose and in carbon dioxide foam, and purification by these means is currently being attempted.

Dr. Horvath is continuing his work on proteins of muscle in normal and diseased states, and has calculated total solids, total protein, non-protein solids (i.e. fat), non-collagenous proteins, collagen, water-soluble proteins, myosin, alkali-soluble proteins, non-protein nitrogen, electrolytes, and tissue water. He finds differences in the normal and dystrophic muscle analyses are reflected by connective tissue and fat, and by an increase in sodium and chloride in dystrophic specimens. He finds there is a relative increase of myosin and decrease in alkali-soluble proteins in most cases. These changes seem to be independent of the remaining muscle mass. The water-soluble proteins appear to be increased relative to other proteins in most dystrophic samples and an inverse relationship is indicated between the remaining muscle mass and the percentage of water-soluble proteins in the muscle on the other hand. He concludes that samples of dystrophic muscle not only contain less muscle and more connective tissue and fat than normal muscle, but that the protein composition of the remaining muscle is different from the normal.

In the study of actin and tropomyosin in normal and diseased muscle, Dr. Klatzo and Dr. Horvath are turning to immunological properties of functionally important muscle proteins. They find that rabbits immunized against serum tropomyosin A, clam tropomyosin A, mammalian myosin, and antisera to human and cat myosin precipitate clam tropomyosin A. No such cross-reaction was found between antisera to chick tropomyosin B on the one hand and clam tropomyosin A or mammalian myosins on the other. Using antibodies

to myosin conjugated with fluorescein, myosin in sections of normal human muscle was clearly and distinctly demonstrated over the fluorescent microscope. Preliminary sections of dystrophic muscle similarly treated showed myosin in residual islands of muscle and a suggestion that in areas of active degeneration myosin-reactive material was present in macrophages. Thus the immunological findings are consistent with the present concepts of the myosin molecule consisting of subunits - Tropomyosin A, B and Actin, the latter can be prepared in a high state of purity than myosin itself, so that it is more suitable for investigational purposes. Since these proteins are also iso-antigenic, the immunological response 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 circulation of the body. Dr. Horvath is continuing the same studies in muscle protein in electrolytes in dystrophic mice obtained from Bar Harbor.

Finally, Dr. Korengold and Dr. Hempp have concluded their studies, which were an attempt to confirm the findings of spirochetes in the cerebral spinal fluid, with patients suffering from multiple sclerosis. Identical material to that used by Ichelson was utilized, and a trip was made to Dr. Ichelson's laboratory to be certain that there were no differences. Twenty-two patients were studied in the out-patient area, at which time spinal fluid was removed. No positive cultures were obtained, and it was felt desirable to terminate the project after this number of studies. This concludes the major findings of the Branch of Medical Neurology.

National Institute of Neurological

Diseases and Blindness

Clinical Research

Medical Neurology Branch

Serial Numbers of Projects:

NINDB-11(c), NINDB-12(c), NINDB-13(c), NINDB-14(c), NINDB-15(c), NINDB-16(c), NINDB-17(c), and NINDB-18(c).

Estimated Obligations for FY 1959

Total: $691,000

Direct: $227,500

Reimbursement: $463,500

1. Medical Neurology Branch
2. Section on Neurological Disorders Services
3. Bethesda, Maryland
4. New Project

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: The Use of a Monoamine Oxidase Inhibitor (JB-516) as an Anticonvulsant Medication for Centrencephalic Seizures.

Principal Investigators: Darwin Prockop
Bushnell Smith

Other Investigators: Andrew Engel
Mark Lane

Cooperating Units: Kristof Abraham, Electroencephalography Branch
John Oates, National Heart Institute

Man Years (calendar year 1958):
Total: 1
Professional: 1
Other: 0

Patient Days (calendar year 1958): 40

Project Description:

Objectives:

Chen et al., have demonstrated in animals that reserpine lowers the threshold for electrically induced convulsions possibly by lowering the amount of brain serotinin (5 hydroxytryptamine) and norepinephrine while Prockop et al., showed that monoamine oxidase inhibitors, which increased the level of serotinin, prevented the tonic extensor phase of electrically induced seizures and generally raised the convulsive threshold to electro shock and pentylene tetrazol.

The objective of the present study is to evaluate the effectiveness of JB-516 ([phenyl isopropyl]-hydrazine) as an anticonvulsant in persons with centrencephalic seizures.

Methods Employed

The centrencephalic seizure with its symmetrical electroencephalographic abnormality (3 per second wave and spike) as well as its clinical frequency of many per day is the type most suitable to evaluation on a short term basis. It may possibly be the one to be most affected by JB-516 because of the higher concentrations of serotinin in the supposed sites of origin of the seizures (Brain stem, Hypothalamus, Thalamic Projections). Patients with centrencephalic seizures will be maintained on the anticonvulsant medication prescribed for them before admission (which in these selected cases will not be completely controlling the seizures) to prevent wide swings in the number of seizures that occur. An equal number of patients will be placed on placebo and on JB-516. The cases to be given JB-516 will be determined arbitrarily by the pharmacist who will have no direct contact with the patients. To determine if the JB-516 produces any inhibition, determination of 5 Hydroxytryptamine in the urine of all patients at the end of one week will be made. Electroencephalographic studies will be made at regular intervals. These will serve as a basis for compilation of effectiveness of medication. Clinical records will be kept of seizures.

Major Findings: It is too early for any results at this time.

Significance to Neurological Research: If the inhibition by the monoamine oxidase inhibitor (JB-516) of the oxidation reaction of 5 hydroxytryptamine to 5 hydroxyindoleactic acid also results in marked inhibition of seizures of the centrencephalic type, new areas of investigation will be opened as to the nature and possible etiology of centrencephalic seizures.

Proposed Course: Ten patients previously studied and known to have centrencephalic seizures, according to the classification of Penfield, will be admitted to the hospital for a period of 6-7 weeks. A baseline period of one to two weeks will be used to evaluate the intensity and number of seizures. Electroencephalograms, level of 5 hydroxytryptamine in the urine and coordinated studies with Drs. Van Buren and Mirsky will be carried. During the remaining five weeks, the patient will receive either JB-516 or placebo in addition to the prescribed anticonvulsant medication. Electroencephalograms will be done at the same hour of the same day each week by the same technician. Clinical course will be closely followed.

A comparison of all data and correlation with the drug that the patient took will be made after the last patient has been studied.

Part B included No

Serial No. NINDB-12(c)
1. Medical Neurology Branch
2. Section on Neurological Disorders Services
3. Bethesda, Maryland
4. New Project

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

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

Principal Investigator: Glenn A. Drager, M. D.

Other Investigator: G. Milton Shy, M. D.

Man Years
Total: 1
Professional: 1
Other: 0

Patient Days: 42

Project Description:

OBJECTIVES:

As a clinical unity orthrostatic hypotension refers to a condition characterized by a significant fall in blood pressure when the patient changes from the recumbent to an erect posture.

The condition was first described by Bradbury et al. in 1925. Since that time approximately 200 reports have appeared in the literature, dealing principally with the clinical sequelae of the disorder, with emphasis on the cardiovascular manifestations. Neurological features have been commented on, and it is stated that neurologic signs are found in at least one-fourth of the cases.

It has been assumed that the site of the lesions are either in an autonomic center or in an efferent pathway, or that it is generalized in the central nervous system, efferent pathway, or nerve endings. This assumption does not explain the manifestations of extra-pyramidal and motor neuron systems, which are often associated with orthrostatic hypotension.

The literature fails to reveal any post mortem studies of the central nervous system of patients with this disorder in which a clinical pathological correlation was made. The present investigation concerns the post mortem findings in the nervous system of a patient who had this disorder and was thoroughly studied from the clinical standpoint.

PATIENT MATERIAL:

One patient, a 54 year old white male, was admitted to the Clinical Center in January, 1957 with a 6-year history of episodes of dizziness, loss of libido, nocturia changes in coordination, speech changes, and a resting tremor of both upper extremities and of the jaw. Anhidrosis had also developed. Orthrostatic hypotension had been present for at least six years.

The patient was completely studied in the hospital for a total of 135 days. Terminally, he developed fever of central origin and expired. The central nervous system was obtained at autopsy.

In addition, one other patient is being studied in the National Institute of Neurological Diseases and Blindness with a similar neurological syndrome associated with orthrostatic hypotension. Other patients with this clinical syndrome have been examined in other Institutes.

METHODS EMPLOYED:

The brain, spinal cord, and autonomic ganglia were removed from the body within a few hours after death and fixed in formalin. The brain was cut and microscopic sections were prepared in the usual manner. The sections were stained or impregnated with technics that demonstrate neurons, glia and lipids. To adequately study this unique opportunity serial sections through the hypothalamus, the III, V, VII, IX, X cranial nerves - dorsal root ganglia, and intermediate cell columns of the cord is necessary. This necessitates the careful study of literally thousands of sections.

MAJOR FINDINGS:

In the spinal cord, pathological changes were found in the intermediolateral cell column and, to a lesser extent, in the ventral cell columns and in Clarke's column. Demyelinization was observed in the fasiculus gracilis. This was marked in the cervical segments of the cord. Gliosis was also present. Lipid, which stained with sudan III, was present in many cells of the spinal cord but was particularly prominate in the ventral horn cells.

In the medulla, degenerative changes were found in many cells. The dorsal nucleus of the vagus demonstrated a marked falling out of the cells. Gliosis was also present around the ventricular gray and in the inferior olives. As in the cord, considerable lipid was found in the motor neurons.

The cerebellum showed a marked decrease in the number of the Purkinjie cells. Many torpedos were present, as well as increased gliosis, and considerable lipid, in the cells of the dentate nucleus. Some diffuse degenerative changes were found in the pons and tegmentum.

Marked degenerative changes were observed in the substantia nigra. Considerable extracellular pigment was present. Diffuse degenerative changes are present in the motor and reticular neurons. Gliosis was found in the central aqueductal gray. Lipid was present in the motor neurons and in the cells of the mesencephalic nucleus of the trigeminal nerve. Changes may be present in the Edinger-Westphal nucleus but these are questionable, and more sections are being prepared for study.

Diffuse degenerative changes have been observed in the larger cells of the corpus striatum and the pyramidal cells of the cortex, however, the study of these areas of the brain have not been completed.

SIGNIFICANCE TO NEUROLOGICAL RESEARCH:

To study and report the degenerative changes found in the central nervous system of a patient with orthrostatic hypotension and the associated neurological syndrome. No clinical pathological study of this syndrome has been made.

PROPOSED COURSE:

To study additional patients with this disorder and, if possible, obtain additional material for pathological study.

Part B included No

NIMH-23(c)
1. Medical Neurology Branch
2. Section on Neurological Disorders Service
3. Bethesda, Maryland
4. Continuation of NIMDB-23(c)

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: The Histopathological and Chemical Investigations of Neuromuscular Disorders

Principal Investigators: G. Milton Shy and Theodor Wanko

Man Years:
Total: .20
Professional: .20
Other: .20

Patient Days: 762

Project Description:

Objectives: The objectives determine (1) variables in DNA in the growth of muscle; (2) endocrinologic and metabolic correlations of disorders of muscle associated with metabolic or cationic disturbances; (3) a study of the value of muscle pathology in determining the etiology of the "Floppy Infant"; (4) electron microscopic study of muscle in the normal and diseased states.

Methods Employed: Recent advances in isotopic procedures and muscle pathology have changed radically as programmed during the past year. The majority of accomplishments to date are found in numbers (2) and (3). A study of 166 cases of myopathic disorders associated with endocrine abnormalities were undertaken during the past year. A list of these is as follows:

Part B included Yes

Thyrotoxic Myopathy	4
Exophthalmic Ophthalmoplegia	7
Hypothyroid	5
Familial Periodic Paralysis	5
Primary Hyperkalemia (Gamstorp-Eulenberg)	36
Central Core Disease	5
Glycogen-Storage (Type II.)	2
McArdle's Disease	1
Late Spontaneous Myopathy	79
Renal Acidosis	2
Hypoparathyroid	2
Hyperparathyroid	3
Addison's Disease	2
Cushing's Disease	4
Amyloid Disease	5
Salt Losing Nephritis	2
Paroxysmal Myoglobinuria	1
Werner's Syndrome	1
Total	166

This is an overall survey, employing chemical studies of muscle biopsies, combined with various metabolic tests with particular reference to hormonal levels of aldosterone, gonadotropin, corticoids, ketosteroids, TSH, etc. A summary of the findings in these disorders has been presented at the meeting of the Association for Research in Nervous and Mental Diseases. Of particular interest is the intensive study in two patients with Familial Periodic Paralysis. In this, aldosterone levels were determined by the double isotope derivative methods. Intracellular cations on muscle removed both before and during attacks were also studied, as was the pathology of muscle before and during attacks, and microelectrode recordings of single muscle fibers in vivo before and during attacks. K^{42} turn-overs were also studied in this disorder.

Twenty-three cases of infantile neuromuscular disease associated with hypotonia were also studied in reference to the pathology, electromyography, and clinical course. From this, five different types of disorders were found in a disease state which has been recently grouped into but one disorder.

The recent finding that DNA is probably inert in non-proliferating cells has led to the utilization of tritium (H^3) labelled thymidine as a powerful tool for the study of regeneration of muscle. The electron-microscopy studies during the past year have been limited by Dr. Wanko to normal human muscle obtained at biopsy.

Major Findings: In Familial Periodic Paralysis it was found that there was a large increase of intracellular fluid, the exact composition of which is still entirely unknown. The cationic concentration, however, demonstrates that potassium concentration is not increased in this disorder. To maintain such a concentration in the face of increased intracellular fluid means that potassium must enter the cells from the extracellular fluid; the paralysis, however, cannot be due to increased potassium in that the concentration is the same as, or less than, in the normal cell. This has been confirmed by microelectrode recordings showing the resting potential to fall with 71 $\pm$ 11 mv. Our present conception is that the paralysis in this disorder is due to the mechanical distension of the cell membrane and not due to high cationic values. Studies on aldosterone, using double isotope derivative methods, have demonstrated that

aldosterone is not a factor in this disorder, as postulated by Conn. This is confirmed by the fact that there is not a potassium diuresis before the attack. The other studies of endocrine abnormalities have been summarized in the Tables presented to the Association for Research in Nervous and Mental Diseases.

Five distinct entities have been implicated in the "Floppy Infant" by Drs. Greenfield, Cornman, and Shy. These are Werdnig-Hoffmann's disease, Congenital Muscular Dystrophy, Central Core Disease, Benign Congenital Myotonia, and Arthrogryposis. This phase of the study has been completed and the paper will appear in the December issue of Brain. The electron microscopic findings in normal adult human muscle have been summarized by Dr. Wanko, and appear in the December Proceedings of the ARNMD.

It is of interest to note that the onset of late proxysmal myopathy in the male is associated with neoplasia in 90 percent of the cases, but in only 10 percent of the females. This is based on a study of 79 cases, including 33 males and 46 females.

Proposed Course of the Project: The growth of muscle will be one of the primary interests of this study in the coming year, using tritium (H^3) labelled thymidine for studies of DNA with autoradiography. Dr. Wanko will continue the electron microscopic studies of diseased muscle.

Significance to Neurological Research: The correlation of anatomical, chemical and metabolic abnormalities associated with disorders of striated muscle as well as further insight into the growth of muscle, gives a powerful correlative understanding of muscle in disease and in the norm.

Serial No. NINDB-13(c)

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

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

2. Greenfield, J.G., Cornman, T. and Shy, G.M. The Prognostic Value of the Muscle Biopsy in the Floppy Infant. To be published in Brain, December, 1958.

Serial No. NIMDB-14(c)
1. Medical Neurology Branch
2. Neurological Disorders
3. Bethesda, Maryland
4. New

PHS NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Pathological Study of Intramuscular Motor and Sensory Nerve Endings in the Normal and in Neuromuscular Diseases.

Principal Investigator: G. R. Haase, M.D.

Other Investigator: None

Cooperating Units:

Man Years
Total: 1
Professional: .5
Other: .5

Patient Days: 25

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. Various investigators, i.e., Schwenn (Deutsches Archiv fur Klinisch Medicin, 70:193, 1901); Schiefferdecker (Deutsches Zeitschrift fuer Nervenheilkunde, 25:1); and Camp (JAMA, 48:1230, 1907) have reported changes in the muscle and specifically in the muscle spindle occurring in cases of Parkinson's disease. Coers (Acta Neurologica et Psychiatrica Belgica, 55:741, 1955) reported changes occurring in the terminal fibers and in the motor endplate in a number of different diseases, including myotonia of either the congenital or dystrophic variety, in "amyotonia", in polymyositis and in various neurogenic lesions. The objectives of the present study are to determine whether these reports can be verified or refuted and whether any additional changes can be discerned by histological means.

Method Employed: The methods consist in the use of intravital injection of methylene blue at the time of biopsy as indicated by Coers, and the use of the acetyl cholinesterase stain as modified by Coers and finally in the employment of silver stains, in particular the modification indicated by Winkelmann.

Patient Material: The material is usually obtained in the course of routine biopsies, excepting for such variations as are imposed by the methylene blue method. In all these pathological specimens, routine staining methods are also employed by the Section of Neuropathology. Control material has been obtained from a number of cases dying of diseases not related to the neuromuscular system. Finally, material has been obtained from various laboratory animals.

The patient material utilized includes predominantly those patients admitted with neuromuscular disease in the course of studies concerning the general pathology in various disorders. Several patients with Parkinson's disease were admitted for the primary objective of obtaining muscle specimens.

Major Finding: The study is still too much in its beginning to permit definite conclusions. Some of the difficulties in the interpretation are due to the great variabilities of these structures in the normal. The occurrence of axonal regeneration in neurogenic diseases has been verified. The other abnormalities which have been described have not been verified so far.

Part B included. ☐ Yes ☐ No

1. Medical Neurology Branch
2. Section on Neurological Disorders Service
3. Bethesda, Md.
4. Continuation of NINDB-2

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: The Physiology and Pharmacology in Myasthenia Gravis

Principal Investigator: G. Milton Shy

Other Investigators: William Matthews

Man Years:
Total: .10
Professional: .10
Other: .10

Patient Days: 560

Project Description:

Objectives: To continue studies of intracellular resting potentials in myasthenia gravis to determine the extent of depolarization or hypopolarization of the muscle membrane.

Methods Employed: Consist of intrafiber recordings with microelectrodes in the single muscle fibers of afflicted muscle in myasthenic man. After much trial and error, the Bak Unity Gain Amplifier has been utilized as optimal with a constant current sent back into the grid of the cathode follower. This latter allows constant sampling of the condition of the probing electrode.

Major Findings: But a few muscle fibers have been successfully penetrated to date in myasthenic man. As anticipated in the 1957 Report, this has become a difficult and time-consuming procedure. Results of present records would indicate that in myasthenic man we have been successful in penetrating about only ten fibers.

Course of the Project: The course of the project will resolve around the overcoming of the difficulties listed above. Many hundreds of recordings must be made to determine a statistically significant resting potential. At the present time this has not been feasible. Studies will continue, however, over the next year.

This project may be abolished if it is found that the results are unreliable or the techniques so difficult as to make any conclusions difficult to interpret.

Part B included No

Serial No. NINDB-16(c)
1. Medical Neurology Branch
2. Section on Neurological Disorders Services
3. Bethesda, Maryland
4. New Project

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: A Study of Progressive Parenchymatous Degeneration of the Central Nervous System.

Principal Investigators: Bushnell Smith, M. D. and Glenn Drager, M. D.

Other Investigators:

Cooperating Units:

Man Years (calendar year 1958):
Total: 1
Professional: 1
Other: 0

Patient Days (calendar year 1958): 92

Project Description:

Objectives:

Progressive parenchymatous degeneration of the central nervous system refers to a group of patients described by Heidenhain, Meyer, Leigh & Bagg; Pallis and Spillane; Jones and Nevin; as well as Foley and Denny-Brown in which they (age range 40-55 years) show progressive involvement of the central nervous system with amaurosis, personality changes, myoclonus, ataxia and finally a comatose state. The onset is insidious and the clinical course is a progressive deterioration of the functions of the nervous system.

A 51-year old white female was admitted to the National Institute of Neurological Diseases and Blindness after 2½ months of such an illness. Myoclonus, cortical blindness and stupor were noted on admission. During the 92 day hospital stay, the patient continued to have myoclonus and became progressively more decerebrate with opisthotonic posturing until death, which occurred approximately six months after the onset of this condition. Post mortem examination was performed.

Studies of the brain are being undertaken (a) to establish a possible etiology for this particular illness, (b) to establish the anatomical structures of the central nervous system that have been pathologically affected in this illness, (c) to correlate these findings with the clinical data and the findings from a brain biopsy obtained during the third month of the disease, and (d) to correlate the findings (clinical and pathological) with pertinent cases in the literature.

Methods Employed:

The brain and the spinal cord were removed approximately eight hours after death and fixed in formalin. The brain was cut and examined for gross pathology after ten days of fixation.

The spinal cord, brain stem, cerebellum and cerebrum will be sectioned and stained with the routine techniques for demonstrating morphological changes in the nervous system. In addition, special stains will be employed to demonstrate inclusion bodies and lipids of the neurons.

Major Findings:

Gross examination of the brain has shown the entire cerebral cortex to be affected. In addition the caudate (and possibly other basal ganglia), the cerebellum, the pons and the spinal cord all show changes.

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 is controversial. The clinical course of this patient from beginning to end has been well documented. A cortical biopsy was obtained during the third months of the disease. The pathological finding of the biopsy when compared with the 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.

-3-

Proposed Course of Project:

To make a clinical pathological correlative study of the nervous system of a case of progressive parenchymatous degeneration of the central nervous system. This will entail a detailed study of the nervous system employing the usual routine stains and special techniques for demonstrating inclusion bodies and lipid content of neurons.

Part B included No

1. Medical Neurology
2. Neurological Disorders Service
3. Bethesda, Maryland
4. NINDB-24(c)

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Spirochetes and viral antigens and their relation to the spinal fluid and blood of multiple sclerotic patients.

Principal Investigator: Marvin C. Korengold

Other Investigators: Edward Hampp, A. Sabin and Robert Huebner

Man Years:
Total: .20
Professional: .10
Other: .10

Patient Days: 22

Project Description:

Objectives: To attempt to isolate spirochetes in the spinal fluid of multiple sclerotic patients. Samples of spinal fluid and blood were obtained from 22 well-screened multiple sclerosis patients and referred to Dr. Huebner's laboratory for special viral studies. Samples of blood were obtained from 22 well-screened multiple sclerosis patients and sent to Dr. Sabin's laboratory for special viral determinations against the antigen obtained by Dr. Sabin from the USSR.

Methods Employed: The method of Ross Ichelson, as described in the Proceedings of the Society for Experimental Biology and Medicine, May, 1957, will be used to attempt to isolate spirochetes from the spinal fluid of multiple sclerotic patients. A special laboratory procedure and chemical media has been described by Miss Ichelson and an attempt to duplicate this procedure will be followed. 15 cc. of spinal fluid will be obtained from 30 patients of well-documented multiple sclerosis (based on the criteria of other multiple sclerosis projects). The fluid will be transferred to Dr. Hampp's laboratory where special procedures will be undertaken to attempt to isolate any possible spirochetes. Each patient's spinal fluid will be studied under the Ichelson medium as well as under various other standard spirochetal mediums currently in use for other purposes.

Patient Material: Patients were obtained from the previously well-screened patients with multiple sclerosis.

Major Findings: Twenty-two patients with multiple sclerosis were admitted to the Clinical Center Out-Patient Department for special spinal fluid studies. Samples of spinal fluid were sent to Dr. Hempp's laboratory on all patients. The specific techniques as described and used by Miss Rose Ichelson were duplicated, and in no instance, were any spirochetes found. It was felt after 22 negative determinations, that further patient studies would not be necessary. The project was, therefore, concluded.

Significance to Program of Institute: The results of this study indicate that no spirochetes can be implicated in the etiology of multiple sclerosis. These studies have now been completed.

Part B included Yes [ ] No [X]

1. Medical Neurology Branch
2. Section on Biophysical Applications
3. Bethesda, Maryland
4. Old No. NINDB-20(c)

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Investigations in Localization of Cerebral Neoplasia by Isotopic Detection

Principal Investigator: G. Milton Shy

Other Investigators: Robert Bradley and William Matthews

Cooperating Units: Oak Ridge National Laboratory
Oak Ridge, Tennessee

Man Years:
Total: .50
Professional: .50
Other: .50

Patient Days: 483

Project Description:

Objectives: In the 1957 Annual Report this Project was listed as an attempt to localize cerebral neoplasia. Its value has since been determined, and the primary objective now has been to determine what percentage of accuracy this technique offers.

Methods Employed: This has been a systematic study of collimation upon highly sensitive large sodium iodide crystals which are juxtaopposed at 180° from a moving electronic scanner. Since the last report, a coincidence circuit has been added to the gamma spectrometer.

Patient Material: Over 200 patients now have been scanned These patients are from NINDB, the National Cancer Institute, the National Navy Medical Center, Walter Reed Hospital, Mount Alto Veterans Hospital, and referring neurosurgeons in the surrounding area.

Major Findings: In these 200-odd cases an accuracy confirmed of 87 percent was found - 14 of these patients had normal contrast studies.

Proposed Course of the Project: Now that the usefulness of this procedure has been determined, this apparatus, in its entirety, will be transferred to the Central Diagnostic X-ray Department, who will now undertake this as one of their service functions. The Unit also is supervising the construction of an identical apparatus at Johns Hopkins University.

Significance to Neurological Research: This procedure, in demonstrating its usefulness, has relieved one of the major hazards in the treatment of intracranial tumors, namely, contrast studies with the hazard to patient life and the necessity of being followed by immediate operation.

Part B included Yes

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Shy, G.M., Bradley, R.B. and Matthews, W.B.: External Collimation Detection of Intracranial Neoplasia with Unstable Nuclides. Published by E. & S. Livingstone Ltd., Edinburgh, Scotland, 1958.

National Institute of Neurological

Diseases and Blindness

Clinical Research

Medical Neurology Branch

Section on Neuroradiology

Serial Number of Project:

NINDB-19(c).

Estimated Obligations for FY 1959

Total: $20,500

Direct: $19,900

Reimbursement: $600

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Metal Chelates as Possible Contrast Media for Myelography

Principal Investigators: Giovanni Di Chiro, M. D. and Martin Rubin, Ph.D.

Other Investigators: None

Cooperating Units: Diagnostic X-Ray Department, C.C. and Georgetown University

Man Years: (calendar year 1958) Patient Days: None
Total: 2
Professional: 1
Other: 1

Project Description:

Objectives: To find a new roentgenopaque contrast medium for myelography. None of the now available contrast media is, in fact, satisfactory. Heavy metals have a marked x-ray opacity. Several of those are more radiopaque than barium and iodine, elements present in the standard roentgenographic contrast media now in clinical use. Heavy metals exert, however, a profound toxicity in living systems. Chelation is a relatively old method to make metal ions chemically inactive and therefore possibly non-toxic for living organisms. Objective of the present project has been to find whether or not different metal chelates are suitable as roentgenopaque contrast media, and more specifically as roentgenopaque contrast media for myelography.

Methods Employed: The essential properties of a useful radiopaque medium includes: 1. high radiopacity; 2. low systemic and local tissue toxicity; 3. pharmacodynamic silence; 4. prompt and complete elimination. In experiments in vitro the radiopacity of a relatively large group of chelated compounds has been tested. For this purpose a board of non x-ray opaque material with small, equal wells to hold the test solutions was used. This board with

the different solutions was x-rayed using standard technical factors. A standard dark room technique was used. The chelated compounds were tested against commonly used iodinated contrast media. Different concentrations of the various chelated compounds were tested in order to determine the concentration for the optimal opacity. Once determined the radiopacity in vitro, the radiopaque chelates were used in vivo and so administered to dogs and rabbits. The compounds were injected intravenously, given per mouth, injected intracardiacally, and injected in the cisterna magna. Urographies, phlebographies, gastro-intestinal studies, angiocardiographies, and myelographies were so obtained. The quantity of the chelated compound injected was varied mainly according to the concentration of the metal in the chelated solution, its x-ray opacity previously proved in vitro, and the animal's weight. Accurate toxicologic studies have been carried out to date only for few of the substances under study. No autopsic study was done in the experimental animals after the x-ray studies had been performed. Here follows a list of the compounds tested:

1. Lead EDTA
2. Lead Cyclohexyl EDTA
3. Lead DTPA
4. Cadmium EDTA
5. Cadmium 544[A]
6. Cadmium Cyclohexyl EDTA
7. Zinc EDTA
8. Cobalt EDTA
9. Cerium EDTA
10. Copper EDTA
11. Nickel EDTA
12. Barium EDTA
13. Bismuth EDTA

Abbreviations: EDTA -- Ethylenediaminetetraacetic acid
Cyclohexyl EDTA - 1, 2 -Diaminocyclohexane (N, N[1] - tetraacetic acid)
DTPA -- Diethylenetriaminepentaacetate
544A -- N, N[1] - (2 - hydroxycyclohexyl) ethylene-diaminediacetic acid

Concurrently, a group of experiments concerned with the metabolism and distribution of injected lead EDTA was carried out by one of the investigators to study the general problem of the quantitative fate and tissue distribution of the administered metal. Lead EDTA administered at a dose level of 10 mg/kg appears in the urine to the extent of 85 - 89% of the injected dose within two days. The fecal excretion of the lead EDTA following its intravenous administration is below 2%. The lead retained in the animal (some 10 - 15% of the injected dose),

is deposited in its major portion (about 50%) in the liver. Some 20% of the residual lead EDTA was found in the bone marrow. Small traces of the injected lead EDTA were found in other tissues and organs: kidneys, heart, lungs, muscles. This group of experiments shows that at least for what the lead EDTA is concerned, the retention of the metal is not to be discounted despite the high urinary excretion. This is particularly true if we consider that the quantities of metal chelates that are to be injected for contrast purposes are high.

Major Findings: As it could have been expected several of the tested metal chelates have shown good radiopacity, useful for diagnostic x-ray purposes. The higher the atomic number of the metal present in the chelated solution, the higher the opacity was found to be. The experiments in vivo show that studies of good diagnostic quality may be obtained, as far as x-ray contrast and detail are concerned, with radiopaque metal chelates. 10 cc. of lead DTPA 8% injected into the cisterna magna of dogs gave excellent myelographic pictures with outlining of the spinal cord. However, the acute toxicity of the tested metal chelates in myelography as well as in most of the other x-ray examinations carried out proved to be high. Death occurred shortly after the chelated compound had been injected into the cisterna magna. While therefore the metal chelates seem by the present study to be interesting as far as their radiopacity is concerned, on the other hand this study shows that the systemic and local tissue toxicity of these compounds is too high. We cannot therefore at present recommend for clinical use the metal chelates which we have tested.

Significance to Neurological Research: Despite their good radiopacity and other characteristics which would make the metal chelates tested good contrast media for myelography, their general and local toxicity is too high to suggest them to be used in clinical myelography.

Part B included: Yes [X] No

Serial No. NINDB-19(c)

PHS--NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards and Publications

Publications other than abstracts from this project:

Rubin, Martin and Di Chiro, Giovanni: Metal Chelates as Possible Contrast Media. (To be published in the Annals of the New York Academy of Sciences, Section of Biology with reference to a presentation at a meeting held by this society on Radiopaque Contrast Agents, October 24 - 25, 1958.)

Honors and Awards Relating to this Project:

None.

National Institute of Neurological

Diseases and Blindness

Clinical Research

Medical Neurology Branch

Section on Clinical Applied Pharmacology

Serial Numbers of Projects:

NINDB-20(c), NINDB-21(c), NINDB-22(c), NINDB-23(c), and NINDB-24(c).

Estimated Obligations for FY 1959

Total: $39,500

Direct: $32,400

Reimbursement: $7,100

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

PHS-NIH
Individual Project Report
Calendar Year 1958

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

Cooperating Units: None

Man Years (calendar year 1958): Total: .66 Professional: .33 Other: .33

Patient Days (calendar year 1958):

Project Description:

OBJECTIVES: To determine the function of specific and non-specific cholinesterase in relation to the blocking of transmission in between nerve and muscle.

METHODS EMPLOYED: The standard Warburg manometric technique is used to estimate muscle and blood cholinesterases. A cross circulation preparation is used in which the blood of one animal is made to perfuse one leg of another animal. The blood and tissue cholinesterases of the two animals are manipulated by use of either di-isopropylfluorophosphate (DFP) or isopropyl methyl fluorophosphate (sarin) so that neuromuscular blocking action of other compounds can be observed with high plasma esterase activity and low muscle esterase activity or low plasma and high muscle activity. Activity of the following three types of compounds have been studied in relation to neuromuscular block and inhibition of cholinesterase; (a) non-depolarizing, (b) depolarizing-not metabolized by cholinesterase, (c) depolarizing-metabolized by cholinesterase.

MAJOR FINDINGS: The neuromuscular blocking activity of a non-depolarizing compound, d-tubocurarine, is reduced or prevented by selective inhibition of

muscle cholinesterase. Inhibition of the plasma cholinesterases greatly prolongs the neuromuscular blocking of depolarizing compounds which are destroyed by plasma esterases. The selective inhibition of muscle cholinesterase prolongs the neuromuscular block produced by a depolarizing compound, decamethonium, which contains no ester group and thus is not capable of destruction by the muscle cholinesterase. The selective inhibition of muscle cholinesterase also prolongs the block produced by succinylcholine, a blocking compound which contains ester groups, and thus could conceivably be metabolized by muscle cholinesterase. Since the prolongation of the blocking activity of succinylcholine occurred to the same extent as the prolongation of the block produced by decamethonium, it appears that succinylcholine is either not metabolized by muscle cholinesterase or metabolized at a rate which does not influence its blocking actions.

The experiments of this project emphasize that inhibition of plasma cholinesterase can greatly prolong the neuromuscular blocking activity of depolarizing compounds which are destroyed by plasma esterase. In this situation, inhibition of muscle cholinesterase plays a minor role in relation to the total neuromuscular block produced. These experiments also emphasize that when neuromuscular blocking compounds are not metabolized by plasma cholinesterase the inhibition of muscle cholinesterase markedly modifies the blocking activity of all of the types of blocking compounds investigated. Blocking activity of non-depolarizing compounds is decreased. On the contrary, the blocking activity of depolarizing compounds is increased.

SIGNIFICANCE TO PROGRAM OF INSTITUTE: This project will furnish additional information on the metabolism of clinically useful neuromuscular blocking compounds and cholinesterase inhibitors. It may, in addition, contribute to an increased understanding of the physiological processes related to myasthenia gravis and its treatment by use of inhibitors of cholinesterase.

PROPOSED COURSE OF PROJECT: This research is to be continued until the objectives stated above have been fulfilled.

Part B included: No

PHS-NIH
Individual Project Report
Calendar Year 1958

1. Medical Neurology
2. Clinically Applied Pharmacology
3. Bethesda, Md.
4. Continued (NINDB-2(c))

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

Cooperating Units: None

Man Years: (calendar year 1958)
Total: .66
Professional: .33
Other: .33

Patient Days: (calendar year 1958)

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: Alternate direct and indirect stimulation of the gastrocnemius or the anterior tibial muscle of the rat has been used. The effect of nerve stimulation has been eliminated by administration of a neuromuscular blocking compound that does not affect the contractility of the directly stimulated muscle. Contractile responses were recorded either from a damped strain gauge which markedly restricts external shortening or from an optical system activated by an isotonic lever. Recordings were made with a cathode ray oscilloscope.

The optical-isotonic lever system was constructed by Jay B. Wells. The depolarizing properties of these compounds have been investigated using the isolated frog sartorius muscle and a travelling fluid electrode.

MAJOR FINDINGS: Previous findings were stated in some detail in the 1957 NINDB Annual Report (3(c)). More recent experiments have shown that the block

to direct stimulation which occurs with succinylcholine and decamethonium is not due to merely increasing muscle compliance. Added compliance in series with the muscle does not give contractile response similar to those obtained when block to direct stimulation is induced by succinylcholine or decamethonium.

When the block to direct stimulation which occurs with succinylcholine is observed with isotonic recording of contraction, a method which reduces to a great extent the role of the elastic element of the muscle, less shortening and a reduced velocity of shortening occurs. This also indicates the reduction in contraction is not increased compliance.

A prolonged latency between stimulation and the start of contraction has also been observed following the administration of depolarizing blocking compounds.

Compounds which reduce the contractile response of directly stimulated muscle also depolarize the muscle membranes. The depolarization has a spatial distribution in the frog sartorius muscle which extends throughout the muscle except for a small area at each end. The depolarization could occur from widely scattered end plates throughout the muscle or from temporal spread of the depolarization from the motor end plate.

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 may contribute to an increased understanding of the physiological processes related to myasthenia gravis.

PROPOSED COURSE OF PROJECT: To continue to investigate the contractile reducing and muscle membrane depolarizing properties of the neuromuscular blocking compounds.

Part B included: Yes

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

"The Contractile Response of Directly Stimulated Muscle after Administration of Neuromuscular Blocking Compounds". Accepted for publication in The Journal of Pharmacology and Experimental Therapeutics, Richard L. Irwin and Jay B. Wells.

Honors and Awards relating to this project: None.

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

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Study of muscle cholinesterase and its inhibitors.

Principal Investigator: Richard L. Irwin

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

Cooperating Units: Central Intelligence Agency and U. S. Army, Office of Surgeon General

Man Years (calendar year 1958):
Total: .66
Professional: .33
Other: .33

Patient Days (calendar year 1958):

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 travelling fluid electrode system used by Patt, J. of Physiol., 111:408.

MAJOR FINDINGS: The cholinesterase of muscle homogenates, made from muscle previously perfused with saline to remove blood, hydrolyzes acetylcholine more rapidly than it hydrolyzes methacholine and markedly more rapidly than it hydrolyzes benzoylcholine or butrylcholine. The enzyme is inhibited by excess of substrates and with acetylcholine shows an optimum substrate concentration in the range of a 5×10^{-3} molar concentration. Optimum concentrations for substrates other than acetylcholine are higher. The findings are all in accord with the belief that muscle cholinesterase is chiefly of the true or specific type. Since benzoylcholine and butrylcholine are hydrolyzed by muscle homogenates at measurable rates, the possibility exists that muscle contains a small amount of non-specific type esterase. A cholinesterase preparation of high purity is needed for further investigation of the substrate specificity of muscle cholinesterase. Obtaining a purified cholinesterase from muscle presents a formidable, although feasible, problem inasmuch as the esterase content of muscle is low and the amount of other proteins in the muscle is high.

The depolarizing properties of the inhibitors of muscle cholinesterase which are used in the treatment of myasthenia have been examined. Neostigmine has been observed to depolarize muscle membranes markedly at a molar concentration of 10^{-3}. In contrast to this, pyridostigmine failed to show depolarizing activity at this concentration. This finding is of interest since these two compounds are both effective in amelioration of the symptoms of myasthenia. Edrophonium produces only slight depolarization at a 10^{-3} molar concentration.

Galanthamine, a recently isolated alkaloid obtained from galanthus Woronowi (Amaryllidaceae), has been introduced into clinical medicine for treatment of myasthenia gravis and other neurological disorders; Annotatsii o Novykh Lekarstvennykh, (Notes on New Medicinal Agents), E. D. Sedova, Medgiz, Moscow, 1956. This

compound is of particular interest since it is markedly different chemically from other compounds currently in use in the treatment of myasthenia gravis. Galanthamine is a phenanthrene derivative and is not a carbamic ester. We have investigated the inhibitory properties of the compound and have found that the molar concentration of galanthamine which causes 50% inhibition of muscle cholinesterase is 6×10^{-6}. The value for the inhibition of plasma cholinesterase is the same. Twenty per cent inhibition occurs at 1×10^{-6} molar and 85% at 1×10^{-4} molar. Similar determinations have been made using physostigmine and neostigmine. Both of these compounds inhibit muscle cholinesterase at lower concentrations than does galanthamine. The in vitro rate of inhibition is more rapid with galanthamine.

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: Our inability to obtain an adequate supply of galanthamine has hindered further study of this drug. The experimentation will be continued when a supply is available. Other compounds will be studied.

Part B included: No

Serial No. NIMDB-23(c)
1. Medical Neurology
2. Clinically Applied Pharmacology
3. Bethesda, Md.
4. New

PHS-NIH
Individual Project Reports
Calendar Year 1958

Part A.

Project Title: A study of naturally occurring choline esters.

Principal Investigator: Richard L. Irwin

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

Cooperating Units : None

Man Years: (calendar year 1958)
Total: .10
Professional: .05
Other: .05

Patient Days: (calendar year 1958)

Project Description:

OBJECTIVES: An increasing amount of evidence is accumulating indicating that choline esters other than acetylcholine occur as natural constituents of biological systems. The physiological and pharmacological significance of the choline esters other than acetylcholine is largely unknown. Certain choline esters produce their physiological and/or pharmacological action by depolarization of membranes. The objectives of the present study are: (a) to determine to what extent the choline esters which are found in biological systems, and related compounds, depolarize tissue membrane, (b) to relate the depolarizing properties of these compounds to their stimulation and/or blocking activity of synapses, (c) to study the metabolism of these compounds by tissue enzymes.

METHODS EMPLOYED: The travelling fluid electrode technique is used to measure depolarization of isolated frog sartorius muscles. Microelectrodes will be used to determine resting membrane potentials. Standard manometric techniques are used to study metabolic activity.

MAJOR FINDINGS: In high concentrations (10^{-3} molar) butyrlcholine, benzoylcholine, imidazoleacrylcholine, and imidazolepropionylcholine resemble acetylcholine in their depolarizing properties. Methacholine, a synthetic compound, which resembles acetylcholine in its biological action but differs from it chemically by having a substituted choline moiety does not depolarize muscle membranes at 10^{-3} molar concentration. Plasma from myasthenia patients have been observed to metabolize imidazoleacrylcholine at about the same rate as plasma from non-myasthenic human beings.

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: The project is in an early phase. Further study will be made of the compounds mentioned above. Other choline esters will be obtained and studied. Emphasis will be given to those compounds which are natural constituents of animal tissue. The chromatographic techniques of Bannister and Whittaker (The Journal of Physiology, 121:55) will be used to identify the active compounds present in tissue. Patient material will be used where applicable.

Part B included: No

PHS-NIH
Individual Project Report
Calendar Year 1958

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

Part A.

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

Principal Investigator: Richard L. Irwin

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1958):
Total: .10
Professional: .05
Other: .05

Patient Days (calendar year 1958):

Project Description:

OBJECTIVES: Recent work by Dr. Kenneth Zierler of Johns Hopkins University has shown that aldolase decreases in muscles with intact membranes when they are incubated in fluid high in potassium. The concentration of potassium used are of the order which depolarizes muscle membranes. The objective of this study is to determine to what extent in vivo depolarization of muscle membrane by drugs affect the efflux of aldolase and other enzymes from muscles.

METHODS EMPLOYED: Enzyme content of muscles are determined by the methods used by Zierler, Am. J. Physiol. 1958, 193:534; Lowry et al., J. B. C. 1954 207:19; and Sibley and Lehniger, J. B. C. 1949, 177: 859.

In the in vivo portion of the experiments, pairs of muscles are used in both experimental and control determination.

MAJOR FINDINGS: NONE.

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: As fulfill the objective stated.

Part B included: No

National Institute of Neurological

Diseases and Blindness

Clinical Research

Medical Neurology Branch

Section on Clinical Neurochemistry

Serial Numbers of Projects:

NINDB-25(c), NINDB-26(c), NINDB-27(c), NINDB-28(c),
NINDB-29(c), NINDB-30(c), NINDB-31(c), NINDB-32(c),
NINDB-33(c), NINDB-34(c), NINDB-35(c), NINDB-36(c).

Estimated Obligations for FY 1959

Total: $160,500

Direct: $84,100

Reimbursement: $76,400

Serial No. NINDB-25(C)
1. Medical Neurology
2. Clinical Neurochemistry
3. Bethesda, Md.
4. Same as 57-NINDB-9(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 Investigator: Dr. Donald B. Tower
Other Investigators: Mr. E. L. Peters
Cooperating Units: Drs. R. O. Brady and B. W. Agranoff, NINDB Section on Lipid Chemistry. Dr. G. Ashwell, NIAMD Lab. of Biochemistry.
Man Years: Patient Days: 0
Total: 1.0
Professional: 0.5
Other: 0.5
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, summarized in publication #1 (Part B.).

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

Major Findings: (1) Studies on the mechanism of action and the effects on incubated slice metabolism of 2-deoxyglucose were completed. The following results and conclusions were forthcoming:
(a) 2-deoxyglucose prevents cellular glucose utilization by inhibiting the hexokinase step primarily due to depletion of available ATP required for this step. The possibility that the 2-deoxyglucose-6-phosphate formed, which cannot be further metabolized, also blocks the glucose-6-phosphate to fructose-6-phosphate step could not be ruled out, but appears to be less important than the effect on hexokinase. Under anaerobic conditions it was possible to overcome the block in glucose utilization by 2-deoxyglucose with addition of either ATP or glucose-6-phosphate to the slices. No effect of these additions on aerobic metabolism was obtained, presumably due to their failure to penetrate slices which under aerobic conditions exhibit less membrane permeability.

"[illegible] of the glucose-6-phosphate dehydrogenase of oxidative shunt pathway. This finding was checked by incubating control and inhibited slices with glucose-1-C^{14} and glucose-6-C^{14}, determining utilization, $C^{14}O_2$ and C^{14}-lactic acid production. The ratios of $C^{14}O_2$ and C^{14}-lactate from C-6 compared to C-1 samples were 1.0 in both cases, whereas C-6/C-1 will be less than 1.0 if the shunt pathway utilizes a significant portion of the labelled glucose (e.g. in liver it is 0.3). This finding is consistent with reported low levels of brain TPN, which is the necessary coenzyme for the shunt pathway and indicates that the shunt pathway is relatively unimportant in cerebral cortex.

(c) 2-deoxyglucose inhibition not only results in marked decrease in glycolysis but also in oxidative metabolism. With glucose-U-C^{14} less $C^{14}O_2$, less labelling of the free amino acid pool and less C^{14}-lactic acid are obtained Under aerobic conditions with 10 mM 2-deoxyglucose glucose utilization and labelling of these components were reduced to one-third of control slices. These findings complement the previously reported[57-NINDB-8(C)] depletion of energy-rich phosphates, ATP and creatine phosphate, in inhibited slices.

(d) From the studies with C^{14}-labelled glucose the distribution of glucose utilized by normal slices to various intermediates could be estimated:

Glycolysis (lactate)	70%
Amino Acids (glutamate)	22%
Respiratory CO_2	7%
Other intermediates, lipids and proteins	1%

From the oxygen uptake (85 μM./g./hr.) it is clear that the 30% of the latter accounted for as amino acid and respiratory CO_2 almost exactly balances the oxygen uptake, assuming 6 moles of the latter per mole of glucose oxidized. This calculation is consistent with recent reports by Geiger on C^{14}-glucose metabolism by perfused cat brain in vivo where 20% appeared as amino acids and 30% appeared as $C^{14}O_2$. It is also consistent with his conclusions and those by this laboratory from studies on γ-aminobutyric acid [see 58-NINDB-30(c)] that non-glucose substrates, such as amino acids, normally support oxidative metabolism by brain and that they are repleted subsequently by part of the glucose utilized. Also our findings in this study agree well with similar previous studies by others using labelled substrates in vitro.

(e) Thus, it may be concluded with Geiger that brain possesses a versatile system for support of oxidative metabolism in which not only glucose but non-glucose

that glucose serves as the principal or sole substrate for oxidative metabolism of brain, since lack of glucose makes repletion of the non-glucose intermediates impossible and energy-production rapidly fails. The studies with 2-deoxyglucose clearly demonstrate this not only by the depletion of ATP and creatine phosphate but also by the deleterious effects on glutamic acid and electrolytes in such inhibited slices. Both these systems depend upon energy production from glucose oxidation. When that fails glutamic acid levels fall profoundly to about 30% of normal. The electrolyte picture can be summarized as follows:

	(μEq./g.)		(%)	
	Potassium	Sodium	Non-Chloride Space	
Normal-Initial	46	100	37	
1 hr. Incub.	92	59	48	
2-DG-1 hr. Incub.	62	84	37	

Thus, inhibited slices fail to extrude excess sodium and reconcentrate potassium in the normal manner. It may be significant that this type of defect in electrolyte metabolism is also encountered in incubated slices of epileptogenic cerebral cortex.

(2) Detailed studies of electrolyte metabolism of incubated slices of epileptogenic cortex from a variety of experimental animal preparations. The previously demonstrated abnormalities demonstrated for human epileptogenic slices [see 57-NINDB-8 (C)] were also found in cortical slices from cats with seizures induced by 3-methyl-3-ethylglutarimide (Megimide) and by methionine sulfoximine. Little deviations from normal was observed for samples from cats with thiosemicarbazide seizures. Since all seizure preparations including the latter are associated with defects in glutamic and γ-aminobutyric acid metabolism, this discrepancy in the electrolyte disturbances with thiosemicarbazide may prove significant. On the one hand it may indicate that electrolyte disturbances are secondary to other more fundamental disturbances, perhaps in energy metabolism, and on the other it suggests that disturbances of glutamic acid and γ-aminobutyric acid metabolism may be primary events in the seizure process, perhaps through effects on energy metabolism.

The ability to determine chloride levels accurately on aliquots of all these samples by the amperometric procedure of Cotlove et al. (N.H.I.) has added greatly to the significance of the studies on electrolytes. The Cotlove instrument makes it feasible to determine the relatively low tissue levels accurately and reproducibly

on as little as one-tenth of the total sample, so that each sample can be analyzed simultaneously for potassium, sodium and chloride. From tissue and medium contents the chloride and non-chloride tissue spaces can be reproducibly calculated. In normal slices these spaces regularly behave during incubation as follows:

	Solids	Non-Chloride Space	Chloride Space
Initial	16%	35	49
1 hr. Incubation	16	48	36

The final space distribution is almost identical with that found for *in vivo* biopsy samples. Furthermore the changes cannot account for the electrolyte changes (extrusion of sodium, reconcentration of potassium) observed for normal slices, thus providing further evidence that the latter are metabolically-dependent. Finally these studies again confirm work of others that the swelling (gain of weight) of normal and epileptogenic slices during incubation is confined to the chloride space.

Calculation of the electrolyte concentration per litre of non-chloride space water at the end of slice incubation can be derived from the above data and may be summarized as follows:

	Potassium	Sodium
Normal	100	30
Epileptogenic	140	80

The significance of the excess sodium concentration in epileptogenic slices is not known. The effects of Nembutal anesthesia *in vivo* upon subsequent behavior of electrolytes in incubated slices of normal cat cortex have been studied. No differences from the behavior in unanesthetized slices have been found. This is in marked contrast to significant effects of anesthesia on subsequent slice metabolism of bound acetylcholine and of glutamic acid. The findings for electrolytes are contrary to what might have been anticipated from such results as well as from *in vivo* observations, e.g. by Woodbury, that certain narcotics stabilize neuronal membranes and affect ionic fluxes across them. Woodbury's most striking results occurred with Dilantin and Diamox which have not been evaluated here.

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 the above studies the procedures developed in the course of the 2-deoxy[illegible] studies will be applied to epileptogenic samples to investigate glucose utilization and energy production and maintenance. The electrolyte studies will be amplified by studies of effects of hypoxia and of various anti-convulsant agents. An attempt to study ion fluxes between incubation medium and slices, using K^{42} and Na^{24} and a γ-ray spectrometer, will be made. If successful with normal slices, applications to the defect in epileptogenic slices will follow.

Part B Included: Yes.

Serial No. NIMDB-2510

PHS - NIH
Individual Project Report
Calendar Year 1958

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: in press.

2. Tower, D. B.

The Evidence for a Neurochemical Basis of Seizures.

pp. 301-348 in Baldwin, M. Et al. (Eds.) Temporal Lobe Epilepsy, Springfield, Thomas, 195

3. Tower, D. B.

The Neurochemistry of Convulsive States.
in: Folch, J. (Ed.) Chemical Pathology of the Nervous System, (3rd International Neurochemical Symposium) London, Pergamon, in press.

4. Tower, D. B.

Glutamic Acid Metabolism in Mammalian Central Nervous System.
in: Brücke, F. (Ed.) Symposium on Biochemistry of the Central Nervous System (IV International Biochemical Congress), London, Pergamon, in press.

Honors and Awards:

Appointed to Editorial Board of Biochemical Pharmacology.

Serial No. NINDB-26(c)
1. Medical Neurology
2. Clinical Neurochemistry
3. Bethesda, Md.
4. Same as 57 NINDB-16 (C)

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A:

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

Principal Investigator: Dr. Beni Horvath

Other Investigators: Mr. J. B. Proctor

Cooperating Units: None.

Man Years:

Total: 0.6
Professional: 0.3
Other: 0.3

Patient Days: 762

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 under Project #58-NINDB-35(c) and 36(c) In addition to muscle samples from normal animals, samples from a strain of mice in which a disease resembling muscular dystrophy is inherited are being used. Controls for these mice are provided by normal littermates.

Patient Material: None.

Major Findings: Because of the physico-chemical characteristics observed for the control and "dystrophic" mouse samples [57-NINDB-15(C)] it was deemed advisable to carry out nitrogen determinations on the various fractions of mouse muscle homogenates, as in 58-NINDB-36(c) before proceeding further. Changes in the muscles of the "dystrophic" mice are similar to but of lesser magnitude than those observed in biopsy specimens of human dystrophic muscle. There is an increase of total solids and non-protein solids, indicative of fat replacement of muscle. There is also an increase of Na and decrease of K, as found in human dystrophic muscle. The relative proportions of water-soluble proteins, myosin and alkali-soluble proteins do not show remarkable changes, but so far only muscles in the early stages of degeneration have been studied.

During the summer a brief visit was made to the Roscoe B. Jackson Memorial Laboratories at Bar Harbor, where this strain of mice was discovered and is being maintained and studied genetically. The observations of investigators there relative to the genetic aspects and to successful propagation were gone into in detail. Cooperation of the Bar Harbor group was strengthened by this interchange of information.

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 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: Yes.

PHS - NIH
Individual Project Report
Calendar Year 1958

Part B:

Publications:

Horvath, B.

Muscle Proteins in Dystrophy
Neurology 8 (Suppl. 1): 52 (1958).

Honors and Awards: None.

Serial No. NINDB-27(c)
1. Medical Neurology
2. Clinical Neurochemistry
3. Bethesda, Md.
4. Same as 57-NINDB-6-(C)

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A:

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

Principal Investigator: Dr. Donald B. Tower

Other Investigators: Mr. E. L. Peters, Dr. M. Baldwin, Dr. C. Ajomne-Marsan, Dr. I. Klatzo

Cooperating Units: Dr. R. O. Brady, NINDB Section on Lipid Chemistry (Liquid Scintillation Counting); Dr. R. W. Albers, NINDB Lab. of Neuroanatomical Sciences.

Man Years:

Total	1.0
Professional	0.5
Other:	0.5

Patient Days: 0

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, summarized in publication #1 (Part B.).

Patient Material: From NINDB patients admitted for other purposes.

Major Findings:

(1) A series of studies on slices of cat cerebral cortex incubated with L-glutamic acid-U-C^{14}, L-glutamine-U-C^{14}, γ-aminobutyric acid-1-C^{14}, L-aspartic acid-U-C^{14}, DL-asparagine-2,3-C^{14}, D-glucose-U-C^{14}, Sodium Pyruvate-3-C^{14}, 2-pyrrolidinone-2-C^{14}, and α-ketoglutaric acid-1,2-C^{14} has been carried out. Findings by previous investigators using some of the above isotopically-labelled compounds have been confirmed and extended. Starting with either glucose or pyruvate, the order of labelling in amino acids is first glutamic acid, followed by glutamine, γ-aminobutyric acid, aspartic acid, α-alanine and serine. α-ketoglutarate equilibrates so rapidly with glutamic acid that its metabolic behavior is equivalent to the latter for all practical purposes. Glutamic acid is rapidly metabolized to glutamine, γ-amino-butyric acid, aspartic acid and α-alanine in that order.

Glutamine is rapidly converted to glutamic acid and then thru the same pathways. γ-aminobutyric acid is rapidly metabolized with labelling appearing in aspartic acid, glutamic acid and glutamine indicating its active entry into the Krebs cycle. Pyrrolidinone is slowly hydrolyzed to γ-aminobutyric acid. Similarly asparagine is slowly metabolized to aspartic acid. Aspartic acid is rapidly utilized with activity appearing in glutamic acid, lactic acid, glutamine and γ-aminobutyric acid in that order. Metabolism of aspartate to lactic acid is compatible with the following reaction sequences:

Aspartic Acid → Oxalacetic Acid

↓ - CO_2

Lactic Acid ⇄ Pyruvic Acid

This pathway is known in bacteria, plants and certain animal tissues but has not been reported before in brain. It has considerable significance since it would mean that aspartic acid could prime the Krebs cycle by providing both oxalacetate and acetyl-Coenzyme A (from pyruvate) in the absence of the latter from glycolysis. The foregoing studies indicate how active the components of the glutamate-aspartate amino acid group are in metabolic participation in the Krebs cycle. Concurrent determination of $C^{14}O_2$ liberated during these experiments confirmed this conclusion.

(2) Glutamic Acid, glutamine, γ-aminobutyric acid and free ammonia metabolism was studied in incubated slices from various non-cortical areas of cat brain: subcortical white matter, thalamus (total), caudate nucleus, and cerebellar cortex. Levels and metabolic behavior in all gray or neuronal areas were similar to that previously observed in cerebral cortex, but white matter exhibited extremely low levels and little change on incubation for glutamic and γ-aminobutyric acids while white matter glutamine was not greatly different from cerebral cortex. These findings compare favorably with levels for many similar areas in rat brain determined by other methods by Waelsch.

(3) Levels of glutamic acid, glutamine and γ-aminobutyric acid in subcortical white matter and in cerebral cortex of the cat brain were determined. Using the calculations of Elliott and Heller it could be estimated from these levels that at least 85 per cent of cortical glutamic and γ-amino-butyric acid content was associated with neurons while only about 50 per cent of the glutamine appeared to be neuronal in location. (This finding is consistent with interpretations by Waelsch based on in vivo isotope studies for a different "compartmentation" for glutamine.) In addition cat cerebral cortex was fractionated by the Brody and Bain technique and the cellular loci of these amino acids determined by analysis of the fractions obtained. The majority of glutamic and γ-aminobutyric acids was associated with fraction R_3

or the mitochondrial fraction, whereas glutamine was distributed almost equally between that fraction and the combined R_1 + R_2 fractions which contain cell debris, axon fragments, nuclei, etc. No content of any of the three amino acids was detectable in R_4, the microsomal fraction. The presence of most of the glutamic and γ-aminobutyric acid of cortex in mitochondria is compatible with their close association with the Krebs cycle and oxidative metabolism which are mitochondrial functions exclusively. The significance of considerable glutamine in a non-mitochondrial, non-microsomal fraction is not clear and will require further study.

(4) Extensive studies were carried out on the nature of the inhibition of glutamine synthesis associated with methionine sulfoximine intoxication of cats. The preliminary findings reported in 1957 were confirmed that slices of cortex from cats with seizures induced by methionine sulfoximine show decreases in levels of glutamic acid and γ-aminobutyric acid during incubation both of which can be corrected to normal levels by added L-methionine 10 mM. Such additions have no effect on inhibited glutamine synthesis in such slices. Addition of ammonium chloride 10 mM to normal cat cortex slices during incubation caused two to three-fold increases in glutamine levels at the expense of glutamic acid (-an *in vitro* effect entirely comparable to *in vivo* findings recently reported from Greenstein's laboratory in N.C.I.) A presumably secondary effect in these incubated slices was lowered levels of γ-aminobutyric acid. However, addition of NH_4Cl to slices of cortex from methionine sulfoximine-intoxicated cats failed to stimulate any rise in glutamine levels, which remained near zero. But when both L-methionine and NH_4Cl were added to such slices a significant increase in glutamine levels of these slices was obtained. It would appear that the inhibition of glutamine synthesis by methionine sulfoximine is primarily an interference with the ammonia moiety, possibly by the imine group on the toxic compound, but that adding only NH_4Cl is ineffective in overcoming the block unless adequate levels of glutamic acid are available to be amidated to glutamine. These findings do not shed any light on the mechanism producing lowered glutamic acid levels except to indicate that they relate in some way to inhibition of methionine metabolism by its antimetabolite.

(5) The effects of 3-methyl-3-ethylglutarimide (Megimide) on glutamic acid metabolism were extended to include γ-aminobutyric acid. Slices from cats with seizures induced by Megimide showed levels of γ-aminobutyric acid significantly lower than normal. Similar studies on slices from cats with seizures induced by thiosemicarbazide (an inactivator of pyridoxal phosphate) showed not only very low γ-aminobutyric acid levels but also very low levels of glutamic acid.

(Killam had reported the former finding, but had not found the latter to be true, presumably because he failed to quick-freeze his brain biopsy samples from such animals.) (6) Incubation of normal cat cortex slices with 40 mM malonate produced some very unusual findings. Malonate is known to competetively inhibit succinic dehydrogenase and the effect of this concentration on oxygen consumption of slices (50% reduction) was reconfirmed here. These same slices exhibited normal production of lactic acid so that no interference with glycolysis or glucose utilization occurred. In the presence of malonate slice levels of both glutamic acid and γ-aminobutyric acid rose to double normal values. This observation is important from two standpoints. First Weil-Malherbe had found malonate caused reduction of oxygen uptake in both homogenates and slices of brain but an accumulation of succinate only in homogenates. At the time it was not clear why slices did not also show succinate accumulation. Our data suggest that in the whole cell preparation it is glutamate and γ-aminobutyrate rather than succinate which accumulate and require a study of the relationships among these three components of Krebs cycle metabolism. Secondly since studies in another project from the section (58-NINDB-30(c) indicate that γ-aminobutyric acid may be an important Krebs cycle substrate, the effect of an inhibitor which blocks the next step beyond in producing an accumulation of γ-aminobutyrate and its precursor glutamic acid is most suggestive confirmatory evidence.

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 studies. Particular attention will be paid to aspartic acid metabolism, utilizing a new, unpublished microanalytical method made available to us recently by Dr. O. H. Lowry. Very little is known about its metabolic role in brain, but the preliminary indications obtained in this project during the past several years suggest that it may be as interesting and important as glutamic acid has proved to be.

Part B, Included: Yes.

Serial No. NINDB-2714

PHS - NIH
Individual Project Report
Calendar Year 1958

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: in press.

2. Tower, D. B.
The Evidence for a Neurochemical Basis of Seizures.
pp. 301 - 348 in Baldwin, M. et al (Eds.) Temporal Lobe Epilepsy. Springfield, Thomas, 1958.

3. Tower, D. B.
Discussion [Clinical and Pathological Aspects of Toxicity from "Agenized" Proteins and Methionine Sulfoximine].
pp. 288-295 in Ibid. 1958.

4. Tower, D. B.
Glutamic Acid Metabolism in Mammalian Central Nervous System.
in Brücke, F. (Ed.) Symposium on Biochemistry of the Central Nervous System (IV International Biochemical Congress), London, Pergamon, in Press

5. Tower, D. B.
The Neurochemistry of Convulsive States.
in Folch, J. (Ed.) Chemical Pathology of the Nervous System (3rd International Neurochemical Symposium), London, Pergamon, in Press

6. Tower, D. B.
The Neurochemistry of Glutamine and Asparagine
in Brady, R. O. and Tower, D. B. (Eds.) Symposium on Neurochemistry of Nucleotides and Amino Acids (American Academy of Neurology), New York, Wiley, in Press.

Honors and Awards:

Appointed to Editorial Board of Biochemical Pharmacology.

Serial No. NINDB-28(a)
1. Medical Neurology
2. Clinical Neurochemistry
3. Bethesda, Md.
4. Same as 57_ NINDB-4(C) and 57-NINDB-5(C).

PHS - NIH
Individual Project Report
Calendar Year 1958

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. Peter Rowley, Dr. Guy McKhann, Dr. Bushnell Smith, Dr. C. Ajmone-Marsan, Mr. E. L. Peters

Cooperating Units: None.

Man Years: Patient Days: 75

Total: 1.0
Professional: 0.5
Other: 0.5

Project Description:

Objectives: To assess the effectiveness of various amino acids and related compounds in the glutamate-aspartate group for control of epileptic seizures complemented with studies of the in vivo metabolism of the various compounds in use.

Methods: See 57-NINDB-4 and 5(C).

Patient Material: Admissions to NINDB wards specifically for this project, plus patients admitted for other purposes.

Major Findings: As indicated in 57-NINDB-5(C), patients remaining on L-asparagine have been or are being discontinued or when appropriate switched to γ-aminobutyric acid. Data from the cooperative study of patients on L-asparagine have been collected from all but one clinic and are being processed and tabulated. The final report on this study will be drafted early in 1959.

Patients on γ-aminobutyric acid have continued to do well. One case became seizure free after three months on the compound compared to multiple daily seizures previously. On stopping γ-aminobutyric acid her seizures returned and have again been abolished by re-starting γ-aminobutyric acid. Several other patients are experiencing much better control on γ-aminobutyric acid than on L-asparagine. One of these, followed for four years on the latter with sustained improvement over his previous levels of control, is now almost free of seizures. One patient has experienced little change in seizure frequency after starting

on γ-aminobutyric acid, but has been able to reduce other medication significantly. Definite improvement in control has been observed in patients with petit mal absence type seizures and in those with generalized convulsions equally. It is clear that in this small group of patients γ-aminobutyric acid has proved relatively effective. The significance of this observation is difficult to evaluate until studies now in progress in another project (58-NINDB-30(c))clarify the questions of whether or not systemic γ-aminobutyric acid crosses the blood-brain barrier and whether it functions as an important substrate for cerebral oxidative metabolism.

In one case several attempts were made to administer γ-aminobutyric acid intravenously during EEG recordings. The solution had been autoclaved, check for pyrogens and rapidly injected intravenously into unanesthetized dogs in a dose of 4 mM/kg. body weight with no untoward effects. Peak blood levels in one dog were 10 μM./ml. of serum. Previous studies on anesthetized dogs [56-NINDB-97(C)] demonstrated no significant changes in B.P., respiration, EKG or EEG following such injections Yet when 1/200 of this dose of the same solution was slowly injected intravenously into the patient there was immediate agitation, flushing, hyperpnea, and drop in diastolic blood pressure. On repeated study, with injections of saline interspersed without effect, the same phenomena could be reproduced. Recovery occurred within 5-10 minutes. Despite reports by Elliott that such occurrences can be ignored and large dose safely injected, it would seem that pursuit of this type of study is too potentially dangerous to be justified.

Studies on a patient with "pyridoxine dependency" and the interrelationships of pyridoxine and γ-aminobutyric acid metabolism and function in the central nervous system are sufficiently unique that they form a separate project (see 58-NINDB-29(c)).

Until data on γ-aminobutyric acid have been more fully studied, trials of 2-pyrrolidinone and β-alanine have been deferred. However, studies with 2-pyrrolidinone-2-C^{14} with slices of cat cortex incubated *in vitro* indicate that cerebral tissues can open the pyrrolidinone ring to yield γ-aminobutyric acid, but the rate is relatively slow - about 15 per cent of a 4 μM/g. dose of specific activity 1.2 μC/μM was hydrolyzed during 1 hour's incubation of cortical slices. This finding coupled with the probable effects of blood-brain barrier *in vivo* suggests that 2-pyrrolidinone is unlikely to be a practical precursor form of γ-aminobutyric acid to use clinically. *In vitro* studies with β-alanine demonstrate a definite inhibition of oxygen uptake by cortical slices incubated with it. Albers has suggested that if β-alanine is metabolized

by brain it would yield malonic acid which is a known inhibitor of succinic dehydrogenase. Despite a favorable report by Williams at Emory that β-alanine exhibits clinical efficacy against seizures, the *in vitro* data makes its use questionable.

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: To continue studies discussed above.

Part B Included: Yes.

PHS - NIH
Individual Project Report
Calendar Year 1958

Part B.

Publications:

1. Tower, D. B.

Pyridoxine and Cerebral Activity

Nutrition Rev. 16: 161 (1958)

2. Tower, D. B.

The Neurochemistry of Convulsive States in Folch, J. (Ed.) Chemical Pathology of the Nervous System (3rd International Neurochemical Symposium) London, Pergamon, in press.

3. Tower, D. B.

The Neurochemistry of Glutamine and Asparagine in Brady, R. O. and Tower, D. B. (Eds.). Symposium on Neurochemistry of Nucleotides and Amino Acids (American Academy of Neurology) New York, Wiley, in press.

Serial No. NIMDB 29
1. Medical Neurology
2. Clinical Neurochemistry
3. Bethesda, Md.
4. New

PHS - NIH
Individual Project Report

Calendar Year 1958

Part A.

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

Principal Investigator: Dr. Guy M. McKhann.

Other Investigators: Dr. D. B. Tower; Dr. C. Ajmone-Marsan

Cooperating Units: Dr. Louis Sokoloff, NIMH Section on Cerebral Metabolism; Dr. R. W. Albers, NINDB Lab. of Neuroanatomical Sciences; Dr. Olaf Mickelsen, NIAMD Lab. of Nutrition; Dr. D. B. Coursin, St. Joseph's Hospital (Lancaster, Pa.)

Man Years: Patient Days 20

Total: 0.4
Professional: 0.4
Other: 0

Project Description:

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

Methods: Pyridoxine deficiency can be induced in experimental animals either by dietary means or by use of pyridoxine antagonists, such as thiosemicarbazide. Since seizures result from the full blown deficiency state, regardless of method of induction, their appearance is taken as the end-point for studies. Effects of the deficiency is evaluated by in vitro determinations of cerebral amino acids, especially glutamic acid and γ-aminobutyric acid, measuring levels and investigating metabolic pathways of these compounds in normal controls and deficient samples as outlined in 59-NINDB-30(c)

The human counterpart under study is a condition originally described by Hunt et al. in 1954 as "pyridoxine dependency." A half-dozen such cases are now recognized in the U.S., representing a condition in which seizures occur unless large daily doses of pyridoxine are provided. Such patients are studied by the Krypton^{85} method for measuring cerebral metabolism developed by Sokoloff and by recent analytical procedures for pyridoxine and metabolites developed by Coursin.

Patient Material: Obtained from NINDB patients admitted specifically for this study and those admitted for other purposes.

Major Findings: The original case of "pyridoxine dependency" reported in 1954 by Hunt et al. has been restudied. The patient now age 7 years is still dependent, regularly developing seizures within 72 hours of omission of her regular daily dose of 10 mg. of pyridoxine. Typical EEG abnormalities at such times were repeatedly observed and could be abolished within 30-60 seconds by intravenous pyridoxine-HCl 15 mg. Concurrent subjective and objective improvement in the patient's condition was dramatic and reproducible.

During a typical period of pyridoxine depletion cerebral metabolism was measured by the Krypton^{85} technique and the effect of intravenous pyridoxine-HCl 15 mg. observed during the same observation period. Results are tabulated as follows:

	Depleted State	After I.V. Pyridoxine
CBF(ml./100 g./min.)	63	70
O_2 Consumption(")	3.3	4.4
A-V O_2 difference (Vol.%)	5.26	6.23
Cerebral R.Q.	0.85	0.96

Since this patient is both mentally retarded and subject to seizures she must be compared to analogous groups of children previously studied by Kennedy and Sokoloff. These groups exhibited values for cerebral blood flow and oxygen consumption lower than those for comparable normal children, but in only an occasional instance was blood flow or R.Q. as low as in this case and in only one was oxygen consumption so low. The values obtained after pyridoxine repletion are similar to those reported by Kennedy and Sokoloff for their groups. On the other hand the decreased oxygen consumption during the depleted state in this case is similar to the situation reported by Sokoloff for hypoglycemic subjects where an obvious substrate deficiency exists. Thus, the interpretation tentatively put upon the data obtained in this case is that during pyridoxine depletion a deficiency of a substrate for cerebral oxidative metabolism existed which was promptly corrected by pyridoxine administration. Since pyridoxine deficiency appears to affect γ-aminobutyric acid metabolism primarily and since that compound appears to be a significant substrate for cerebral oxidative metabolism [see 58-NINDB-30(c)], this case may represent an example of γ-aminobutyric acid deficiency with consequent reduction in oxidative metabolism. The latter reduction by about 25% is compatible with *in vitro* estimates of 15% of total oxygen uptake being due to γ-aminobutyric acid metabolism if the respective *in vitro* and *in vivo* levels of metabolic activity are taken into consideration.

The nature of the defect in pyridoxine nutrition in cases of "pyridoxine dependence" is important to an understanding of the mode by which pyridoxine is handled in the body. Samples of blood, urine, etc. from this patient have been obtained during periods of depletion and repletion for determinations of levels of pyridoxine, pyridoxal, pyridoxamine, pyridoxal phosphate and 4-pyridoxic acid. These samples have been frozen and await analysis until the microfluorimetric procedures developed by Coursin have been thoroughly proved.

Complimentary studies on experimental animals are under way. Production of dietary deficiencies in kittens has been attempted repeatedly by Dr. Nickelsen during this year. A satisfactory artificial diet has now been achieved so that suitable animals may be available shortly. Chemically-induced deficiencies using thiosemicarbazide, have been produced and preliminary studies on cerebral samples in vitro carried out, complementing those in 58-NINDB-30(c) . Significantly low levels of γ-aminobutyric acid and of glutamic acid have been found in such samples.

Significance of Project: The association of a seizure state with a specific defect in cerebral oxidative metabolism would provide a long sought solution to the problem of why no such defect has been demonstrable in the past and to the possible bases for biochemical abnormalities clearly present in seizure states (disturbances in electrolyte and amino acid metabolism) which seemed most readily explicable in terms of defects in oxidative metabolism. The inability to find such defects in previous attempts may be due to (1) the less widespread dysfunction as e.g. in focal seizures and/or (2) the much lower level of oxidative metabolism in vitro compared to that in vivo such that a fractional defect could be obscured in the absence of in vivo activity demands. Thus, a study of this type could provide a very valuable key to understanding of these problems.

Proposed Course: To continue studies outlined above notably (1) in terms of pyridoxine metabolism in the body, (2) on experimental animal material in vivo and in vitro and (3) if available, with similar "pyridoxine-dependent" patients.

Part B Included: No.

[illegible]-57(c)
1. [illegible]
2. Clinical Neurology
3. Bethesda, Md.
4. Same as 57-NINDB-7 (C)

[illegible] - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: The Metabolism of γ-Aminobutyric Acid in Neural Tissue.
Principal Investigator: Dr. Guy M. McKhann
Other Investigators: Dr. D. B. Toney, Mr. E. L. Peters.
Cooperating Units: Dr. R. Wayne Albers, NINDB Lab. of Neuroanatomical Sciences (Collaboration)

Man Years: Patient Days: 0

Total:	0.9
Professional:	0.8
Other:	0.1

Project Description:

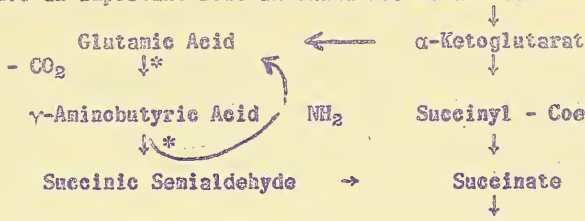
Objectives: To study the metabolsim of γ-aminobutyric acid in neural tissues, to study factors affecting such metabolism, and to deduce therefrom the significance of this compound in neural metabolism and function.

Methods: The fluorimetric method for determination of γ-aminobutyric acid is described in 57-NINDB-7 (C). An enzymatic method, using an enzyme system from Pseudomonas, is being adapted, based on reports by Jakoby, but is still in exploratory stages. In vitro studies on γ-aminobutyric acid metabolism in incubated brain slices and isolated mitochondria are carried out by established methods applied in this laboratory. Detailed studies of metabolic pathways involved utilize γ-aminobutyric acid - 1 - C^{14}, L-glutamic acid - U - C^{14} and α-ketoglutaric acid - 2, 3-C^{14} using slices or homogenates of brain tissue with isolation of individual components by ion exchange and chromatographic techniques and counts of activity by gas flow and liquid scintillation counters.

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

Major Findings: (1) In experiments using γ-aminobutyric acid, succinic semialdehyde, or glutamic acid as sole substrate, the oxygen consumption of incubated slices of cat cerebral cortex was supported to the same extent as when glucose was used as substrate. Similarly when esterification of phosphate was studied with cat cortex mitochondria by the Brody and

Bain technique, P/O ratios (moles of phosphate esterified per atoms of oxygen consumed) with γ-aminobutyric acid or succinic semialdehyde as substrate were identical with those obtained when glutamate orpyruvate were the substrates. These findings indicate that γ-aminobutyric acid can function as a substrate of cerebral oxidative metabolism. Because of its position in a shunt pathway around the α-ketoglutarate to succinate step of the Krebs cycle (see diagram), it may have an important role in oxidative metabolism:



[* - steps catalyzed by Vitamin B_6 as pyridoxal phosphate]

(2) In incubated slices of cortex from cats with seizures induced by methionine sulfoximine, 3,3-methylethylglutarimide (Megimide), or thiosemicarbazide, the levels of γ-aminobutyric acid are low and decrease further during incubation. Preliminary data on human epileptogenic cortex slices are similar. The levels of γ-aminobutyric acid obtained in normal control slices are higher than those reported by Roberts using a chromatographic method of estimation. The possible reasons for the discrepancies are being investigated using the specific enzymatic method adapted from Jakoby. One of the convulsants cited above, thiosemicarbazide, is a Vitamin B_6 (pyridoxine) antagonist which has been shown by others to inactivate the coenzyme form, pyridoxal phosphate, to produce a chemical deficiency. Preliminary experiments indicate that in the presence of thiosemicarbazide the ability of γ-aminobutyric acid to support cerebral oxidative metabolism is inhibited.

(3) Since control of seizures in animals due to a variety of convulsant agents as well as those in clinical patients has been observed with oral or parenteral γ-aminobutyric acid, the previous claims by others that systemically-administered γ-aminobutyric acid does not penetrate the blood-brain barrier are being reinvestigated. Blood levels rise sharply upon administration and fall promptly, indicating rapid distribution and metabolism with little or no spillage into the urine. In some human cases definite rises of cerebrospinal fluid levels have been observed. Using C^{14} - labelled γ-aminobutyric acid, preliminary evidence for penetration across the blood-brain barrier of mice has been obtained, using constant infusions of high doses to compensate for the relatively small cerebral blood flow/hepatic blood flow ratio

present in rodents.

(4) Simultaneous determinations on the same tissue sample *in vitro* of the rate of metabolism of γ-aminobutyric acid and oxygen uptake indicate that metabolism of γ-aminobutyric acid can account for approximately 15 per cent of the total oxygen uptake. This estimate compares favorably with observations reported in 58 - NINDB - 29(c) on a pyridoxine-dependent patient where the *in vivo* fraction of cerebral oxygen consumption possibly attributable to γ-aminobutyric acid metabolism was 25 per cent.

Significance of Project: In creasing evidence from this study and reports by other investigators indicates that γ-aminobutyric acid has an important role in cerebral metabolism. The shunt pathway (glutamate - γ-aminobutyrate) appears to be active and important in cerebral oxidative metabolism and is significantly involved in certain dysfunctions of the brain such as seizures. The possibility that this pathway for γ-aminobutyric acid metabolism, which is unique to the brain, may exert a regulatory action on a critical portion of oxidative metabolism and hence on energy production warrants careful and detailed investigation both in terms of normal function and of seizure states.

Proposed Course: To continue studies outlined above.

Part B. Included: Yes

Serial No. NINDB-30(c)

PHS - NIH
Individual Project Report
Calendar Year 1958

Part B.

Publications:

McKhann, G. M. and Tower, D. B.
Gamma-Aminobutyric Acid: A Substrate
for Oxidative Metabolism of Cerebral Cortex.
Am. J. Physiol. 196: in press

Honors and Awards: none

Serial No. NINDB-31(c)
1. Medical Neurology
2. Clinical Neurochemistry
3. Bethesda, Md.
4. Same as 57-NINDB-17 (C)

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Microchemical Determination of Acetylcholine.
Principal Investigator: Dr. William C. Curtis.
Other Investigators: Mr. Lamar Kennerson; Mr. J. W. Phoenix
Cooperating Units: None.
Man Years: Patient Days: 0

Total:	0.2
Professional:	0.1
Other:	0.1

Project Description:

Objectives:) See 57-NINDB-17 (C).
Methods:)

Major Findings: All attempts to eliminate gross interference encountered when applying the acetylcholine-boron-flavanol reaction to biological materials have proved unsuccessful. Since such interference renders this reaction useless for fluorimetric determination of acetylcholine in biological samples this project has been discontinued until such time as more information on the kinetics and mechanisms of the reaction become available.

Part B Included: No.

Serial No. NINDB-32(c)
1. Medical Neurology
2. Clinical Neurochemistry
3. Bethesda, Md.
4. Same as 57-NINDB-19(C)

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Physico-chemical studies of Human Cerebrospinal Fluid.

Principal Investigator: Dr. William C. Curtis

Other Investigators: Mr. L. Kenerson, Mr. J. W. Phoenix

Cooperating Units: Dr. W. A. Zisman, Surface Chemistry and High Polymer Section, NRL.; Dr. John H. Seipel, Mt. Alto V.A. Hospital; Dr. D. B. Coursin, St. Joseph's Hospital (Lancaster, Pa.)

Man Years: Patient Days: 0

Total: 1.5
Professional: 0.5
Other: 1.0

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 under major findings.

Patient Material: Obtained from NINDB patients and outside sources (see cooperating units) admitted for other purposes. Pooled samples collected at random used for preliminary studies and specific samples from individual patients with verified neurological disorders used for subsequent work. In addition fresh human brain samples obtained at autopsy, as soon as possible after death, used as sources for various components under study.

Major Findings: Preliminary investigations of suitable procedures for isolation, analysis and their control have been necessary. Two approaches have been adopted: (1) study of a model protein, guinea pig serum asparaginase, a globulin with easily assayable enzyme activity as an indicator of the effects of isolation and analytical procedures upon the natural state of the protein; and (2) application of surface-chemical techniques for separation and isolation of micro quantities of lipids and proteins in cerebrospinal fluid.

The studies on guinea pig serum asparaginase detailed in previous reports [see 57-NINDB-19(C)] have been completed except for a few additional confirmatory studies on electrophoretic and ultracentrifuge data now in progress. The partially purified enzyme preparation contains at least two macromolecular (?protein) contaminants which have defied attempts at separation by electrophoretic or ultracentrifugal means. The enzyme protein can be quantitatively adsorbed on modified cellulose and in carbon dioxide foam. Purificati by these means is currently being attempted.

The difficulties encountered with this relatively simple problem illustrate the necessity for more sensitive and discriminating isolation techniques, particularly for cerebrospinal fluid where proteins and lipids are present in trace amounts and the amounts of fluid available are so limited. Hence attention has been directed toward application of surface-chemical techniques to these problems.

Progress in fractionation of separable surface active fluid constituents in unchanged form has been made, using selective adsorption in foams or at interfaces between immiscible fluids. Appreciable quantities of fluid protein are definitely removed in carbon dioxide foams. Air foams proved much less effective. If xanthoproteic acid determinations on trichloracetic acid and phosphotungstic acid filtrates of the fluid provide valid estimates of polypeptide content, then polypeptides are also removed in the foam. Significant amounts of lipids not bound to protein are removed by adsorption at benzene-fluid interfaces while protein-bound lipids are apparently not removed. All fluid lipids are concentrated at chloroform-fluid interfaces.

Considerable time and effort has been devoted to devising suitable procedures for identification and quantitation of lipid constituents so isolated. Qualitative identifications of total unbound lipids, free and bound cholesterol, cerebrosides, sphingomyelin and the various phospholipids have been achieved by the chromatographic methods of Hack using suitable color reactions. Quantitative estimates have been possible in some cases and are in process of development now.

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: To continue studies along lines outlined above.

Part B Included: No.

Serial No. NINDB-33(c)
1. Medical Neurology
2. Clinical Neurochemistry
3. Bethesda, Md.
4. Same as 57-NINDB-18 (C)

PHS - NIH
Individual Project Report
Calendar Year 1958

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; Mr. J. W. Phoenix.
Cooperating Units: Dr. W. A. Zisman, Surface Chemistry and High Polymer Section, N.R.L.

Man Years: Patient Days: 0
Total: 1.5
Professional: 0.5
Other: 1.0

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 report 56-NINDB-95(C)

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

Major Findings: Surface tension of urine in relation to other parameters has been evaluated. A strong time-dependence was found with characteristics suggesting at least two physical processes, one an initial rapid one and the other a longer and slower process. From this observation it is obvious that in urine there is no such thing as surface tension per se but only surface tension at a particular time. Effects of temperature (not significant), of pH (observation of minima), and of adding various presumed surface-active agents such as albumen (no effect) and bile salts (pronounced lowering of tension) were studied. However, it was obvious that surface tension measurements alone are too gross to reveal more than the overall phenomenon of adsorption and in addition are difficult to reproduce from one operator to another because of the time-dependence.

The possibility that surface tension measurements could be replaced by optical measurements, which intrinsically are more closely related to the ultrastructure of an interface, has been explored. Preliminary crude measurements of this sort have been made at the Naval Research Laboratories and indicate the

method has merit. Exploration of this approach is continuing.

Meanwhile attention has been turned to fractionation of the difficultly dialysable urinary constituents, which contribute to surface active behavior, by adsorption on solid surfaces (adsorption column resins and the like), in foams and at interfaces between urine and water-immiscible liquids. Convenient, simple analytical procedures have been developed to evaluate the effectiveness of these surface-chemical methods of fractionation. Such work is now in progress.

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: To continue investigations along lines outlined above. Progress must necessarily be expected to be slow because of the requirements to develop suitable analytical and control methods, to explore a large number of possible surface-chemical techniques, and to clarify the theoretical bases for observed data and phenomena.

Part B Included: No.

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

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Cerebral Protein Metabolism and Turnover in Tissue Slices incubated in vitro.
Principal Investigator: Dr. JohnL. Wherrett (Guest Worker)
Other Investigators: Dr. D. B. Tower, Mr. E. L. Peters.
Cooperating Units: Dr. Heinrich Waelsch, New York State Psychiatric Institute (Advice and Isotopically-labelled Materials).
An NIH unit for Mass Spectrograph Analysis (to be arranged later).

Man Years:
Total: 0.4
Professional: 0.4
Other: 0

Patient Days: 0

Project Description:

Objectives: To determine whether slices of cerebral tissues 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: Standard incubated slice techniques developed in this laboratory will be used for cat cerebral cortex. Multiple slice samples of pooled weight 1.5-2.0 grams will be incubated with L-Glutamine-U-C^{14} and L-Glutamine-Amide N^{15}, separately and in combination with L-Glutamic Acid-U-C^{14} and with L-Aspartic Acid-U-C^{14}. Slices and incubation media will be separated, the slices homogenized in trichloracetic acid to yield a supernatent fraction containing the free amino acids and a precipitate or crude protein fraction.

The free amino acid fraction will be assayed for glutamic acid by methods previously applied in this laboratory to obtain total content of each of these free components. Counts of C^{14} activity in each free amino acid pool will be obtained after chromatographic separation by methods now in use in the laboratory to obtain the specific activities in each free amino acid pool. N^{15} specific activities will be obtained by mass spectrograph analysis (arrangements to be concluded later for this phase of the study).

The crude protein fraction will be purified by the method of Siekevitz and Potter as adapted by Waelsch to remove nucleic acids and lipids. The resulting pure protein fraction will be weighed, dissolved in thioglycollic acid and/or NaOH (as specified by Waelsch) and reprecipitated to check total C^{14}-activity as remaining constant and hence truly present in the proteins. The protein fraction will then be enzymatically hydrolyzed using a preparation from hog pancreas, as developed by Barry specifically for glutamate and aspartate amino acid groups. After dialysis and concentration by lyophilization, the free amino acids derived from the proteins will be separated chromatographically and counted as above, and aliquots of the free amino hydrolysate will be assayed for glutamine, glutamic acid, asparagine and aspartic acid as above. Thus specific activities both in terms of protein and in terms of specific amino acid contents of the protein can be calculated.

If preliminary experiments on slices incubated 1-2 hours prove successful, rates of amino acid incorporation can be obtained by using a series of incubation times. From these data plus the activity in the free pools, turnover rates of the amino acids in the proteins can be estimated.

Major Findings: This project has just begun, since Dr. Wherrett came to the laboratory in September, 1958. Basic techniques have been mastered in reproducible fashion on slices without added isotope down to the purified protein stage. It has proved feasible to carry out the assay of free amino acids in the slices on the trichloracetic acid supernatent after removal of the latter by ether extraction so that these data can be obtained on the same samples from which the protein fraction is obtained. Protein yield of 7% of the wet weight of the slices have been obtained, in good agreement with theoretical yields by calculation.

Significance of Project: Studies by Richter with S^{35}-methionine, by Waelsch with C^{14}-lysine and C^{14}-leucine and by Sporn and Dingman with C^{14}-proline indicate that *in vivo* amino acids are very rapidly incorporated into cerebral proteins and that the turnover of the latter is comparable in rate to that of liver proteins. It is practically not feasible to conduct such studies in man due to limitations on the level of C^{14} which can be administered. Therefore, a successful demonstration of *in vitro* amino acid incorporation into cerebral tissue proteins and their turnover using animal samples would make it possible to apply this technique to human neurosurgical brain samples and thus provide some data on man both for normal samples and for those with disease processes such as seizures. Since no data on the most prevalent and metabolically active amino acids (glutamic acid, glutamine and aspartic acid) are available even *in vivo*, it is felt that the use of them initially will carry additional value of itself.

Proposed Course: To continue the studies as outlined under Methods above.

Part B Included: No.

Serial No. NINDB-85(c)
1. Medical Neurology
2. Clinical Neurochemistry
3. Bethesda, Md.
4. Same as 57-NINDB-14 (C)

PHS - NIH
Individual Project Report
Calander Year 1958

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. J. B. Proctor.
Cooperating Units: Dr. K. Laki, LPB, NIAMD.
Man Years: Patient Days: 0
Total: 0.6
Professional: 0.3
Other: 0.3

Project Description:

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

Methods: Actin is prepared by the procedure of Mommaerts. Tropomyosin A is prepared by the method of Laki from Clam muscle and by the method of Kominz from rabbit myosin. Tropomyosin B is prepared by the method of Bailey. Myosin is prepared by the method of Szent - Gyorgyi. These muscle proteins are obtained in a high state of purity. Rabbits are immunized by repeated injections of these proteins with use of skin tests to assess the response of the animals. Sera are collected from immunized animals and are used in precipitin reactions. The globulin fraction of such sera is conjugated with fluorescein, and the conjugated antibodies are used as specific stains for muscle proteins (antibody-antigen complex) on sections prepared from biopsy material and studied under the fluorescence microscope according to the technique originally devised by Coons.

Patient Material: Muscle biopsy specimens are obtained from NINDB patients admitted for other purposes.

Major Findings: Sera of rabbits immunized against clam Tropomyosin A precipitate mammalian Myosin. Antisera to human and cat Myosin precipitate clam Tropomyosin A. No such cross-reaction was found between antisera to chicken Tropomyosin B on the one hand and clam Tropomyosin A or mammalian Myosins

on the other. Actin had previously been found to be iso-antigenic by Horvath and also by Kesztyus. This finding was reconfirmed using Actin which is 99% pure. In addition Tropomyosin A, Tropomyosin B and Myosin were now also found to be iso-antigenic. Some of the animals immunized with muscle proteins appeared to show weakness and stiffness of muscles and exhibited muscle lesions on microscopic examination.

Using antibodies to Myosin conjugated with fluorescein, Myosin in sections of normal human muscle was clearly and distinctly demonstrated under the fluorescence microscope. Preliminary sections of dystrophic muscle similarly treated showed myosin in residual islands of muscle and a suggestion that in areas of active degeneration myosin-reactive material was present in macrophages.

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. To investigate the nature of the muscle lesions observed. 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.

Serial No. NINDB-35(c)

PHS - NIH
Individual Project Report
Calendar Year 1958

Part B.

Publications:

1. Klatzo, I., Horvath, B. and Emmart, E. W.

   Demonstration of Myosin in Human Striated Muscle by Fluorescent Antibody.
   Proc. Soc. Exp. Biol. Med. 97: 135 (1958)

2. Laki, K., Horvath, B. and Klatzo, I.

   On the Relationship between Myosin and Tropomyosin A.
   Biochim. Biophys. Acta 28: 656 (1958)

Honors and Awards: None.

Serial No. NINDB-30(C)
1. Medical Neurology
2. Clinical Neurochemistry
3. Bethesda, Md.
4. Same as 57 NINDB-15(C)

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A:

Project Title: Alterations of Actomyosin Tensile Strength and Muscle Proteins in Neuromuscular Diseases.

Principal Investigator: Dr. Beni Horvath

Other Investigators: Dr. G. M. Shy, Mr. J. B. Proctor

Cooperating Units: None

Man Years: Patient Days: 0

Total:	0.8
Professional:	0.4
Other:	0.4

Project Description:

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

Method: See 1957 report [NINDB-15(C)] for methods previously developed. Additional procedures in use are the following. Muscle samples are homogenized in water and aliquots of the homogenate are extracted respectively with (1) trichloracetic acid, (2) water, (3) Edsall's solution (0.6M KCl with carbonate-bicarbonate buffer), and (4) 0.05 N NaOH. Samples of the homogenate diluted with water serve for determination of total solids (by a micromethod standardization on normal muscle) and total nitrogen. Nitrogen content of the four extracts and total nitrogen are determined by direct Nesslerization (a spectrophotometric procedure adapted for this purpose and standardized in this laboratory on normal muscle). Extract (2) is also utilized for determination of K, Na, and Cl. These procedures permit triplicate analyses for all components on 0.3 gram of dystrophic muscle.

Major Findings: The determinations detailed under Methods permit calculation of the following components for each specimen: (1) total solids, (2) total protein, (3) non-protein solids (fat), (4) non-collagenous proteins, (5) collagen, (6) water-soluble proteins, (7) myosin, (8) alkali-soluble proteins, (9) non-protein nitrogen, (10) electrolytes (Na, K, Cl), and (11) tissue water. Comparison of normal and dystrophic muscle analyses reflect the wasting of muscle and replacement of muscle by connective tissue and fat as indicated by increases in (1), (3), (5) and Na and Cl in dystrophic specimens while all other components are lower in dystrophic specimens. Values

obtained on dystrophic specimens for protein fractions (6), (7) and (8) indicate a relative increase of myosin and decrease of alkali-soluble proteins in most cases. These changes seem to be independent of remaining muscle mass, estimated from (4), and also of the kind of tissue replacing muscle. Water-soluble proteins appear to be increased relative to other proteins in most dystrophic samples and an inverse relationship is indicated between remaining muscle mass, estimated from (4), on the one hand and the percentage of water-soluble proteins in the muscle on the other. It is concluded that samples of dystrophic muscle not only contain less muscle and more connective tissue and fat than normal muscle, but the protein composition of the remaining muscle is different from normal.

Significance of Projects: This project is part of an integrated effort, involving many disciplines, directed toward elucidation of the nature of myopathies such as muscular dystrophy, myotonia and myasthenia gravis. One possibility is some abnormality of muscle protein, and this project is designed to investigate that possibility.

Proposed Course: To continue these studies on biopsy specimens obtained from patients with other types of muscle disease, and to extend the study of muscular dystrophy samples by physico-chemical investigations of the protein fractions here determined by the general approaches previously adopted [See 57 NINDB-15(C)].

Part B included: Yes

Serial No. NINDB-36(c)

PHS - NIH
Individual Project Report
Calendar Year 1958

Part B:

Publications:

Horvath, B. and Proctor, J. B.
Muscular Dystrophy: Quantitative Studies on the Composition of Dystrophic Muscle.

Proc. Assoc. Res. Nerv. Ment. Dis. in press (1958).

Honors and Awards: none.

ANNUAL REPORT
OPHTHALMOLOGY BRANCH
NATIONAL INSTITUTE OF NEUROLOGICAL
DISEASES AND BLINDNESS

Calendar Year 1958

The past year brought several changes in the staff of investigators in the Ophthalmology Branch with the arrival of new visiting scientists, Dr. van Alphen, Dr. Tansley, Dr. Lele and Dr. Tasaki, and the departure of other research workers, as Dr. Dodt and Dr. Gouras. Dr. Dodt's six month stay ended all too early in February of this year and was felt as a serious loss. The severe strain on the only senior fulltime ophthalmologist, a strain resulting from the growth of the clinical program, was considerably relieved by the appointment of Dr. van Alphen as Associate Ophthalmologist. His experience in medical and surgical ophthalmology and his interest in research provided the possibility to assist young ophthalmologists in the out-patient service, in the operating room and on the wards, and also to discuss certain laboratory procedures with them. His and my tasks in patient care as well as the tasks of the nursing staff, were greatly facilitated by the concentration of eye patients on one floor. The advantages of this system will be fully realized when the necessary organizational changes have been completed. The help of Dr. O'Rourke, Consultant to the Branch, in this endeavor, is greatly appreciated. The commendable performance of Clinical Associates, their unusual medical background and their devotion to assignments made it possible to absorb a considerably increased patient load, to employ new time-consuming methods of examination and to increase clinical investigative work. That these men have time to carry out these multiple duties and laboratory studies is much to their credit.

The individual project reports have been more detailed than in previous years. It is not necessary, then, to cite specific results of the reported investigations, important as they may be, but convey an overall impression. It is obvious from the reports that the general trend of laboratory and clinical investigation proceeds along a steeply ascending limb. This observation refers both to the quality and quantity of research activities. -- There can be little doubt in my mind that Dr. Fuortes and the co-workers in his section are leading the way in their exploring work on problems of vision. This seems true in regard to the quality of the contributions as well as their bearing on basic general problems of sensory physiology. It is always amazing to me that such excellent work could be accomplished in so short a time. -- In a related field

Dr. Dodt's research in spectral sensitivity of the retina by physical methods of examination is outstanding and the offspring of his thoughts are reflected in studies on color vision so ably conducted by Dr. Copenhaver and Dr. Gunkel -- Dr. Katharine Tansley, eminent physiologist from the Institute of Ophthalmology in London, is at work to utilize the facilities for examination of spectral sensitivity and flicker fusion frequency on animal species with pure cone retinae in order to demonstrate clearly a separation of photopic responses from contaminating scotopic influences. -- The clinically important studies on patients with various types of degenerative retinal disease by electroretinography is being continued in the same laboratory and advanced by technical developments of adaptometry and perimetric light sense studies (Dr. Gunkel). These physical and psychophysical methods combined with spectral sensitivity studies allow for a most comprehensive workup of diagnostic problems. In the three other major projects of the Branch laboratory investigations on a basic level have also been linked to clinical problems.

These projects are (1) physiology and pathology of the intraocular pressure, (2) cataract, and (3) uveitis.

(1) Physiology and pathology of the intraocular pressure. Of great promise is the elegant technique originally devised by Dr. Lele for studies on sensory receptors in the cornea when applied to investigations of afferent discharges travelling in posterior ciliary nerves in response to small intraocular pressure changes. Dr. Lele and Miss Grimes have already demonstrated that such discharges can be obtained from all mixed nerves in isolated eye nerve preparations and that positive results are more frequent than they have been in the in vivo work reported last year. It was also shown in this study that the pressure induced discharges differ distinctly from those produced by touch. The whole theory of nervous regulation of the intraocular pressure has received significant support from these results. -- In view of this, an anatomical examination of posterior ciliary nerves started early this year is of particular interest and timely. This study was greatly facilitated by the modification of Christensen's silver technique by Miss Grimes for gross demonstration of nerves so that it can be extended to systematic examination of species differences.

It was pointed out last year that Dr. Macri's studies on the elasticity of the eye, the effect of extraocular muscle on the elasticity, and his aqueous humor outflow experiments have been helpful in evaluating the rigidity factor in tonographic studies. Systematic recordings of the pressures in venous channels near their exit on the surface of the eye carried out by Dr. Macri are intriguing. The

demonstration by casts of the connections between anterior chamber (trabecular area) and episcleral veins and the interconnections between these vascular beds and other ocular venous systems should have repercussions in debates on aqueous humor dynamics. These preliminary results as well as Dr. Macri's observations on the lowering of the pressure in the vortex vein of cats by Diamox may well have clinical implications. - - Dr. Cohan's approach to use angiography for demonstration of intraocular vessels also might provide information pertaining to problems of intraocular pressure regulation. He cannulates the anterior ciliary vein and injects radio-opaque material in this vessel. With laminagraphic techniques he obtained clear pictures of the intraocular venous system in the living animal.

It is partly on the basis of reported laboratory results that the clinical glaucoma problem has been expanded. Cross fertilization between laboratory and clinical studies is expected to increase when sufficient pharmacological data are available and confirmed. At present the unique opportunity to admit glaucoma patients for prolonged periods of time with the availability of necessary instruments permit diagnostic studies in doubtful cases and the determination of the relative value of diagnostic procedures (Dr. Paton and co-workers).

Cataract. The cataract project received great impetus from the excellent work of Dr. Wanko and Miss Gavin, who showed by electron microscopic studies the fine morphology of normal lens tissue elements and of changes in cells and fibers in initial stages of cataract development. It is the first time that the ultrastructure of the lens--a most difficult tissue to handle--has been investigated with a reliable technique, and it seems that the information obtained will change the concept of cytopathology based on light microscopic observations. Of considerable general interest are observations of a low density element in the epithelial cells and the lens fibers and the analysis of these fine structural particles by ultracentrifugation and chemical methods. This work, conducted by Dr. Resnik and Dr. Wanko, approaches the state in which morphological elements might be chemically identified. The studies in the electron microscope laboratory have been extended to other areas of the eye, particularly the ciliary processes, but this investigation is in an initial stage. Furthermore, the same investigators conducted studies on biopsy material of normal and dystrophic skeletal muscles. The changes observed in material obtained from patients with myotonic dystrophy have been described and tissue specimens of other neuromuscular diseases will be subjected to electron microscopic examination.

Light microscopic examinations have been carried out on two types of experimental cataracts which demonstrated dramatically the difference of cataract formation produced by different cataractogenic agents when studied on a cellular level. Although the value of such observations for the differential diagnosis of human cataract is definite, in vivo and histological findings do not give any clues for medical therapy in these two types of cataract -- It is possible that such clues are forthcoming when tissue and organ culture methods have rendered more reproducible results than obtained at present. The tissue culture work on the lens met with difficulties due to the complex nutritional requirements of this material, but efforts are being made to proceed in this line (Mr. Caravaggio).

Related to the cataract problem are outstanding studies on proteins of the lens by Dr. Resnik. Ultracentrifugation, electrophoresis, spectrophotometry, equilibrium dialysis and viscosimetry are employed to establish the characteristics of alpha crystallin. Dr. Resnik's values of molecular weight, diffusion co-efficient and apparent specific volume are most satisfactorily confirmed in the studies of Orekhovich. The future will show whether the chemical data on alpha crystallin and the influence of environmental factors on the protein have applicabilities to studies on transparency of the lens.

Dr. Kuhlman worked with elaborate microchemical techniques on the enzymatic systems of the lens, but the main part of this important investigation deals with the corneal epithelium. With regard to the lens problem he confirmed that hexokinase and glutathione reductase was unaffected by the exposure of the eye to 1,000 R-rays, despite the presence of morphological changes seen at the time intervals examined. The results obtained on corneal epithelium were particularly noteworthy with respect to the lactic dehydrogenase activity in the rabbit which exceeded ten times that reported in other tissues or species. -- In radioactive tracer experiments Dr. Kuhlman demonstrated the importance of lactate for corneal metabolism and showed that the stroma is capable of oxidating lactate better than glucose. The direct oxidative shunt plays an integral part in corneal metabolism as shown previously by other techniques.

(3) Uveitis. Uveitis is one of the most frequent ocular diseases, of which still very little is known. The investigative efforts of the Branch in previous years have been geared to the toxoplasma problem and this situation has not changed essentially in the last year. This is explained by the apparently high

incidence of toxoplasma infections as a cause of uveitis and further by opportunity to have the undivided cooperation from Dr. Jacobs' laboratory, who has contributed so greatly to the knowledge of this disease. It seems, then, that clinical and laboratory studies on this subject promise to be most rewarding. -- Dr. Kaufman, in cooperation with Dr. Jacobs' laboratory, has carried out studies on the virulence of strains of toxoplasma gondii and shown in a beautiful study the dependence of invasiveness on virulence and that of susceptibility of the organism for Daraprim action on the growth rate of strains; that is, slow growing organisms are more resistant to the chemotherapeutic agents than rapid growing strains. -- Distribution studies on Daraprim (Dr. Kaufman) conducted on humans and laboratory animals show that the drug does scarcely enter the aqueous humor from the blood but that it reaches apparently levels in the retina comparable to those in the serum. Dr. Kaufman also has demonstrated that an initial high dose of Daraprim results rapidly in a satisfactory serum level, which then can be maintained with the usual smaller daily doses. The clinical implications are obvious. The problem of increased toxicity by a high loading dose requires further study. -- Dr. O'Rourke continued his studies on the association of recurrence rate of uveitis and abnormalities in the peripheral utilization of thyroid hormone. It seems, on the basis of observations on about 30 patients, that uveitis cases utilize the circulating thyroid hormone at a shower rate than normals but the clinical material is not sufficient to draw more definite conclusions or to proceed to therapeutic trials. -- Dr. van Alphen's project on immunological relations of ocular tissues, now conducted with lens capsule and lens protein, might lead to immunological studies connected with the uveitis problem. The results obtained on antigen antibody responses on animals immunized with lens capsule and lens protein were negative so far, inasmuch as cataract could not be produced under the conditions of the experiments. It should be stressed that Dr. van Alphen's experience with immunological work fills a need of the Branch and has many potential ramifications.

A small number of investigations cannot be grouped into the four main areas of research efforts. (1) Dr. Kaufman collected a number of patients with the main ocular sign of cottony vitreous opacities interfering with vision but without signs of hemorrhage or uveitis. Vitreous aspiration proved that the opacities were due to amyloid deposits. These observations carry considerable weight as they suggest that the disease, difficult to diagnose when unexpected, can be recognized on the basis of a simple biomicroscopic examination which might point to the diagnosis of other members of the family afflicted with primary familial amyloidosis.

In another clinical study Dr. O'Rourke demonstrated the relative reliability of transscleral counting at selected exposed sites of the globe of P^{32} emissions for the diagnosis of malignant melanoma of the choroid. The usual transconjunctival counts of the limbus may give negative results. -- To this group of investigations also belongs Dr. van Alphen's study on interrelations of optic elements in the human eye as a basis for a theory of refraction anomalies and an experimental study to determine the role the tension of the choroid may play in the growth of the globe. Measurements in the perichoroidal space indicated that the pressure there is several millimeters less than the intraocular pressure. Such data might also be of interest for understanding the development of choroid detachment. -- Finally, in the tissue culture laboratory, Dr. Wolf and Dr. Aronson cooperated in a study on staining living pigment cells of the eye with acridine orange. The results are of great interest, since they can provide a way to distinguish between living and dead cells in tissue culture. Other aspects of this problem are being discussed by Dr. Wolf in his project report.

The attached list of publications could be supplemented by seven papers which are expected to be cleared and accepted by professional journals within this year. These papers deal partly with subjects presented at the Eastern Section meeting of the Association for Research in Ophthalmology November 21-22, 1958. The list does not include certain clinical studies which are forthcoming as for instance "Retinopathy in Hypoalbuminemia" (Dr. Aronson); "Skin and Choroid Melanoma" (Dr. Paton); and "Angoid Streaks and Sickle Cell Disease" (Dr. Paton).

Participation of members of the staff in scientific meetings was gratifying. In fact contributions by the Ophthalmology Branch as a single unit ranked first in the overall activity of three meetings of the Association for Research in Ophthalmology this year. There are several reasons for the apparent upswing in the research activity. The most decisive factor seems to me is the continuity of a program in laboratories headed by eminent section or laboratory chiefs who have a permanent status. Second, the stimulation from visiting scientists (Drs. Bornschein and Dodt) who introduced areas of research, provided fertile soil for further work. A third cause of a favorable score--which is fully realized--is the capability, diligence and unselfishness of medical officers as well as the sound knowledge, and loyalty of a highly qualified technical staff

and last, not least, the continuous support in all administrative and secretarial tasks by competent, efficient and devoted office workers.

One deterrent to the efforts of the Branch research efforts is well known; that is the short term assignment of Clinical Associates. Another restraining factor is seen in the misfortune that it was impossible, for reasons beyond the Institute's control, to recruit a section chief for the tissue culture laboratory. It is hoped that further attempts will meet with success. At the end of this report I would like to express my deep gratitude for the opportunity to be associated with such a selected group of qualified, dedicated and honest workers and being advised and helped in matters pertinent to the prosperity of the Branch and the welfare of its members by the Directors of the Institute.

Ludwig von Sallmann, M. D.
Chief, Ophthalmology Branch
National Institute of Neurological
Diseases and Blindness

PUBLICATIONS
Ophthalmology Branch
1958

1. Aronson, Samuel B., II, and Shaw, Richard: Corneal crystals in multiple myeloma, A.M.A. Arch. Ophth. (in press).

2. Dodt, E.; Copenhaver, R.M., and Gunkel, R.D.: Photopischer Dominator und Farbkomponenten im Menschlichen Elektroretinogramm, Pflugers Archiv., 267:497-507, 1958.

3. Dodt, E.; Copenhaver, R.M., and Gunkel, R.D.: Electroretinographic measurements of the spectral sensitivity in albinos, negroes and whites, A.M.A. Arch. Ophth. (in press).

4. Fuortes, M.G.F.: Electric activity of cells in the eye of limulus, Am. J. Ophth., 46:210-223 (Pt. II) 1958.

5. Fuortes, M.G.F.: Generation, conduction and transmission of nerve impulses, Arch. ital. Biol., 96:285-293, 1958.

6. Fuortes, M.G.F.: Generation of nerve impulses in receptor organs. A summary of the annual Bishop Lecture. EEG Journal (in press).

7. Fuortes, M.G.F.: Initiation of impulse in visual cells of Limulus, J. of Physiol. (in press).

8. Goodman, George, and Gunkel, Ralph D.: Familial and adaptometric electroretinographic studies in retinitis pigmentosa. Am. J. Ophth., 46:142-178 (Pt. II) 1958.

9. Gouras, Peter: Electric activity of toad retina, Am. J. Ophth., 46:59-72 (Pt. II) 1958.

10. Gouras, Peter: Spreading depression of activity in amphibian retina, Am. J. Physiol., 195:28-32, 1958.

11. Kaufman, Herbert E.: Primary familial amyloidosis, A.M.A. Arch. Ophth. (in press).

12. Kaufman, H.E.; Remington, J.S.; and Jacobs, Leon: Toxoplasmosis: The nature of virulence, Am. J. Ophth., 46: 255-261 (Pt. II) 1958.

13. Kaufman, H.E.; Melton, M.M.; Remington, J.S.; and Jacobs, L.: Strain differences of toxoplasma gondii, J. of Parasitology (in press).

14. Remington, J.S.; Jacobs, L.; Melton, M.M.; and Kaufman, H.E.: Research Note: Chronic toxoplasma infection in a human uterus. J. of Parasitology (in press).

15. Remington, J.S.; Jacobs, L.; and Kaufman, H.E.: Studies on chronic toxoplasmosis: The relation of infective dose to residual infection and to the possibility of congenital transmission. Am. J. Ophth., 46:261-268 (Pt. II) 1958.

16. Kuhlman, R.E., and Resnik, R.A.: Quantitative histochemical changes in the development of the rat lens and cornea. Am. J. Ophth., 46:47-55 (Pt. II) 1958.

17. Macri, F. J.; Wanko, T.; and Grimes, P.A.: The elastic properties of the human eye, A.M.A. Arch. Ophth (in press).

18. Macri, F.J.: Outflow patterns of the cat eye, Am. J. Ophth. (in press).

19. Macri, F.J.; Wanko, T.; and Grimes, P.: The effect of extraocular muscle contraction on the elasticity of the eye. A.M.A. Arch. Ophth. (in press).

20. Macri, F.J.: Some aspects of aqueous dynamics, Glaucoma, Trans. Third Conf. Jan. 8, 9, and 10, Josiah Macy Jr. Foundation, Ed. Frank J. Newell (in press).

21. Dekaban, A.; O'Rourke, J.; and Cornman, T.: Abnormalities in offspring related to maternal rubella during pregnancy, Neurology 8:387-392, 1958.

22. von Sallmann, L.; Fuortes, M.G.F.; Macri, F.J.; and Grimes, P.: Study of afferent electric impulses induced by intraocular pressure changes. Am. J. Ophth., 45:211-220 (Pt. II) 1958.

23. von Sallmann, L.: The role of the central nervous system in the regulation of the intraocular pressure. Trans. Glaucoma Symposium, Liege, Belgium, Sept. 3-5, 1958 (in press).

24. von Sallmann, L.: Studies on morphology, physiology and pathology of the lens epithelium. Trans. XVIII International Congress of Ophthalmology, Brussels, Belgium, September 7-12, 1958 (in press).

25. von Sallmann, L.: Early lenticular lesions resulting from ionizing radiation. Trans. Am. Acad. Ophth. and Otol. (in press).

26. von Sallmann, L.: Aspects of nervous influences on the intraocular pressure, Glaucoma, Trans. Third Conf., January 8-10, Josiah Macy Jr. Foundation, Ed. Frank J. Newell (in press).

27. Wanko, Theodor, and Gavin, M.A.: The fine structure of the lens epithelium. An electron microscopic study. A.M.A. Arch. Ophth., 60:868-879, 1958.

1958 Presentations
Ophthalmology Branch

Symposium on Electrophysiology of the Visual System, Bethesda, Maryland, January 16, 17, 1958:

Gouras, Peter. Electrical Activity of Toad Retina.

Goodman, George. Familial Adaptometric and Electroretinographic Studies in Retinitis Pigmentosa.

Dodt, Eberhard. Physical Factors in the Correlation of ERG Sensitivity Curves with Visual Pigment.

Eastern Section Meeting, Association for Research in Ophthalmology, Bethesda, Maryland, January 17, 18, 1958:

Maeri, Frank J. The Distensibility of the Human Eye.

Kuhlman, Robert E. Quantitative Histochemical Changes in the Development of the Rat Lens and Cornea.

Wanko, Theodor. Electron Microscope Study on the Lens Epithelium

Third Conference on Glaucoma of the Josiah Macy, Jr. Foundation, Princeton, New Jersey, January 8, 9, 10, 1958:

Maeri, Frank J. Some Aspects of Aqueous Dynamics.

von Sallmann, L. Discussion of Afferent Discharges in Posterior Ciliary Nerves in Response to Eye Pressure Changes

XVIII International Congress of Ophthalmology, Brussels, Belgium, Sept. 8-12, 1958:

von Sallmann, L. Studies on Morphology, Physiology and Pathology of the Lens Epithelium.

Goodman, George. Electroretinography in Night-Blinding Diseases.

Glaucoma Symposium, Liege, Belgium, Sept. 3-5, 1958:

von Sallmann, L. The Role of the Central Nervous System in the Regulation of the Intraocular Pressure.

Cataract Symposium, Strasbourg, France, Sept. 15-16, 1958:

von Sallmann, L. Informal discussion on Lens Chemistry.

Washington Society for Electron Microscopy, Fourth Meeting, March 14, 1958:, Washington, D. C.:

Wanko, T. The fine Structure of Lens Epithelium.

American Academy of Ophthalmology and Otolaryngology, Chicago, Illinois October 12-16, 1958:

von Sallmann, Ludwig. Early Lenticular Lesions Resulting from Ionizing Radiation.

Eastern Association for Electroencephalographers, Ste. Adele, Canada, Feb. 27 - March 1, 1958:

Gouras, Peter. The Similarity Between Leao's Phenomenon and Spreading Retinal Depression.

24th Proceedings of the German Physiological Society, Munich, Germany, May 27-31, 1958:

Dodt, Eberhard. Photopischer Dominator und Farbkomponenten im menschlichen Elektroretinogram.

Annual Bishop Lecture, St. Louis, Washington University, April 11, 1958:

Fuortes, M. G. F. Generation of Nerve Impulses in Receptor Organs.

National Meeting, Association for Research in Ophthalmology, San Francisco, California, June 23-27, 1958:

Fuortes, M. G. F. Electrical Activity of the Cells of the Eye of the Limulus.

Macri, Frank J. The Elastic Properties of the Human Eye.

Eastern Section Meeting, Association for Research in Ophthalmology, New York, New York, Nov. 21-22, 1958:

Kaufman, H. E. Pharmacology of Daraprim.

Resnik, Robert E. Small, Cytoplasmic Elements in Lens Fibers. Integrated Biochemical and Electron Microscopic Observations.

Resnik, Robert E. Lens Proteins II. The Effect of pH on Alpha Crystallin.

Wanko, Theodor. Electron Microscope Study on Normal Lens Fibers.

Copenhaver, Richard M. Spectral Sensitivity (Distribution) of Color-Defective Individuals Determined by Electroretinography.

Goodman, George. Electroretinography in Night-Blinding Disorders.

*Only the first authors are mentioned.

National Institute of Neurological

Diseases and Blindness

Clinical Research

Ophthalmology Branch

Serial Numbers of Projects:

NINDB-37(c), NINDB-38(c), NINDB-39(c), NINDB-40(c), NINDB-41(c), NINDB-42(c), NINDB-43(c), NINDB-44(c), NINDB-45(c), NINDB-46(c), NINDB-47(c), NINDB-48(c), NINDB-49(c), NINDB-50(c), NINDB-51(c), NINDB-52(c), NINDB-53(c), NINDB-54(c), NINDB-55(c), NINDB-56(c), NINDB-57(c), NINDB-58(c), NINDB-59(c), and NINDB-60(c).

Estimated Obligations for FY 1959

Total: $882,000

Direct: $335,300

Reimbursement: $546,700

Serial No. NINDB-37 (c)
1. Ophthalmology Branch
2. Cytology and Histopathology Section
3. Bethesda, Maryland
4. Same as NINDB-58 (c)

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Studies on Diet and Drug Induced Experimental Cataract

Principal Investigator: Ludwig von Sallmann, M.D.

Other Investigators: Patricia Grimes, B.A.
Leo Caravaggio, M.S.
Eleanor M. Collins

Cooperating Units: Dr. Mary Elizabeth Reid, Laboratory of Nutrition and Endocrinology, NIAMD

Man Years (calendar year 1958):
Total: .10
Professional: .05
Other: .05

Patient Days: 67

Outpatient Visits: 44

Project Description:

Objectives: To extend the knowledge of cataractogenesis first by studying types of experimental cataract other than those examined in the past. Second, by using on the material new methods of examination as stereomicroscopy at low power, electron microscopy, microchemical procedures and other techniques. Specifically to follow the clinical and cytological changes produced by a toxic agent (mimosine) and those resulting from an amino acid deficiency diet.

It is expected that the data collected from these two forms of experimental cataract will be useful in the understanding and prognosis of the human complicated and vitamin deficiency cataract.

Methods Employed:

Mimosine Cataract: The experiments reported last year were supplemented by (1) administration of the toxic compound locally instead of by feeding; (2) studying the effects of high pyridoxine and niacin levels of the toxicity of the mimosine diet; (3) subjecting the experimental lens to electron microscopic examination (Dr. Wanko); and (4) applying Lowry's histochemical technique to small portions of the lens (Dr. Kuhlman). The young rat was the experimental animal.

Tryptophan Deficiency Cataract: Guinea pigs were used which were fed on a synthetic diet developed by Dr. M. E. Reid. The animals received either a diet containing as a basal constituent 0.1% tryptophan or diets with additional small quantities of tryptophan ranging from 0.2 to 0.1%. The eyes were studied biomicroscopically and used for histological examination for six, eight and fourteen weeks after the five day-old animals were put on the diet.

Major Findings:

Mimosine Cataract: The unique histologic picture of this cataract with the selective damage of the cells of the germinative zone in the earlier stages and the proliferation of these cells in a circumscribed area have been described last year. The combination of lens damage with conjunctival, corneal and anterior uveal changes suggests the local use of the compound in the form of frequent instillation of ½% solution into the conjunctival sac. This treatment completely fails to produce any of the surface changes or signs of lens damage as they are seen in mimosine-fed animals.

The chemical structure of mimosine shows a certain similiarity with that of the vitamin pyridoxine, and to a lesser extent, to that of niacin. To examine the possibility that in this case cataract formation is connected with an antivitamin effect of the toxic compound, high levels of vitamins are administered in an effort to protect the experimental animals against the effect of mimosine. Such treatment does in no way alter the ocular and systemic mimosine effects.

Electron microscopic examinations (Dr. Wanko) show conspicuous development of the rough surfaced endoplasmic reticulum in the equatorial cells, distention of cystic space between the membranes of the reticulum, dispersion of aggregates of ribonucleoprotein granules and accumulation of abnormal, fine granular material in the cell nucleus. The departure from the normal, then, implicatesboth nucleus and cytoplasm.

The investigation of certain enzymes (dehydrogenases) have not led to conclusive results and require further studies.

Tryptophan Deficiency Cataract: Contrary to other studied types of experimental cataract all tissue components of the equatorial zone remained unaffected by the cataractous process. The architecture of this area is well preserved even where lens cortex and lens nucleus are destroyed. To a great extent the first changes are seen in the peri-nuclear zone around the anterior pole of the lens nucleus. Progressive decomposition of fibers spreads later to the surface of the lens along the sutures. Superficial lens fibers succumb to the destruction weeks after the changes in the deep cortical layers have appeared. Whereas the epithelium does not undergo degenerative changes initially, in advanced stages it proliferates at circumscribed sites to form multilayered plaques or knobs. These changes resemble the epithelial proliferation observed in galactose and alloxan cataract.

Further observations were made in a series of guinea pigs which were fed in Dr. Reid's laboratory diets supplemented only with the D-isomer of tryptophan. Animals were compared with others, fed equivalent levels of L-tryptophan and of a D L mixture. Although the supplement levels covered a wide range, the clinical examination did not reveal clear-cut differences in the utilization of the D-isomer by itself or when fed simultaneously with the L form. The histological examination carried out on all lenses confirmed the clinical results.

Significance to Program of Institute: Information on cataractogenesis based on histological changes and the sequence of the development of these changes of variously induced lens lesions is limited. Many types of experimental cataract (other than radiation cataract) have not been subjected to a comprehensive study utilizing newer methods of examination. Cataract therapy as a medical and a surgical problem cannot be contemplated without the knowledge of the cytopathology of cataract. The two examples, mimosine and tryptophan deficiency cataract, show clearly to which extent cataract formation might vary from one to another type indicating that the prognosis (recovery or progression) depends on the involvement of the germinative epithelium. Clinical observations of experimental cataract have an obvious application for the differential diagnosis of lens opacities in the human.

Proposed Course of Project: It is planned to continue the studies of ultrastructure in incipient stages of experimental cataract and make concentrated efforts to investigate pertinent

aspects of the cytologic problem by tissue and organ culture techniques. Human lens material will be studied histologically and cytologically when it becomes available in quantity.

Part B included Yes [XX] No [ ]

Serial No. NINDB-37 (c)

PHS - NIH
Individual Project Report
Calendar Year 1958

Part B. Honors, Awards and Publications

Publications other than abstracts from this project:

von Sallmann, Ludwig: Studies on morphology, physiology and pathology of the lens epithelium, Trans. XVIII Internat. Cong. Ophth., Brussels, Belgium, 1958 (in press)

Honors and Awards relating to this project: None

Serial No. NIHDB-38 (c)
1. Ophthalmology Branch
2. Cytology and Histopathology Section
3. Bethesda, Maryland
4. Same as NINDB-74 (c)

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Study of Submicroscopic Structures of Lens Tissue Components by Phase Contrast Microscopy

Principal Investigators: Ludwig von Sallmann, M.D.
Leo Caravaggio, M.S.
Samuel Aronson, M.D.

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1958): Patient Days: None
Total: .10
Professional: .05
Other: .05

Project Description:

Objectives:

1) To establish the growth pattern of normal lens epithelium of various species in tissue and organ culture after a technique has been developed which is better adapted to the requirements of this tissue than that previously used.

2) To study in continuing cultures the effect of medium composition, cataractogenic substances and nutritional deficiencies.

3) To investigate the nature and prevention of injurious effects of this tissue.

4) To expand the investigations to other tissues of the eye.

Methods Employed: A Paul chamber adapted to phase contrast microscopy and rat tail collagen, an optically more homogeneous substrate than plasma clot, are used in morphological studies on outgrowth from lens epithelium explants of embryonic chick, newborn chick and rabbit and adult rabbit. The major portion of this work has been directed towards the study of variation in cell form occurring in vitro. The medium used has been a combination of balanced salt solution (Hanks 85%), horse serum (10%) and chick embryo extract (5%). A study of the effect of various media combinations on the growth of lens epithelium in vitro has recently been initiated. It is hoped that a medium will be formulated in which lens epithelium will grow in a manner more predictable and analogous to that in vivo. Preliminary attempts have been made to cultivate lens epithelium without the benefit of a collagen substrate, i.e. directly on glass surfaces. The success of this technique would permit the growing of a continuous culture and the use of replicate culture techniques for quantitative determination of the effects of cataractogens and nutritional deficiencies.

Major Findings: Thus far, the cultivation of rat, chick and rabbit lens epithelium in the Paul perfusion chamber has proven successful in regard to the initiation and maintenance of outgrowth. In adult rabbit outgrowth from the explant usually begins in about seven days. Cultures in the Paul chamber have been successfully maintained for periods up to one month, while cultures on cover-slips in roller tubes have been maintained for up to two months in a chemically defined medium (Eagle's) containing as little as 5% horse serum.

Individual cells in cultures under phase contrast microscope examination have shown a great amount of morphological variation, ranging from totally round forms to extremely elongate and multi-processed cells. Although the study on the growth effect of various media is in a preliminary stage, there is some evidence to indicate that the elongate forms are more constantly produced in cultures containing chick embryo extract, while cells grown without this media component grow in sheets more analogous to the in vivo condition.

Significance to Program of Institute: It has been established that certain types of cataractous lenses are due to the formation of abnormal lens fibers by physically damaged or metabolically altered cells from the germinative zone of the lens epithelium. This information has been obtained from the study of fixed and stained tissue from in vivo experimentation.

While this type of investigation has significantly increased our understanding of the cataractogenesis, it has certain inherent limitations.

The use of phase microscopy and tissue culture techniques enable the direct observation of the effects of cataractogenic agents on the living cells. Artifacts of the histological method are eliminated and the cell responses that escape histological detection are recorded by time-lapse cinematography for further study.

Proposed Course of Project: Once a basically sound growth pattern has been determined, it will be possible to subject cultures to perfusion with cataractogenic agents (e.g. dinitrophenol, mimosine, alloxan, galactose) and x-irradiation for study by time-lapse cinematography and histochemical methods.

In a continuing study of media effects on growth, an attempt will be made to adapt lens epithelium to grow in a chemically defined medium. Success in this technique would enable nutritional studies to be carried out for lens epithelium as have been done for HeLa and L-strain fibroblasts. The study of tryptophan deficient cataract would also fall in this category.

Part B included Yes ☐ No ☒

Serial No. NINDB-39 (c)
1. Ophthalmology Branch
2. Physiology Section
3. Bethesda, Maryland
4. Same as NINDB-57 (c)

PHS - NIH
Individual Project Report
Calendar Year 1958

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 1958): Patient Days: None
Total: .10
Professional: .05
Other: .05

Project Description:

Objectives: The in vivo studies reported last year dealing with the search for IOP (intraocular pressure) receptors and for afferent pathways signalling changes in IOP show that in positive experiments discharges could be obtained from either one, a few, or all isolated and tested posterior ciliary nerves. Although damage of individual nerves during preparation can explain the irregularity of these results, it is felt that the employed surgical procedure does not permit differentiation of somatic, parasympathetic or sympathetic nerves and that for interpretation of results, an anatomical study is necessary. The scope of this work then is:

1) to define a technique which allows a selective and stable silver impregnation of the nerves in the orbit for satisfactory dissection. The extremely small dimension of the anastomotic branches obviate their demonstrability in unstained preparations.

2) to study the extent of fusion between fifth nerve and post-ganglionic third nerve fibers and to demonstrate histologically, the fiber distribution in the so-called short and long ciliary nerves.

3) to compare the anatomical and histological characteristics of posterior ciliary nerves in various species.

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 before fixation in formalin avoiding damage to the underlying nerves. For staining, the fixed material is 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: With the original Christensen technique preparations are obtained which lose differentiation after a short period of time, since exposure to light causes all tissues to gradually turn brown. The selective staining of nerves according to the new technique is stable to light and the material may be worked with for many hours before loss of differentiation begins. Such preparations are well suited for photography, as the pictures show clearly the whole course and ramifications of the ciliary nerves, and are excellent for demonstration.

The patterns of nerve distribution differ in cats and monkeys. Preparations from these two species have in common the fact that the nerves close to the globe are generally mixed nerves, that is, they contain fifth and third nerve fibers. In the cat, however, fine branches can be demonstrated which travel along a tortuous course to the posterior pole of the eye as distinct bundles without fusion with any of the post-ganglionic branches of the ciliary ganglion. Such isolated "long" ciliary nerves are not observed in the monkey orbit. The ciliary ganglion in this species receives three or four very fine branches of the fifth nerve. Whether they undergo synaptic connections is not known. Such branches joining the ganglion are absent in the cat. Fusion of fifth nerve fibers and "short" ciliary nerves occurs at various sites and at various distances from the ciliary ganglion in both species. The number of the anastomoses also differs greatly from animal to animal and from eye to eye. Nerves arising in the ciliary ganglion which travel directly to the eye without joining with branches of the fifth nerve have not been observed in either species, but the segregation of fibers within mixed nerves and subsequent branching might give rise to a few nerves which enter the scleral coat as purely parasympathetic nerves. Accompanying the long ciliary arteries in the horizontal meridian of the globe course nerves which supposedly contain only fifth nerve and sympathetic fibers. It is shown that these mixed nerves also carry post-ganglionic parasympathetic fibers.

Significance to Program of Institute: The renewed interest in nervous influences on the intraocular pressure and a possible central nervous mechanism playing a part in the regulation of this pressure has resulted in studies reported from this laboratory in the past years and repeated in laboratories abroad. Demonstration of discharges led off from ciliary nerves in response to small IOP changes bring into focus the importance of identifying the nervous pathways which conduct such signals. The present study provides information on the nature and distribution of these nerves in two species.

Proposed Course of Project: It is planned to extend the anatomical and histological studies to human autopsy material.

Part B included Yes [XX] No [ ]

Serial No. NIMDB-39 (c)

PHS - NIH
Individual Project Report
Calendar Year 1958

Part B. Honors, Awards and Publications

Publications other than abstracts from this project:

von Sallmann, Ludwig: The role of the central nervous system in the regulation of the intraocular pressure, Trans. Glaucoma Symposium, Liege, Belgium, Sept. 3-5, 1958 (in press).

Honors and Awards relating to this project: None

Serial No. NINDB-40
1. Ophthalmology
2. Physiology Section
3. Bethesda, Maryland
4. Same as NINDB-57 (c

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Study of Afferent Electric Impulses Induced by Intraocular Pressure Changes.

Principal Investigator: P. P. Lele, M.D.

Other Investigators: Patricia Grimes, B.A.

Cooperating Units: None

Man Years (calendar year 1958): Patient Days: None
Total: .10
Professional: .05
Other: .05

Project Description:

Objectives: The role of nervous mechanisms in the regulation of intraocular pressure is, as yet, not clearly understood. The existence of such a mechanism can be postulated only if both the efferent and the afferent pathways can be determined. Electrical stimulation of certain regions in the diencephalon of the cat has been reported to cause occasional alterations in the intraocular pressure without concomitant changes in systemic blood pressure and pupillary reactions. On this evidence an efferent pathway originating from or passing through the diencephalon has been suggested. The presence of an afferent pathway signalling the level of, or the changes in, the intraocular pressure (IOP) would strengthen the concept of a nervous mechanism for the regulation of IOP. von Sallmann et al. obtained some informative data in their study of afferent impulses induced by IOP changes, but a large proportion of their experiments gave negative results.

In this present investigation, this problem is being re-examined using different techniques, originally developed

for a study of the sensory receptors in the cornea of the cat. In view of the complexity and the variability of the anatomical arrangement of the nerves supplying the globe of the cat and the necessity of adequate exposure of the nerves in an untraumatized condition, the eyeball together with the nerves and blood vessels is resected from the animal and used as an isolated organ preparation.

Methods Employed: The animal, under light pentobarbital anesthesia, is sacrificed by air-embolism. Craniotomy is performed and the roof of the orbit resected. The contents of the orbit, with retrobulbar tissue and the trigeminal ganglion, is carefully and quickly dissected from the animal.

The isolated eye is kept immersed in warm, oxygenated Krebs-Ringer-Glucose (K.R.G.) solution. The extraocular muscles and connective tissue are excised, carefully avoiding trauma to any of the nerves. The dissection of the ciliary nerves from the optic nerve and connective tissue sheaths is facilitated by injection of a solution of Hyaluronidase in K.R.G. solution.

Two 22 S.W.G. hypodermic needles, connected by short lengths of saline columns to a pressure transducer and a micro injection apparatus respectively are inserted at the 3 and 9 o'clock positions at the limbus into the anterior chamber. The eyeball and the needles are securely mounted on a transparent plate of acrylic resin.

The nervous activity is examined by successively placing each of the dissected nerves onto a pair of platinum electrodes which feed through an R.C. coupled preamplifier into one channel of dual-beam cathode-ray-oscillograph (C.R.O.). The pressure transducer, connected to the needle in the anterior chamber, is fed through a carrier amplifier to a pen recorder and the second channel of the C.R.O. Photographic records of the oscilloscope traces are made at different film speeds.

Major Findings: Branches of the ophthalmic division of the fifth nerve going to the globe (long ciliary nerves) did not show any spontaneous electrical activity, but in every instance, afferent impulses were evoked when the cornea and/or the bulbar conjunctiva were mechanically stimulated. Neither spontaneous nor evoked activity was recorded from short ciliary nerve originating from the ciliary ganglion and entering the globe. Responses obtained from ciliary nerves of mixed origin were essentially similar to those from the long ciliary nerves. The preparations remain active for at least 12 hours if the K.R.G. solution is kept oxygenated and maintained at pH 7.4 and between 30° and 37°C.

All long and mixed ciliary nerves tested showed a response to increase of IOP. In each instance there is a sharp but transient increase in the frequency of impulses, lasting as long as the IOP rises, the maximum frequency being proportional to the rate and the height of the rise of IOP. In approximately 60% of the nerves examined, the activity was sustained while the IOP was maintained at a steady elevated level. The frequency of sustained activity was, in general, dependent upon the increase in IOP. Every eye tested has shown this type of sustained response in one or more of the nerves. This impulse discharge is quite separate and distinct from responses to acute mechanical deformation (touch) which can be seen as momentary discharge superimposed on the sustained discharge. Similar observations were made in an eyeball excised from a monkey.

Significance to Program of Institute: The results of the present experiments have important implications in the study of intraocular pressure regulation and may be useful in interpreting the mechanism of some types of increased IOP in man (glaucoma). The isolated eye preparation with functionally viable neuro-muscular apparatus would be ideally suited for studies on the ciliary body (effects of drugs, nerve stimulation, etc), elasticity of the eyeball and evaluation of local anesthetics.

Proposed Course of Project: A systematic quantitative investigation of the effect of IOP rises on the afferent nerve activity by isolating single active units is being undertaken. A comparative study of the effects of raising the IOP in different ways (e.g. external pressure, injection of fluids into the ophthalmic artery and injections into the vitreous) on the afferent impulse discharges, is contemplated. Efforts will be made to localize the site of origin of the sustained afferent activity. Attempts will be made to confirm the essential findings in experiments with the eyeball in situ.

Part B included: Yes [XX] No [ ]

PHS - NIH
Individual Project Report
Calendar Year 1958

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

von Sallmann, Ludwig; Fuortes, Michelangelo G.F.; Macri, Frank J.; and Grimes, Patricia: Study of afferent electric impulses induced by intraocular pressure changes. Am. J. Ophth., 45: 211-220 (Pt. II) 1958.

Honors and Awards relating to this project: None

S[illegible] NINDB-40
1. Ophthalmology Branch
2. Pharmacology Section
3. Bethesda, Maryland
4. Same as NINDB-60 (c)

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Effects of Muscle Relaxants on IOP* and Extraocular Striate Muscles

Principal Investigator: Frank J. Macri, Ph.D.

Other Investigator: Patricia Grimes, B.A.

Cooperating Units: None

Man Years (calendar year 1958): Patient Days: None
Total: 1.0
Professional: .5
Other: .5

Project Description:

Objectives: To determine the mechanism by which decamethonium and succinylcholine affect the IOP.

A probable mechanism by which the elasticity of the cat eye was altered by either decamethonium or succinylcholine (as reported last year) appeared to be one mediated by the spasm of extraocular muscles induced by these agents. The problem was studied by inducing extraocular muscle spasms by other means:

1) stimulation of the third nerve intracranially and

2) rigor mortis.

* Intraocular Pressure

The [illegible] determined under [illegible] conditions, [illegible] and compared to those obtained on living [illegible]. The eyes were then enucleated and similar measurements [illegible]. In order to determine whether a diffuse outside pressure could simulate the effects produced by contraction of extraocular muscles, a rubber chamber was constructed such that constant outside pressures could be exerted on the enucleated eye. Elasticity measurements were made under these conditions and compared with those previously obtained.

Methods Employed: Cats anesthetized with pentobarbital Na were used in this study. When the effect of rigor mortis on the elasticity of the eye was studied, the animals were killed by means of an overdose of the anesthetic agent.

The elasticity of the eye was determined by multiple infusions of fixed volumes of saline into the anterior chamber and by recording the intraocular pressure levels before and after each infusion. The data were then plotted as volume versus IOP. These elasticity curves formed the basis for comparison of the elastibity of the eye under the various experimental conditions.

Major Findings: Spasm of the extraocular striate muscles induced by third nerve stimulation or rigor mortis produced changes in the elasticity of the eye similar to those obtained after the administration of decamethonium or succinylcholine. This effect could be abolished by resection of the extraocular striate muscles or enucleation. Since the three different methods of inducing muscle spasm produced similar changes in the elasticity of the eye, it appeared unlikely that factors other than the muscles would be involved. The normal elastic values obtained after resection of the spastic muscles or after enucleation supported this concept. The primary influence on the eye brought about by muscular contraction was one of a diffusely exerted outside pressure. This was demonstrated by experiments in which the eye was enclosed in a chamber filled with saline. The pressure of the chamber was raised to various levels and the elasticity determined. The effects on elasticity of the eye, under these conditions, were practically identical with those obtained by muscle spasm.

Significance to Program of Institute:

1) To serve as a guide for cautious clinical use of these muscle relaxants in intraocular surgery.

2) To increase our knowledge of the pharmacology of these agents.

3) To add to the understanding of the physiology of the extraocular striate muscles and their effects on the intraocular pressure.

Proposed Course of Project: To determine if these agents affect the vasculature of the eye by the compressive action of the muscles.

Part B included: Yes [XX] No [ ]

Serial No. NINDB-41 (c)

PHS - NIH
Individual Project Report
Calendar Year 1958

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Macri, F.J.; Wanko, T., and Grimes, P.: Effects of extraocular muscle contraction on the elasticity of the eye. A.M.A. Arch. Ophth. - in press.

Honors and awards relating to this project: None

1. Ophthalmology Branch
2. Pharmacology Section
3. Bethesda, Maryland
4. Same as NINDB-59 (c)

PHS - NIH
Individual Project Report
Calendar Year 1958

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 1958):
Total: 1.0
Professional: .5
Other: .5

Patient Days: 334

Outpatient Visits: 59

Project Description:

Objectives: To test various drugs including the new atraxics as well as older compounds for their ability to influence intraocular pressure. This approach may contribute to the medical treatment of patients with glaucoma. The results of this investigation may also shed some light on the mechanisms by which the intraocular pressure is regulated.

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.

Patient Material: Patients with wide and narrow angle glaucoma, particularly borderline cases, are admitted in greater numbers than in previous years. Laboratory observations made on aqueous outflow mechanisms, scleral rigidity and the action of locally or systemically introduced drugs are utililized in the clinical studies.

Major Findings: It was reported last year that a method for the determination of aqueous outflow devised in this laboratory produced results compatible with those reported in the literature, i.e., outflow was proportional to the IOP. The flow rate in the cat was approximately 0.30 cmm/min/mm.Hg. Since that time, however, a much larger series of determinations has been carried out. It now appears that there is a second, biphasic outflow pattern which is not proportional to the IOP throughout the pressure range examined (usually from 20 mm. to 80 mm. Hg.). The biphasic curve was characterized by a very fast outflow at the lower IOP levels (approximately 1.5 cmm/min/mm.Hg) which then inflected at pressure levels between 35 and 50 mm. Hg. to a rate of approximately 0.3 cmm/min/mm. Hg. Such an outflow pattern could be induced by the parenteral administration of Diamox. Here the outflow was changed from a monophasic to a biphasic one.

It has been reported that the pressure in the veins to which the aqueous humor flows is essentially constant and independent of IOP changes. This hypothesis has been accepted for the situation in humans and has been applied by many workers to that in the cat, rabbit and monkey. The outflow pressure is the difference of pressure values between the two ends of the outflow channels, i.e., intraocular (trabecular) and venous (episcleral). It became quite important, therefore, to study the venous pressures on the surface of the eye.

Three veins (anterior ciliary, long posterior ciliary and vortex) in the cat can be cannulated for pressure determination. Plastic casts were made to illustrate the complicated anatomic relationship of the various venous vascular beds. The cast material was injected into the anterior chamber under continuous pressure until many of the episcleral vessels were grossly seen to be filled. The material was allowed to harden, then the tissue was digested away. The aqueous outflow channels were thus demonstrated in their course from the trabecular area to the Circle of Hovius. Pressure readings in the anterior ciliary and vortex veins, and those of the intraocular pressure,

appeared almost identical under resting conditions. However, if the IOP was either raised or lowered, the venous pressure fell. Thus, it is shown that induced changes of the IOP can alter the venous pressure. A summary of the conditions affected by changes in the venous pressure follows:

1) The biphasic outflow patterns became much more pronounced when outflow pressure was calculated as IOP-venous pressure.

2) Arterenol, acetylcholine, histamine, hexamethonium Br, sympathetic and parasympathetic nerve stimulation all produced changes in the venous pressure which paralleled the changes of the IOP.

3) Trauma of the eye induced identical elevations of the IOP and venous pressure.

4) Diamox lowered both the IOP and venous pressure.

The effect of Diamox on the vortex venous pressure was also determined in the monkey. In this species there are no anastomotic connections between the vortex and episcleral veins (into which the aqeuous veins feed). The pressure in the vortex vein when measured under resting conditions was found to be 10 to 20 mm. Hg. higher than that of the IOP. Diamox was capable of lowering this pressure as well as decreasing the IOP.

Significance to Program of Institute:

1. (a) The calculations of "facility of outflow" in man are based on the assumption that outflow of aqueous is proportional to the intraocular pressure and also that the episcleral venous pressure is 11.0 mm. Hg. in both normal and glaucomatous eyes. The effective outflow pressure then equals the IOP minus 11.0 mm. Hg. In the experiments on anesthetized cats neither of these two conditions appear fully met.

(b) Glaucoma is thought to be caused in most instances by a diminished outflow and a relatively constant inflow - the net effect being an increase of IOP. In cat experiments the intraocular pressure and venous pressure were nearly equal to each other, a result which casts some doubt on the correctness of this hypothesis. However, the difficulty to interpret the experimental results and to apply them to the human eye is fully realized.

2. Diamox has been shown to lower the IOP in glaucoma by the reduction of aqueous inflow. That this drug is also capable of reducing the venous pressure in the eyes of cats and monkeys indicates that another mechanism may play a part in this lowering of IOP. These observations point once more to the necessity of studying carefully the role of the vascular bed in the maintenance of the IOP.

Proposed Course of Project: It is planned to continue studies on the IOP with other pharmacologic agents. A more intensive attempt will be made to investigate the mechanism of Diamox action on the eye.

Part B included Yes [XX] No [ ]

Serial No. NINDB-42 (c)

PHS - NIH
Individual Project Report
Calendar Year 1958

Part B. Honors, Awards and Publications

Publications other than abstracts from this project:

Macri, Frank J.: Outflow patterns of the cat eye, Am. J. Ophth. (in press)

Macri, Frank J., Wanko, Theodor, and Grimes, Patricia A.: The elastic properties of the human eye, A.M.A. Arch. Ophth. (in press)

Macri, Frank J.: Some aspects of aqueous dynamics, Glaucoma, Trans. Third Conf., Jan. 8, 9 and 10, 1958, Josiah Macy, Jr. Foundation. Ed. Frank J. Newell. (in press)

Honors and awards relating to this project: None

Serial No. NIND[illegible]
1. Ophthalmology Branch
2.
3. Bethesda, Maryland
4. New Project

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Clinical Glaucoma Study

Principal Investigators: David Paton, M.D. and
Ludwig von Sallmann, M.D.

Other Investigators: Herbert Kaufman, M.D.,
Richard Copenhaver, M.D. and
Bruce Cohan, M.D.

Man Years (calendar year 1958):
Total: .10
Professional: .05
Other: .05

Patient Days: 334

Outpatient Visits: 59

Project Description:

Objectives:

1) The chief objective of this study is to evaluate suspected cases of early glaucoma to determine through multiple clinical observations and tests those which are most helpful in the diagnosis, prognosis, and adequacy of glaucoma therapy. Because of the intraocular tension, visual field limits and individual response to test situations, a diagnosis of glaucoma is often withheld or quite uncertain with present day techniques. Although one single test will undoubtedly not be found upon which a diagnosis can be based, it is the object of the present study to determine which procedures are most useful, what is their reproducibility, and what retrospective information can be gained in regard to prognosis by carefully following these patients. In particular, the study is concerned with the information gained from tonography in borderline cases of glaucoma.

2) The study is also concerned with physiologically distinct forms of glaucoma such as "low tension", hypersecretion, pigmentary narrow angle, or inflammatory forms of glaucoma. These cases are studied as extensively and in the same manner as the borderline cases which most often consist of chronic simple glaucoma. Here again, the study is primarily concerned with the diagnosis of the disease and the means of differentiating one form from the other.

3) Because of its concern with the value of tonography, the project also includes the performance of whatever necessary surgical procedures are indicated to reduce uncontrolled intraocular pressure. The adequacy of surgical therapy as well as medical means to reduce the pressure is judged in regard to day curve tonography and prevention of further visual field loss.

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 under rather constant environmental conditions and at all times of the day or night. In addition to history and general physical examination, specific tests include tonometry with day curve determinations, applanation tonometry, measurement of depth of anterior chamber, biomicroscopy and gonioscopy, tonography, at frequent intervals, and provocative tests also incorporating tonography. In addition, visual field studies are carefully performed, using the Goldmann projection perimeter and the Gunkel tangent screen. Wherever possible, photographs of the optic discs are obtained. When the patient's untreated intraocular pressure has been observed over a time interval sufficient to obtain either a positive diagnosis or a sizable amount of data, the response to glaucoma therapy is subsequently evaluated and the patients are not discharged until the intraocular tension is satisfactorily controlled on a twenty-four hour basis. Subsequent to discharge, attempts are made to follow each patient at four to six month intervals, at which time tonometry, tonography, and visual fields are again included.

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: At the present time, efforts are being made to instruct a technician in the expert use of the electronic tonometer. If this objective is realized, it is hoped that the project size can be increased, that more extensive tonography can be undertaken and that the increasing size of the followup patient population can be adequately handled by outpatient visits. Furthermore, the need for identical observations on normal control subjects of the glaucoma age group is apparent.

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-44(c)
1. Ophthalmology Branch
2. Microbiology Section
3. Bethesda, Maryland
4. New Project

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: The Ocular Diagnosis and Treatment of Ocular Complications of Primary Familial Amyloidosis.

Principal Investigators: Herbert E. Kaufman, M.D.
Gerard van Alphen, M.D.

Other Investigator: None

Cooperating Units: None

Man Years (calendar year 1958):
Total: .10
Professional: .05
Other: .05

Patient Days: 230

Outpatient Visits: 4

Project Description:

Objectives: (1) To determine whether vitreous opacities reported in occasional cases of primary familial amyloidosis are, in fact, diagnostic of the disease. (2) To elucidate their histochemical characteristics. (3) To evaluate the possibility of vitreous replacement in the therapy of blindness caused by these opacities.

Methods and Materials: The appearance of the vitreous opacities is carefully studied by biomicroscopy, and the presence or absence of systemic deposits of amyloid is determined by skin, muscle and gingival biopsies. The histochemical appearance of amyloid is determined by various histochemical stains. The electron-microscopic appearance has been studied by Dr. Theodor Wanko.

An instrument has been developed which will remove diseased vitreous and replace it, after lavage, by fresh donor

vitreous without appreciably changing the pressure within the eye. Vitreous replacement and lavage has been attempted on one patient with this instrument.

Major Findings: (1) In addition to one patient with primary familial amyloidosis, who was admitted for study of loss of vision and was subsequently diagnosed when systemic symptoms appeared, two other patients with primary familial amyloidosis without systemic complaints have been diagnosed because of the characteristic appearance of their vitreous opacities. In one of these patients, autopsy specimens from a brother on whom the postmortem diagnosis was diffuse arteriosclerosis revealed that, in fact, the cause of the brother's neuropathy, gastro-intestinal disturbance and heart failure was due to amyloidosis. The other patient, who was confirmed as having unsuspected amyloidosis, is from a family in which one brother has neuropathy with muscle wasting, and a third brother has bouts of cardiac syncope. A single skin biopsy of the brother with cardiac syncope was negative.

(2) Vitreous aspiration was carried out on six eyes of four patients with the disease. In four of these, opacities were obtained at the time of aspiration. These opacities were found to have the staining properties of amyloid, whereas control vitreous and opacities in bank vitreous were not found to have these properties.

(3) On one patient, vitreous lavage was attempted with the newly developed instrument. In this patient there was a transient improvement of vision, but this was neither dramatic nor sustained, and was accompanied by transient inflammation.

Patient Material: Patients with vitreous opacities are obtained by physician referral. (Usually these patients have been diagnosed as having uveitis.)

Significance to Program of Institute: Primary familial amyloidosis is often readily diagnosed once it is suspected. The demonstration that the vitreous opacities present in some of these patients are actually comprised of amyloid points up the diagnostic importance of their characteristic appearance. The demonstration of amyloidosis in one family, where it had neither been diagnosed clinically nor on postmortem examination, suggests that the disease may be more widespread than was previously thought. The discovery of two patients with unsuspected amyloidosis confirms that the ophthalmologist is in a position to suggest the correct diagnosis in a syndrome otherwise difficult to identify and may, thereby, permit the correct diagnosis not only in the patient under observation but in other members of the family who may be

ill, but may not have the vitreous opacities. The usefulness of the instrument for vitreous lavage in cases with blinding vitreous opacities must still be evaluated. Certainly it is potentially useful in conditions other than amyloidosis.

Part B included Yes [X] No [ ]

Serial No. NINDB-44(c)

PHS - NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards and Publications

Publications other than abstracts from this project:

Kaufman, H.E.: Primary Familial Amyloidosis, A.M.A. Arch. Ophth. (in press)

Honors and Awards relating to this project: None

Serial No. NINDB-45(c)
1. Ophthalmology Branch
2. Microbiology Section
3. Bethesda, Maryland
4. Same as NINDB-68 (c)

PHS - NIH
Individual Project Reports
Calendar Year 1958

Part A.

Project Title: Study of Toxoplasmosis and Its Therapy

Principal Investigator: Herbert E. Kaufman, M.D.

Other Investigator: Lee A. Caldwell, B.S. (Investigator for Part III)

Cooperating Units: Studies described in Part I and II were done in conjunction with Dr. Leon Jacobs' project on Studies on Toxoplasmosis in the National Institute of Allergy and Infectious Diseases.

Man Years (calendar year 1958):
Total: .10
Professional: .05
Other: .05

Patient Days: 2,201

Outpatient visits: 202

Project Description:

I. LABORATORY STUDIES

Objectives: (1) To investigate differences between virulent and avirulent strains of toxoplasma gondii and the effect of some parameters of virulence on therapy. (2) To study the efficacy of present therapy with Daraprim and sulfa in vivo and in vitro. (3) To explore the metabolism of Daraprim in man and the details of its toxicity.

Methods Employed: Various strains of toxoplasma gondii were maintained in chick embryos and harvested from ground chick embryo chorioallantoic membrane. The organisms were counted in a Neubauer-Levy Counting Chamber and inoculated into standing roller tube tissue cultures of monkey kidney epithelium and human amnion cell monolayers maintained in appropriate media

The time of attachment of the organisms could be controlled by washing out the inoculum at desired intervals. The organisms were permitted to multiply, but cultures could be fixed and stained before cell lysis had occurred. In stained preparations microscopic examination enabled the number of cells invaded and the number of organisms per parasitized cell to be counted. Other cultures were permitted to go on to lysis and the time required for lysis could be correlated with in vivo virulence. The effect of Daraprim on toxoplasmas in vitro was investigated by exposing infected cultures to known concentrations of drug, and then grinding up cultures and inoculating them into mice to determine whether viable organisms could be recovered.

Major Findings: Strains of toxoplasmas that were most virulent for animals also destroyed tissue cultures most rapidly. In the strains studied the virulent RH strain invaded 4-5 times as many cells and multiplied 2-3 times as fast as the less virulent 113 CE strain. The S-5 strain of intermediate virulence was also intermediate in invasiveness and rate of multiplication. It was expected that Daraprim, which is a metabolic antagonist, would be considerably more effective against more rapidly growing organisms. When cultures were exposed to varying concentrations of Daraprim for five days, the slow-growing organisms were shown to be much more resistant to the Daraprim than the rapidly growing organisms. Further studies of a similar nature reveal that appreciable time is required for the organism to be in contact with the cell before invasion takes place, and that chronic infection of tissue cultures can be produced with slow-growing strains. In this chronic state, the organism and the culture seem to be in balance, and damage to the culture is not apparent. When organisms are incubated with serum containing a high titer of dye test antibodies, these organisms are killed by the serum, suggesting that the dye test antibody may, in fact, be toxoplasmocidal.

Patient Material: Cases of granulomatous uveitis are obtained by physician referral. Active cases that suggest a possible toxoplasma etiology by history, morphology, skin test or dye test can be selected.

Significance to Program of Institute: In the past, some cases of ocular toxoplasmosis have been reported that do not appear to respond to therapy with Daraprim and sulfa. The differences between strains elucidated can explain possible differences in the clinical course of toxoplasmosis, as well as the apparent resistance of some infections to therapy.

II. EFFICACY OF THERAPY

Objectives: To determine whether Daraprim, as used clinically, can penetrate into the eye, and, in addition, to

determine whether it is effective against the proliferating form of toxoplasma in the eye.

Methods Employed: To determine whether Daraprim penetrates into the retina, monkeys were given Daraprim, and after a suitable period of time the serum and the retina was analyzed for Daraprim. In addition, guinea pigs were inoculated with proliferating toxoplasmas into the vitreous and were then fed with Daraprim and sulfa. Controls that were inoculated with the organism, but not given drugs, were also maintained. After appropriate periods of time, the eyes of all animals were examined and the brains and inoculated eyes were ground up. The suspension was inoculated intraperitoneally into mice to determine whether live organisms persisted. Dye tests were done on control and treated guinea pigs.

Major Findings: The concentration of Daraprim in the retina of monkeys was comparable to that found in the serum. In guinea pigs treated with Daraprim and sulfa, in almost all cases there were no organisms recovered from the brain and the dye tests were negative. In untreated guinea pigs, organisms were recovered from all brains and all eyes. Chorioretinitis was seen to develop in controls only, and dye tests were positive.

Significance to Program of Institute: Despite the fact that Daraprim has been used in the treatment of ocular toxoplasmosis for many years, many dispute its efficacy. It is surprising that no studies have yet been done to determine whether Daraprim actually penetrates the retina, and no other studies have been done confirming its efficacy on intraocular infection.

III. PHARMACOLOGICAL PROPERTIES AND TOXICITY OF DARAPRIM

Objectives: (1) To investigate the pharmacology of Daraprim,(2) to study the cause of Daraprim toxicity, its manifestations and the best method to safeguard patients from it.

Methods and Patient Material: Serial serum Daraprim determinations were done on patients being treated with Daraprim and sulfa on various regimens. Serial hemotology observations were obtained and were correlated with earlier hemotological studies in the records of patients previously seen in the Institute where the studies were done.

Major Findings: Patients had been treated with 25 mg. of Daraprim orally per day, but on this regimen it was found that when the Daraprim was stopped the compound could be detected in the blood for up to two weeks. This slow decay

and slow rise in drug levels suggested that a loading dose would be desirable followed by a maintenance dose. A regimen was therefore developed which gave rapid stable levels approximately two weeks earlier than the previously used regimen. Wide differences were found in the final concentration of Daraprim from patient to patient. When the drug was stopped, the fall in drug concentration from a given concentration in all patients was similar. Therefore, since elimination was the same, the difference in drug levels must be due to differing absorption of the drug. In those patients who developed hemotologic toxicity, the Daraprim level was significantly higher than in the non-toxic patient.

Significance to Program of Institute: Patients have been treated for many years with Daraprim and sulfa drugs and yet the pharmacology of Daraprim in humans maintained on the drug has never before been studied. It is obvious from these studies that therapeutic regimens different from those previously used might be desirable, and furthermore it appears that the differences in drug levels obtained are due to differences in absorption of the drug from the gastro-intestinal tract. Since toxicity appears to be a function of the absorption of the drug, it may be possible to obtain stable high uniform blood levels with minimal toxicity and minimal variation in absorption by using different salts of Daraprim, such as succinate or glutamate. Early studies by Dr. Leo Gaudette in the National Institute of Allergy and Infectious Diseases suggest that this is, in fact, true. In addition, because of the toxicity of Daraprim it is important to determine whether one parameter of hemotology can be followed or whether it is essential to do complete hemotological workup in all cases of patients on Daraprim. There is no data on the precise nature of the hemotological toxicity in man, and there is no indication in the literature how long the toxicity can remain and how serious it may become.

Proposed Course of Project: Continued studies on the hemotologic toxicity of Daraprim are in progress. In addition, it is hoped that an evaluation of the efficacy of Daraprim in patients treated for chronic ocular toxoplasmosis will be possible.

Part B included Yes [X] No [ ]

Serial No. NINDB-45(c)

PHS - NIH
Individual Project Report
Calendar Year 1958

Part B. Honors, Awards and Publications

Publications other than abstracts from this project:

Kaufman, H.E., Remington, J.S., and Jacobs, L.: Toxoplasmosis: The Nature of Virulence, Am. J. Ophth., 46:255-261 (Pt. II), 1958.

Kaufman, H.E., Melton, M.M., Remington, J.S., and Jacobs, L.: Strain Differences of Toxoplasma Gondii, J. Parasitol. (in press)

Remington, J.S., Jacobs, L., Melton, M.M., and Kaufman, H.E.: Studies on Chronic Toxoplasmosis: The Relation of Infective Dose to Residual Infection and to the Possibility of Congenital Transmission, Am. J. Ophth., 46:261-268 (Pt. II), 1958.

Remington, J.S., Jacobs, L., Melton, M.M., and Kaufman, H.E.: Research Note: Chronic Toxoplasma Infection in a Human Uterus, J. Parasitol. (in press)

Honors and Awards relating to this project: None

Serial No. NINDB-46 (c)
1. Ophthalmology Branch
2.
3. Bethesda, Maryland
4. Same as NINDB-66 (c)

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Thyroid Hormone Turnover in Uveitis

Principal Investigator: James F. O'Rourke, M.D.

Other Investigators: Richard Copenhaver, M.D.
Herbert Kaufman, M.D.
David Paton, M.D.
Bruce Cohan, M.D.

Cooperating Units: Advice and assistance given by Dr. E. Rall of the Clinical Endocrinology Branch, NIAMD.

Man Years (calendar year 1958):
Total: 1.0
Professional: .5
Other: .5

Patient Days: 2431

Outpatient Visits: 206

Project Description:

Interim Note: As indicated in the report for 1957, the emphasis in this project has been placed on two parameters. First, the utilization or turnover of I^{131} thyroxine, administered exogenously, in patients with chronic, relapsing uveitis.

Second, determination of similar factors in normal control patients for comparison with published values.

Objectives: To determine whether there is an association between chronicity and rate of recurrence in uveitis with abnormalities in peripheral utilization of thyroid hormone.

Methods Employed: Patients accepted for the study have been screened to determine the status of thyroid function (basal metabolic rate, serum cholesterol, I^{131} uptake and serum protein-bound iodine).

The half-time of radiothyroxine turnover is calculated from the rate of disappearance of I^{131} hormone from the blood, following intravenous injection. The radioactivity injected is 50 microcuries. Blood samples are drawn daily for 14 days and prepared as 2.0 ml. counting samples. Radioactivity is assayed in a well-scintillation counter and compared with known standards of the injected dose. Half time values are calculated from the counting results and compared for accuracy with values derived linearly from the slope of a semi-log plot of the data. All plotted results are fitted by the method of least squares.

By comparison of counting results with serum levels of protein-bound iodine the following values are derived for each patient:

1. Daily percent of thyroxine pool degraded.
2. Extrathyroidal iodine pool.
3. Micrograms of iodine degraded daily.
4. Correction of all data for age, sex, weight and surface area.

Patient Material and Major Findings: Radiothyroxine turnover studies have been completed in 30 uveitis patients and 5 normal controls.

Data available indicate that in the main the uveitis patients show retarded rate of utilization of circulating thyroid hormone as compared with normal controls. Comparisons based on age, sex, body weight and surface area do not affect this result significantly. The major difference lies in the daily rate of degradation of I^{131} thyroxine and of course, in turnover half time.

Treatment with thyroid hormone has, in a few patients, resulted in correction of these abnormal results.

Studies conducted on 5 normal control patients have given results that agree closely with those of several other investigators.

Significance to Program of Institute: Patients with recurrent uveitis constitute a major clinical problem in the Ophthalmology Branch program. This project is designed to complement other studies that are done in the routine work-up of uveitis patients. It is felt that hormonal imbalance may be an important co-factor related either to susceptibility of patients to the disease or possibly to their patterns of therapeutic response.

Proposed Course of Project: There is need for further turnover studies to be done in additional patients. Beyond this it will be of interest to determine what effects treatment with thyroid hormone has on:

1. Turnover results
2. Clinical features of uveitis in patients previously tested.

Part B included: Yes ☐ No ☒

Serial No. NINDB-47 (c)
1. Ophthalmology Branch
2. Physiology Section
3. Bethesda, Maryland
4. Same as NINDB-65 (c)

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Detection of Ocular Tumor by Isotope Tracer Methods

Principal Investigator: James F. O'Rourke, M.D.

Other Investigators: David Paton, M.D.
Bruce E. Cohan, M.D.
Richard M. Copenhaver, M.D.
Herbert E. Kaufman, M.D.

Cooperating Units: None

Man Years (calendar year 1958):
Total: 1.0
Professional: .5
Other: .5

Patient Days: 313

Outpatient Visits: 98

Project Description:

Interim Note: As indicated in the report for 1957, the course proposed for this project was to continue the study of tumor detection, mainly at the clinical level.

Previously, basic studies indicated that animal tumors may take up relatively more Zn^{65} or I^{131} than P^{32}. These results are not at once applicable to the clinical problem of tumor diagnosis for the reason that a reliable method for localizing foci of gamma energy, within the eye, has not been found. The result is that we continue to depend on tracing the beta emissions of radiophosphorus.

Objectives: To assess the accuracy of tracer methods used for diagnosis of ocular tumor, comparing trans-scleral and trans-conjunctival methods of counting.

Methods Employed: Radiophosphorus uptake studies continue to be done on patients showing evidence of intraocular tumor. The technique presently used is to inject 500 microcuries (adult dose) of sodium radiophosphate, intravenously, and to measure the relative radioactivity of each eye after twenty-four hours, using an end-window Geiger tube.

The initial counting is done trans-conjunctivally. Following this, the appropriate portion of the surface of the globe is exposed, surgically, and trans-scleral counting is done over the area of the fundus lesion.

Major Findings: The advantages of trans-scleral counting, done as a surgical procedure, are indicated by results obtained in several recent attempts. There is evidence that, in the four patients most recently studied, the trans-scleral counting results were correctly positive, although results of the trans-conjunctival method were negative or equivocal. The former method seems to be the present one of choice, although patients available are too few to support this as a definite conclusion.

Significance to Program of Institute: Patients referred to the Ophthalmology Branch as tumor suspects often represent doubtful or unusual cases: the need for continued attempts to improve P-32 counting methods is indicated by several false negative results obtained in this group of patients.

Proposed Course of Project:

1) To continue P^{32} study on patients available with suspected ocular malignancy;

2) to attempt correlation between the locus of highest radioactivity and the position of intraocular malignancy, on histopathologic sections.

Part B included Yes ☐ No ☒

Serial No. NINDB-48 (c)
1. Ophthalmology Branch
2.
3. Bethesda, Maryland
4. New Project

PHS - NIH
Individual Project Report
Calendar Year 1958

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 1958): Patient Days: None
Total: .10
Professional: .05
Other: .05

Project Description:

Objectives:

1) To determine the possible antigenicity of the lens capsule;

2) to produce cataracts immunologically by immunization with lens capsule and lens proteins;

3) to determine whether various tissues of the eye are immunologically related;

4) to find immunological clues for ocular involvement in patients with various allergic dermatoses.

Methods Employed: Lack of sufficient animal space necessitates the use of guinea pigs throughout the experiments. Guinea pigs are immunized with guinea pig lens capsules and

guinea pig lens proteins homogenized with Freund's adjuvant to enhance antibody production. Injections are repeated every two weeks for several months and the eyes of the immunized animals are observed on the slit lamp. Blood and aqueous humor are tested for antibodies by the Ouchterlony method. Other guinea pigs are immunized with calf corneal epithelium, calf vitreous, calf lens capsule, and calf lens protein, respectively. The sera are tested for antibodies with particular regard for reactions of identity or non-identity with the various antigens mentioned.

Major Findings: The sera of guinea pigs immunized with guinea pigs' lens capsule and guinea pigs' lens proteins or with calf lens capsules and calf lens proteins show cross-reactions with corneal epithelium and vitreous and do not react with donor blood, -iris, -retina, or -aqueous. Anti-calf vitreous sera show strong cross-reactions with calf blood and none with the other calf antigens. Anti-calf corneal sera react with corneal epithelium only and not with calf blood or ocular calf antigens. In none of the animals immunized with lens capsules and lens proteins did cataracts appear, although repeated paracenteses were carried out and some lenses were traumatized.

Significance to Program of Institute: The complete knowledge of related antigens in eye tissues is theoretically intriguing and necessary for a better understanding of the response of the eye to disease.

Proposed Course of Project: The nature of the antigen-antibody response between various eye tissues has to be further investigated, particularly whether one deals here with true cross-reactions or reactions between identical or related protein molecules. The lens being the prime example of organ specificity suggests testing of other ocular tissues on organ and species specificity.

It is known that subcutaneous implantation of donor skin in recipient animals will lead to opacification of a corneal graft. It would seem, therefore, that both skin and cornea have related or identical protein molecules, but in the above-mentioned experiments anti-corneal sera did not react with skin. Neither did anti-lens sera react with skin. The obscure immunological relation between skin and cornea and skin and lens seems well worth investigating. If the results of the animal experiments so justify, it is hoped to study patients with certain dermatoses showing ocular manifestations of cornea and lens.

Part B included Yes ☐ No ☒

Serial No. NINDB-49 (c)
1. Ophthalmology Branch
2.
3. Bethesda, Maryland
4. New Project

PHS - NIH
Individual Project Report
Calendar Year 1958

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 1958): Patient Days: None
Total: .10
Professional: .05
Other: .05

Project Description:

Objectives:

1) To complete a statistical analysis about the interrelations of the five optical elements in the human eye as a basis for a theory on refraction anomalies.

2) To test that part of the theory which assumes that the tension in the choroid, by reducing the pressure on the sclera, is a factor in determining the size of the globe. It is clinically known that wherever the choroid is absent, the sclera becomes ectatic. Quantitative confirmation may be obtained by measuring the pressure in the suprachoroidal space and comparing it to the intraocular pressure.

Methods Employed:

1) Statistical analysis. According to preliminary estimates, three factors will explain the correlations between

the five optical elements. It seems desirable to check these estimates on a computer as soon as a factor analysis program for an IBM 650 becomes available.

2) The subscleral pressure (SSP) is measured by inserting a 27 gauge needle in the suprachoroidal space. The needle is connected to a transducer and a pressure head allows the introduction of a droplet of fluid at the tip of the needle. As soon as equilibrium is reached between the SSP and the pressure in the needle, the outflow stops, and pressure changes may be recorded from pressure changes in the droplet. The SSP is compared with the IOP measured by a needle in the anterior chamber.

Major Findings:

1) As the lens power in Stenström's data was calculated from the four other optical elements in the human eye, pseudocorrelations may have been introduced in those correlations in which the lens power is contained as one of the variables. Since the amount of pseudocorrelation cannot be directly estimated, correlation calculations were repeated in Sorsby's material in which the lens power was measured and the axial length computed. As correlations in Stenström's data check well with those of Sorsby, the amount of pseudocorrelation so introduced must be negligible.

2) The SSP measured in 12 eyes appears to be lower than the IOP. The difference amounts to between 2 and 6 mm. Hg. Parasympathetic stimulation (ciliary ganglion) leads to decreased pressure and sympathetic stimulation (cervical sympathetic) leads frequently, but not always, to an increase in pressure.

Several investigators consider the choroid as too fragile to be able to stand pull and pressure. However, by trephining scleral windows in the posterior pole, the choroid bulges out and is considerably retracted on parasympathetic stimulation. A large scleral window causes a large herniation of the choroid but even when overstretched, the choroid is able to stand 90 mm. of IOP before rupturing.

Significance to Program of Institute: The reduction of all correlations between the optical elements of the human eye to three basic factors demonstrates a link between corneal power and axial length, and at the same time, between lens power, depth of the anterior chamber and axial length. An adequate explanation of these factors will mean a step forward towards a theory on refraction anomalies.

Proposed Course of Project:

1) To complete statistical calculations by a factor analysis of Stenström's data; to determine the number and load of the factors underlying the optical elements.

2) To confirm preliminary pressure measurements in the suprachoroidal space by an extremely fine solid type of pressure gauge.

3) To carry out a number of tests on myopic and hypermetropic patients to obtain information on sympathetic and parasympathetic activity in refraction anomalies.

Part B included Yes ☐ No ☒

Serial No. NINDB [illegible]
1. Ophthalmology Branch
2.
3. Bethesda, Maryland
4. New Project

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Intraocular Angiography

Principal Investigator: Bruce E. Cohan, M.D.

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1958): Patient Days: None
Total: .10
Professional: .05
Other: .05

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 aqueous outflow channels and intraocular vessels in an essentially intact eye.

Methods Employed:

1) The aqueous humor of the rabbit's eye is replaced by the same volume of sodium diatrizoate (Hypaque) and pilot studies with conventional laminagraphic and contact laminagraphic techniques are carried out using a 0.3 mm. focal spot tube.

2) After excision of lids, orbital rim, primarily nasally, and the nasal orbital wall, the anterior ciliary vein of the cat's eye is cannulated. Radiographs are taken during hand injection of sodium diatrizoate and also during infusion at varying speeds with a screw-type electrical infusion pump.

3) The same technique as in 2 is used on cats' eyes without removal of lids and bone. 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.

4) The same preparation as in 3 is used in pilot studies of laminagraphy of the cat eye during the retrograde perfusion of the anterior ciliary vein.

Major Findings: The techniques mentioned above have resulted in the successful demonstration of the roentgen-anatomy of the intraocular venous systems in the essentially intact cat eye. The entire ciliary venous system including the iris, ciliary body, choroidal and related intrascleral components are clearly visible on the radiographs. Infusion at varying rates has made possible fractionate intraocular venograms. A number of associated interesting findings have been obtained.

Significance to Program of Institute: It is hoped that the studies mentioned above may lead to better understanding of:

1) The anatomy of the intrascleral venous plexus which is intimately related to the mechanism of aqueous outflow and, therefore, glaucoma.

2) The anatomic pattern and interrelationships of the intrascleral plexus with the vessels of the ciliary body and choroid which are important in the study of many diseases of the eye.

3) The dynamics of the intraocular vasculature.

4) The possibilities for future possible investigation of the intraocular veins in man by angiographic methods.

Proposed Course of Project: It is proposed to complete the studies described in Method 3 and then to continue with

the laminagraphic exploration of the intraocular venographic pattern. An attempt will be made to utilize this technique to study the effect of pharmacological agents and surgical interventions on the intraocular vasculature.

Part B included: Yes ☐ No ☒

Serial No. NINDB-57 (c)
1. Ophthalmology Branch
2. Cytology and Histopathology Section
3. Bethesda, Maryland
4. Same as NINDB 70 and 71

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Electron Microscope Studies on Epithelium, Capsule and the Fibers of the Lens and on the Epithelium of the Ciliary Body and the Optic Nerve

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 1958): Patient Days: None
Total: 1
Professional: .5
Other: .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 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.

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 lens of the anesthetized animal was removed from the eye in situ and immediately immersed in the fixation medium. It was then dehydrated in a graded series of alcohols, dissected, and embedded in methacrylate. Adequate sections were cut on a Servall ultramicrotome and transferred on collodion filmed grids covered with a fine carbon layer.

Major Findings:

1. Normal Lens

Subsequent to the study of lens epithelium, as reported last year, the cortical layers of lens fibers were investigated on rat, rabbit, monkey and calf lenses. The fibers represent elongated, prismatically shaped cells, outlined by dense membranes and separated from each other by a small intercellular space. From the transitional zone of the lens epithelium, which is the equatorial region, to the fibers and hence to deeper layers there is variation in amount and configuration of intracellular elements. A description of these cytoplasmic structures follows.

Nuclei: In the lens epithelium, the nuclei often show marked indentations and the presence of one nucleolus which can be recognized as an irregular aggregate of dense granules. In the bow region of the lens fibers, nuclei are somewhat larger, show fewer indentations, and commonly possess two nucleoli. Here the nucleolar granules are disposed in thick coils, embedded in a lighter matrix. In more central parts, structures of a size between 1 and 2 μ are found in the cytoplasm. They are composed of opaque granular material massed into a body which is outlined by a dense border. The occasional presence of a double membrane boundary and association with elements of the Golgi Complex suggest that these bodies might represent remnants or fragments of nuclei in a state of regressive metamorphosis in deeper lens regions.

Mitochondria: Mitochondria in the lens epithelium gradually increase in number from the center to the equator and range in size from 0.1 to 0.7 μ. In the youngest lens fibers, they attain a length around 1 μ and are frequently seen. In deeper lying fibers and in the axial regions of the lens cortex, they are sparse and occur in the form of large long structures measuring up to 3 μ in length.

Endoplasmic reticulum: Endoplasmic reticulum of the rough surfaced type gradually decreases from the central zone of the lens epithelium towards the periphery. In the youngest lens fibers, it is found in the form of dense profiles with relatively large spaces between its membranous parts. In sections of deeper lying cells, it appears smaller and more vesicular,

and is present neither in the deepest investigated layers nor in the perinxial regions of the lens cortex. There is a gradual increase of small clusters of ribonucleoprotein granules from the youngest to the older lens fibers. The clusters are diffusely dispersed throughout the cytoplasm.

No changes in the appearance of the smooth surfaced type of endoplasmic reticulum are noted in this investigation of different areas of the lens.

Golgi Complex: The Golgi complex is similar in the various regions of the lens investigated. However, in the fibers, it appears more segregated from the nuclear boundary, and in deeper cells its individual components are somewhat dissociated from one another.

Low density elements: In the lens epithelium as well as to a higher degree in the lens fibers, cytoplasmic elements of spherical and filamentous shape with diameters ranging from 100 to 120 Å are observed. They are characterized by low density to electrons and constitute the preponderant cytoplasmic structure in the investigated lens fibers.

In a joint investigation with Dr. R. A. Resnik, lenses were fractionated by ultracentrifugation in order to find a residue which contains these low density elements exclusively. For this purpose six different fractions were obtained, and after fixation, embedding and sectioning, they were studied with the electron microscope. The supernatant of a fraction obtained after centrifugation for 16 hours at 105,000 x g, contained these low density elements exclusively. This fraction was further subjected to morphological studies and to chemical analysis.

2. Experimental cataract

Studies on the effect of cataractogenic agents on the fine structure of the lens are being carried out. At present the following preliminary results are available.

X-Ray Cataracts: Rabbit lenses were observed after exposure of the eye to 1500 rad x-rays. Four days after treatment a few cells in the peripheral zone of the epithelium contained rather long (2 µ) lammellated structures which after a 10 day interval seemed to be transformed into mitochondria. Another finding 10 days after irradiation consists of structural changes in mitochondria; they appear as ballooned, club-shaped, and elongated. In one cell a dense conglomerate of granular and membranous configurations around a lighter homogenous core

was seen opposite the nucleus. In several cells, nuclear material is located outside the boundary of the cell nucleus. Fourteen days after irradiation severe changes can occur in both nuclei and cytoplasm. Nucleoplasm appears agglomerated into dense masses in a lighter matrix. The nuclear membrane appears as a wide band, without double membraned contours and with its continuity interrupted by several large, circular openings. In the cytoplasm, profiles of the endoplasmic reticulum appear larger than normal and a considerable increase in ribonucleoprotein granules occurs simultaneously with an augmentation of low density filaments. Other cytoplasmic elements appear reduced in number. Generally, it may be noted that the transformations in the lens epithelium after irradiation, occur in some areas alternating with apparently normal zones.

Myleran cataracts (2-4-6- weeks): Rat lenses were prepared from animals fed from a diet containing myleran for 2, 4, and 6 weeks. Thus far, observations have been on lenses of animals that were sacrificed after two weeks on the diet. They reveal a great quantity of low density filaments in the cytoplasm of the lens epithelium. In areas beneath the epithelium severe damage can be observed in the form of complete loss of lens fiber cytoarchitecture and the deposition of a dense amorphous substance. Occasionally, posterior parts of lens epithelial cells are included in these foci.

Mimosine cataracts: Rat lenses of animals subjected to a diet containing mimosine for 5, 7, and 14 days have been prepared. Observations on lenses of the first group show that the rough surfaced endoplasmic reticulum is transformed to large cysternes which present a very complicated three dimensional configuration. At the same time mitochondria appear swollen with a displacement of their internal cristae to the periphery. In the nuclei, dense, irregular, deposits can be found at the sits of the nucleoli. The regions observed in the second and third stages of this experiment, in the lens epithelium, do not display considerable changes. Extensive changes, however, are seen in the region of the lens bow in the form of intracellular vacuolization, destruction of several lens fibers with irregular deposits of a dense material.

Mary Ann Gavin in cooperation with B. J. Lloyd, Jr. (NCI) developed a method to improve sectioning of tissue for electron microscopy investigation. In this procedure a knife broken from Vycor brand plate glass is used instead of the conventional kinds of plate glass. Vycor resembles fused quartz in its physical and mechanical properties and has the advantage of cutting satisfactory sections of brittle specimens and generally maintains a usable cutting edge for longer periods.

Significance to Program of Institute: The completed investigations on the cortical lens fibers together with the previous study on lens epithelium provide information on the general fine architecture and cellular organization of the lens of the normal, mature animal. This serves as a basis for comparison in studies on experimental cataracts which have been initiated.

The joint investigation with Dr. Résnik led to the chemical definition of cytoplasmic constituents that are microscopically visible and represent the soluble lens proteins.

Proposed Course of Project: Observations on experimentally-induced cataracts will be continued. The joint investigation with Dr. Resnik will be extended to determine whether soluble lens proteins can be identified as individual morphological entities. Study on developing lens and tissue culture material is contemplated.

3. Ciliary Body

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.

An extensive study on fixation methods has been initiated in order to establish the most suitable means of preserving this structure in vivo and in situ during functional changes induced by sympathomimetic and parasympathomimetic drugs, carboanhydrase inhibitors and the enzyme chymotrypsin. For this purpose, the eyes of anesthetized albino rabbits have been infused through the cannulated anterior chamber with buffered osmium tetroxide of varying concentrations for 30 to 60 minutes. In another series the same infusions will be repeated on eyes where a colobema has been surgically introduced to secure penetration of the fixative into the posterior chamber. A parallel study of histological changes is planned. The specimens thus treated will first be examined under the light microscope.

Major Findings: None

Significance to Program of Institute: Development of an adequate procedure for such a study will also allow a simultaneous investigation of other regions in the eye bordering the anterior and posterior chambers.

Proposed Course of Project: Study of the ciliary epithelium under the aspects indicated in the methods.

III. Optic Nerve.

This project has not been continued in the last year. It will be resumed as completion of other projects permit.

Part B included Yes [XX] No [ ]

Serial No. NINDB-4 [illegible]

PHS - NIH
Individual Project Report
Calendar Year 1958

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Wanko, T. and Gavin, M.A.: The fine structure of the lens epithelium. An electron microscopic study. A.M.A. Arch. Ophth., 60:868-879, 1958.

Honors and Awards relating to this project: None

Serial No. NINDB-52 (c)
1. Ophthalmology Branch
2. Cytology and Histopathology Section
3. Bethesda, Maryland
4. Same as NINDB-72 (c)

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Electron Microscope Studies on Biopsies of Human Muscle Diseases

Principal Investigators: Theodor Wanko, M.D.
G. Milton Say, M.D.

Other Investigators: Mary Ann Gavin, M.S.

Cooperating Units: None

Man Years (calendar year 1958):
Total: .10
Professional: .05
Other: .05

Patient Days:
762

Project Description:

Objectives: To determine by means of the electron microscope detectable changes in various human dystrophic muscle 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% sodium tetroxide for fixation. This is followed by dehydration and, in some cases, by additional impregnation with 1% phosphotungstic acid, imbedding in methacrylate 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:

1) Normal muscle - Specimens of four normal human subjects have been investigated. The results generally are comparable to those obtained from other vertebrates with a suggestion of the presence of two types of myofilaments (primary and secondary). Special attention has been given to the organization of the myofibril at the Z band level. It is felt that a double membraned structure with a tortuous configuration separates the individual sarcomeres. It seems to extend beyond the limit of the myofibril towards the sarcolemma and, on the other side, to adjacent myofibrils. Morphologically, it is barely distinguishable from the sarcoplasmic reticulum; at the present time it cannot be decided whether these two elements are parts of one system. In one respect the human samples appear different from animal tissue. All observed normal and pathological biopsy specimens of adult persons contain a number of cytoplasmic inclusions in muscle cells, capillary endothelial cells and to a lesser degree, in pericytes. These inclusions vary in size between 1 and 2 μ and consist of an agglomerate of vacuoles of various densities and opaque granules.

2) Myotonic dystrophy - Four cases of myotonic dystrophy were studied and the following characteristics observed. In small muscle fibers chains of central nuclei are seen. They are frequently surrounded by filamentous structures arranged in bundles which sometimes display cross bandings that are similar to normal striated muscle. Sometimes they appear in directions perpendicular to one another, suggestive of ring fibers. The small muscle fibers also contain numerous mitochondria, sarcoplasmic reticulum, ribonucleoprotein granules and, in a relatively high number, dense agglomerates described before in normal muscle. In other muscle fibers of apparently normal size the following structural transformations are observed: Increase of ribonucleoprotein granules sometimes concentrated under the sarcolemma comparable to the sarcoplasmic pads known from light microscope findings. Rarefication of mitochondria and changes in their internal organization. A pattern of cross banding at the Z disc which differs from the normal and occasionally assumes the appearance of a double Z disc with a lighter space between two dense contours. Myofilaments can be more widely spaced than normal which might represent a reduction in number. In one case ring fibers were observed.

3) Werdnig-Hoffman's disease - Preliminary observations on a child show club-shaped mitochondria of a large size. Some present an apparently normal configuration while the boundaries of others seem distended and surround areas which lack organizational details.

Significance to Program of Institute: It is possible that significant morphological differences between normal and dystrophic muscles might yield some insight in the cytopathology involved in these diseases.

Proposed Course of Project: Studies on normal muscle will be continued with special attention being given to structural details at the Z disc level, presence of two morphologically distinct types of myofilaments and identification of the dense, heterogeneous agglomerate in muscle fibers and blood vessel walls.

Observations on myotonic dystrophy will be continued Further studies will be carried out on samples of Werdnig-Hoffmann's disease and subsequently on other specimens collected, of familial periodic paralysis, progressive muscular dystrophy and other neuro-muscular diseases.

Part B included: Yes ☐ No ☒

Serial No. NINDB-53 (c)
1. Ophthalmology Branch
2. Cytology and Histopathology Section
3. Bethesda, Maryland
4. Same as NINDB-74 (c)

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Study of Submicroscopic Structures of Ocular Pigment Cells. (Staining of the Living Tissue Culture Pigment Cell by Acridine Orange).

Principal Investigators: M. K. Wolf, M.D. and Samuel B. Aronson, M.D.

Other Investigators: Leo Caravaggio, M.S.

Cooperating Units: Dr. M. K. Wolf, Laboratory of Neuroanatomical Sciences, NINDB, "The Significance of the Acridine Orange Staining of Neurones In Vitro and In Vivo".

Man Years (calendar year 1958): Patient Days: None
Total: .10
Professional: .05
Other: .05

Project Description:

Objectives:

1) to establish the fluorescent image which the living tissue culture cell will display when stained with AO and.

2) to define changes in the image produced by injury.

Methods Employed: Tissue cultures of chick heart fibroblasts, choroid pigment cells and ciliary body pigment epithelial

cells were raised in the Paul Chamber in the presence of staining concentrations of AO and the fluorescent image observed at regular intervals through the lifetime of the cultures. The granulo-kinesis of living pigment cells provided an important criterion for distinguishing between living and dead cells in these experiments.

Major Findings: AO is toxic in tissue culture at the concentration of 1:100,000, but permits continued growth and good cellular health of cultures in a concentration of 1: 1,000,000. The AO has a photodynamic effect in stained cultures rendering them more susceptible to light injury than unstained controls. Healthy AO stained cells when first illuminated show green (orthochromatic) fluorescence predominantly of the nucleus and nucleolus. With continuing illumination the cells become brighter, and red (metachromatic) granules appear in the cytoplasm. At this stage light injury is still reversible. If illumination is continued the nucleolus and the entire cytoplasm acquire metachromatic fluorescence, and at this stage the cell is irreversibly injured. The metachromacy of irreversibly injured cells is like that obtained in fixed AO stained preparations at controlled pH and probably is due to RNA. The metachromatic granules in reversibly injured cells probably are not RNA, because the cells observed do not contain granular aggregations of RNA large enough to produce the image observed.

Significance to Program of Institute: The orthochromatic and metachromatic fluorescence of AO are known to correspond to monomolecular and associated states of the dye respectively. Study of the staining of living cells may provide important information about the chemical state of the components of living cells. The use of the Paul Chamber in these experiments permitted more rigorous definition of criteria for cellular health than has been possible in previous experiments with this dye.

Proposed Course of Project: The experiments are complete and are being prepared for publication.

Part B included Yes [ ] No [XX]

Serial No. NINDB-54 (c)
1. Ophthalmology Branch
2. Chemistry Section
3. Bethesda, Maryland
4. Same as NINDB-75 (c)

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: A Study of the Proteins of the Lens

Principal Investigator: Robert A. Resnik, Ph.D.

Other Investigators: Theodor Wanko, M.D. and Edith Kenton, B.S.

Cooperating Units: None

Man Years (calendar year 1958): Patient Days: None
Total: 1.0
Professional: .5
Other: .5

Project Description:

Objectives: To study the chemistry of the lens proteins, the structure of alpha crystallin, its interactions with other molecules, and its properties in solution.

Methods Employed: The techniques of ultracentrifugation, Tiselius electrophoresis, and spectrophotometry are used to characterize these proteins. Other techniques, such as equilibrium dialysis and viscosimetry also are employed to obtain information concerning the properties of these molecules.

The size and shape of the molecule calculated from the diffusion data indicate that it is an asymmetric molecule with axial ratios of 1:15 or 1:20 depending upon whether it is represented either as a prolite or an an oblite ellipoid of revolution. The viscosity coefficient of 12.1 is in agreement with these general results, indicating that it is a thin, elongated molecule.

The effects of pH, ionic strength and solvent have been studied and suggest that alpha crystallin undergoes transformation in solution as these factors are varied. For example, two components are present in the sedimentation pattern at pH 3.1 in 0.1 M glycine buffer. At this same pH in 0.1 M sodium chloride only one component is seen. In addition, between 4.5 and 7.8 the sedimentation is asymmetric, while above pH 8 it is symmetrical. These data are interpreted as indicating the presence of subunits which under certain conditions exist in equilibrium.

Major Findings: Additional studies indicate that the value for the sedimentation coefficient of alpha crystallin is 17.0×10^{-13}. This value is slightly lower than that reported previously but is based on additional data. The molecular weight of alpha crystallin is 900,000 to 950,000. These values and parameters, previously reported as the diffusion coefficient, and the apparent specific volume, are similar to those reported by Orekhovich et al.

In collaboration with Dr. T. Wanko electron microscopic observation of isolated preparations of this protein and the low density elements seen in sectioned lens fibers has also been carried out. At the present time it is not possible to state whether the elongated structures in preparations of the low density elements are alpha crystallin, one of the other soluble lens proteins, or the results of interactions between these molecules. In any event, the chemical analyses of these low density structures indicate that they are proteins and that the soluble lens proteins alpha, beta, and gamma crystallin are present.

Significance to Program of Institute: It is now possible to assign a definite size and shape to alpha crystallin. Additional data for beta and gamma crystallin are not yet available due to the difficulty of separating various components of these two groups of protein.

The information now available for alpha crystallin may provide some basis to correlate chemical changes in the lens to its transparency.

The low density elements, visualized by the electron microscope, will provide important information about the size and shape of alpha, beta, and gamma crystallin. This may permit an evaluation of the soluble lens proteins, as related to the transparency of this tissue.

Proposed Course of Project: The effects of pH, ionic strength, and solvent will be investigated in greater detail in order to bbtain more information about the effects of these variables or the size and shape of alpha crystallin.

Electron microscopic studies, in collaboration with Dr. Wanko, on electrophoretically isolated lens protein as well as the low density system will be continued.

Part B included Yes ☐ No ☒

Serial No. NINDB-95 (c)
1. Ophthalmology Branch
2. Chemistry Section
3. Bethesda, Maryland
4. Same as NINDB-76 (c)

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: An Investigation of the Enzymatic Systems Present in the Lens, Cornea and Aqueous Humor and their Relation to *in vivo* Tissue Metabolism.

Principal Investigator: Robert E. Kuhlman, M.D.

Other Investigators: Robert A. Resnik, Ph.D.

Cooperating Units: None.

Man Years (calendar year 1958): Patient Days: None
Total: 1.0
Professional: .5
Other: .5

Project Description:

Objectives: This project has been divided into four parts:

1) An investigation of the variation of lactic dehydrogenase in the corneal epithelium of different species. Enzymes closely related to lactic dehydrogenase in metabolic pathways were also studied.

2) Radioactive tracer experiments were undertaken to gain an understanding of the enzyme content in the cornea in relation to its actual glucose catabolism.

3) The effect of moderate doses, 1000 r. of x-rays upon specific enzymes in the lens and cornea was further investigated.

4) Microchemical procedures for the assay of enzymes and metabolites in micro samples of aqueous humor and tear fluid are being developed.

Methods Employed: In parts 1 and 3 of this project the activity of various enzymes was determined in dissected areas of frozen dried sections of lens and cornea using microchemical techniques.

Part 1 - Lactic, malic, glucose-6-phosphate, and iso-citric dehydrogenase, hexokinase, aldolase and glutathione reductase were investigated in corneal epithelium from rat, cat and rabbit.

Part 2 - The oxidative metabolic pathways of the whole and de-epithelialized cornea were studied by the use of glucose 1-C^{14}, glucose 2-C^{14}, and glucose 6-C^{14} and 1-C^{14}, 2-C^{14}, and 3-C^{14} labelled lactate. Procedures modified from those of Bloom and Stetten were used, and the isotopes were counted with the liquid scintallation counter.

Part 3 - The lens and cornea of animals exposed to 1000 r. of x-ray were assayed for their content of glutathione reductase, hexokinase, and isocitric dehydrogenase. Whole lens homogenates, and dissected areas of frozen dried corneal epithelium were used.

Part 4 - Fluorometric assay methods for lactic, malic and glucose-6-phosphate dehydrogenase and lactic acid are being adapted to study biopsy specimens, aqueous humor and tear fluid. Modified Levi-Lang constriction pipettes and special micro-glassware are being utilized.

Major Findings:

Part 1 - Of the three species investigated, rat, rabbit, and cat, the rabbit corneal epithelium had the highest general level of enzymatic activity. Two enzymes of the citric acid cycle, malic and isocitric dehydrogenase were found in all three groups to be present at levels equivalent to those present in the cellular areas of the brain and retina. While all species also contained glucose-6-phosphate dehydrogenase at a level equivalent to brain and retina, aldolase and hexo-kinase were lower. The rabbit corneal epithelium was most unusual by virtue of its very high lactic dehydrogenase act-ivity which was 20 times greater than that in the cat or rat; the values were 10 times greater than those reported in other locations or in other species. Investigations of the

Michaelis constant and pH optimum for lactic dehydrogenase from corneas of the three species, indicate that there is no significant species variation in the characteristics of this enzyme.

Part 2 - The whole cornea oxidizes glucose at a rate approximately one-half that of the rat liver or diaphragm. The prominence of the direct oxidative shunt in corneal metabolism is confirmed. In addition, it has been demonstrated that the cornea is able to oxidize lactate, and may do so even in the presence of glucose. Removing the epithelium reduces the ability of the cornea to oxidize glucose in ranges from 88 to 97%, depending on the position of the C^{14} label, whereas lactate-2-C^{14} oxidation is reduced only 27%, indicating corneal stroma has more capacity for the oxidation of lactate than for glucose.

Part 3 - Though marked morphological changes have been noted to appear in the lens and corneal epithelium following irradiation, there was no alteration in the whole lens content of hexokinase or glutathione reductase. In addition, pure cellular areas from irradiated corneal epithelium showed no alteration in their content of hexokinase, glutathione reductase or isocitric dehydrogenase.

Part 4 - Preliminary investigations of lactic and malic dehydrogenase activity in the aqueous humor from a small group of rabbits indicate much individual variation, but malic dehydrogenase is the more active enzyme (average of 23.9 ml/LH) and lactic dehydrogenase to be less active (1.96 ml/LH). Volumes of 20 µl of sample were used for study and this can be reduced ten-fold. Procedures for glucose-6-phosphate dehydrogenase and lactic acid assay are being developed.

Significance to Program of Institute: Through an understanding of the biochemistry of aqueous humor and corneal metabolism, information pertaining to the mechanism of corneal hydration may be obtained. Such information might be valuable in the understanding of some rare forms of corneal dystrophy. In part 2 of this work the importance of lactate to corneal metabolism is demonstrated, as is the relative distribution of its oxidation between epithelium and stroma.

Further, the failure to find significant enzyme damage to result from therapeutic levels of x-rays is important in the consideration of the mechanism of radiation cataract formation.

Proposed Course of Project: Both the study of enzyme damage resulting from x-irradiation to the lens and cornea and the investigation of the species variation of lactic dehydrogenase and other enzymes present in the corneal epithelium have been concluded. Further investigation using radioactive labelled glutamate and α amino butyric acid will be undertaken to explore the possibility that these substrates also may be important to corneal metabolism.

An effort is also being made to correlate the carbohydrate metabolism of corneal stroma with that of other tissues rich in collagen.

Micro methods for enzyme assays and the determination of intermediates of carbohydrate metabolism in biopsy specimens of cornea and samples of tear fluid and aqueous humor of 2 to 10 ul volume are being developed. These can then be applied to available patient material, and the altered metabolism of pathological states may be investigated.

Part B included: Yes [XX] No [ ]

Serial No. NINDB-[illegible]

PHS - NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards and Publications

Publications other than abstracts from this project:

Kuhlman, R.E. and Resnik, R.A.: Quantitative histochemical changes in the development of the rat lens and cornea. Am. J. Ophth., 46:47-55, 1958.

Honors and awards relating to this project: None

Serial No. NINDB-56(c)
1. Ophthalmology Branch
2. Physiology Section
3. Bethesda, Maryland
4. Same as NINDB-11 (c)

PHS - NIH
Individual Project Reports
Calendar Year 1958

Part A.

Project Title: Electrophysiology of the Eye

Principal Investigator: M.G.F. Fuortes, M.D.

Other Investigators: Peter Gouras, M.D., and
Kyoji Tasaki, M.D.

Cooperating Units: None

Man Years (calendar year 1958) Patient Days: None
Total: 3
Professional: 2
Other: 1

Project Description:

Objectives: The scope of the present investigation has been expanded from a study of the features of the activity of visual nerve cells in the eye to the more general problem of the transducer action of sense organs. There is reason to believe that the processes leading to generation of impulses in receptor organs may be appropriately subdivided in two groups: specific transducer effects, whereby external energy is transformed into a change capable of stimulating nerve cells, and neural processes, whereby nerve cells discharge impulses in a manner related to type and intensity of the stimulus. In some cases (for instance in free nerve terminals) the transducer action is accomplished by the nerve cell itself; in other cases (for instance in the eye) it requires activity of specialized receptor elements external to the neurones producing impulses.

Methods Employed: Experiments are performed by Dr. Furuto on invertebrate eyes (Limulus) and by Drs. Gouras and Tasaki on the vertebrate retina (toad and fish). In addition to the conventional techniques for intracellular recording, a method permitting intracellular application of electric currents is routinely employed. It is therefore possible 1) to test the effects of light; 2) to study the stimulating effects of electric currents; 3) to analyze the interactions between lights and currents. In this way direct information can be obtained on 1) passive properties of visual nerve cells; 2) physical correlates of the processes leading to their activation. In addition, comparison of the effects of light (which stimulate nerve cells through a photochemical action) and those of currents (which stimulate nerve cells directly) provides indirect information on some properties of the transducer effect of visual receptor organs.

Major Findings: Dr. P. Gouras has brought to a conclusion the work initiated last year on the relations between slow electrical waves and impulse activity produced by illumination in amphibian retina. Potentials were recorded by means of metal or glass microelectrodes either from the surface or from deep layers of the retina. The slow electrical activity recorded from the surface is similar to the electroretinogram, presenting a sharp surface negative wave, (similar to the A-wave) followed by a slower surface positive wave (similar to the B-wave). Depth recording shows that both negative and positive waves have maximal amplitude in the ganglion cell layer and that the negative wave is closely related to impulse discharge from these cells. When the electrode is near the receptor layer only a slow positive wave is recorded. These results suggest that both the ganglion cells and receptor cells produce electrical potentials during illumination and may both contribute to the electroretinogram. While performing this research, Dr. Gouras observed that a phenomenon comparable to Leao's "cortical spreading depression" occurs in the excised amphibian retina. A color change occurs in some part of the retina and spreads slowly to greater and greater areas. Ganglion cells at the front of this wave present intense impulse firing which changes into profound depression as the wave progresses upon them. This process is spontaneously reversible, recovery occurring in 5-15 minutes. Dr. Gouras points out that there are some interesting similarities between this phenomenon and Jacksonian epilepsy.

In the eye of Limulus it is found that frequency of impulses discharged in response to natural stimulation (light) is an approximate linear function of the logarithm of light intensity. The frequency of discharge of the same cells in response to depolarizing electric current is, however, a simple

linear function of current intensity. It appears therefore that the logarithmic transformation which is typical in perception (Fechner-Weber law) is exerted in this case by the photochemical processes. Following stimulation with lights of supraliminal intensity, one records from visual cells a sustained depolarization with superimposed impulses. With subliminal intensity of the natural stimulus only a sustained depolarization is recorded. It can be thought therefore that light evokes firing of nerve cells by depolarizing their membrane. It becomes then important to find out how the photochemical processes initiated by light in the photoreceptor structures of the eye evoke depolarization of nerve cells. Analysis of interaction between light and electric currents gives indirect but convincing evidence that the depolarization evoked by light is the consequence of a change of conductance of the nerve cell's membrane, and impedance measurements performed with the bridge-balance method, using slow frequency alternating currents, show directly that a change of membrane conductance occurs during illumination. By contrast, no conductance change can be measured when electric currents of either direction are passed through the membrane. For these reasons it is thought that the change of conductance occurring in nerve cells, following light stimulation, is induced by a chemical agent and it is suggested that this chemical agent is liberated by the photoreceptor structures under the action of light.

It has been found (Svaetichin) that certain structures in the eye of fish respond with a depolarizing potential change to lights of certain wavelengths and with a hyperpolarizing potential change to other wavelengths. This information has great importance for understanding the functional organization of the retina and the processes underlying color vision. Unfortunately, very little work has been done on this problem so far, and nature of the structures generating these color responses are still uncertain. Dr. Tasaki plans now to perform experiments aimed at identifying location and nature of the structures responding to light with hyperpolarizing potentials. Dr. Tasaki joined the unit only last September and has obtained no results so far.

Significance to Program of Institute: The work described may further our understanding of the following processes: 1) Mechanism by which rhythmical trains of impulses are generated by nerve cells following sustained depolarization of their membrane. It appears likely at the present time that different parts of the same cell may be specialized for production of either graded and sustained responses or all-or-none impulses; 2) Mechanisms by which photoreceptors evoke nerve cell depolarization; 3) Function of the retinal elements interposed between receptors and ganglion cell layers.

Proposed Course of Project: In the work on Limulus an attempt will be made to identify the properties of eccentric cells (with a large axon) and retinula cells (with a small axon). Subliminal responses to light will also be analyzed with the purpose to understand some features of the activity of photoreceptors. The work on fish will continue as planned as long as Dr. Tasaki remains with the section.

Part B included Yes ☑ No ☐

Serial No. NINDB-56 (c)

PHS - NIH
Individual Project Report
Calendar Year 1958

Part B. Honors, Awards and Publications

Publications other than abstracts from this project:

Fuortes, M.G.F.: Electrophysiology of the visual system, a symposium (Editor), Am. J. Ophth., 46:1-182 (Pt. II), 1958.

Fuortes, M.G.F.: Generation, conduction and transmission of nerve impulses, Arch. ital. Biol., 96:285-293, 1958.

Fuortes, M.G.F.: The Annual George H. Bishop Lecture, Generation of nerve impulses in receptor organs, EEG Clin. Neurophysiol., 9:71-73, Suppl. 10, 1958.

Fuortes, M.G.F.: Electric activity of cells in the eye of limulus, Am. J. Ophth., 46:210-223 (Pt. II), 1958.

Fuortes, M.G.F.: Initiation of impulses in visual cells of limulus, J. Physiol., - (in press).

Gouras, P.: Spreading depression of activity in amphibian retina, Am. J. Physiol., 195:28-31, 1958.

Gouras, P.: Electric activity of toad retina, Am. J. Ophth., 46:1-182 (Pt. II), 1958.

Honors and Awards relating to this project:

Fuortes, M.G.F. Delivery of the Annual Bishop Lecture at the Washington University Medical Center, St. Louis, Missouri, April 11, 1958, entitled "Generation of Nerve Impulses in Receptor Organs".

Serial No. NIH-57 [illegible]
1. Ophthalmology Branch
2. Physiology Section
3. Bethesda, Maryland
4. New Project

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: ERG Reactions of Pure-Cone Mammalian Retinae

Principal Investigators: Katharine Tansley, D.Sc. and
Richard M. Copenhaver, M.D.

Other Investigator: Ralph D. Gunkel, O.D.

Cooperating Units: None

Man Years (calendar year 1958): Patient Days:
Total: .10
Professional: .05
Other: .05

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 an ark adaptation curves.

Major Findings: None to date.

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 retinae 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 retinae 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 colour 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 colour 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: See Objectives.

Part B included: Yes [ ] No [XX]

Serial No. NIND-58(c)
1. Ophthalmology Branch
2. Physiology Section
3. Bethesda, Maryland
4. Same as NIMDB-63 (c)

PHS - NIH
Individual Project Reports
Calendar Year 1958

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., and Ralph D. Gunkel, O.D.

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1958):
Total: .10
Professional: .05
Other: .05

Patient Days: 280

Outpatient Visits: 96

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 are 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: These studies have in the past yielded significant information of value in the diagnosis of such diseases as retinitis pigmentosa, cerebro-macular degenerations, congenital night blindness, total color blindness and others. In addition, these studies contributed basic information about the differentiation of scotopic and photopic function which is necessary in the study of retinal physiology.

Recently these methods have been applied to color-defective subjects who comprise 8% of the male population. It has been possible to calculate spectral sensitivity curves from the electroretinographic data which have given some insight into the photopic mechanisms which are related to color vision. The defects responsible for the typical color anomalies are demonstrated to be retinal in location rather than in the optic pathways or the cerebral cortex. The spectral sensitivity curves of several types of color defectives were found to be significantly different from the normal which supplies a method of distinguishing between normal and defective color vision which is purely physical and

does not require any subjective response on the part of the patient. The electroretinographic method allows a determination of the type of defect and to some extent the degree of deficiency. By finding the difference between the electroretinal spectral sensitivity curves between normals and color defectives it is possible to obtain information about the color mechanism lost. The difference maximum in the protanope agrees well with the peak absorption of the red-sensitive pigment erythrolabe which Rushton (at Cambridge, England) has found to be absent in the retina of this type of color defective. The sensitivity loss in deuteranopes agrees well with the green-sensitive pigment present in normals and also in deuteranopes and hence suggests an interruption of the electrical impulses from the green-sensitive cones at a retinal level rather than a loss of pigment. As previously stated this represents a new and practical method of investigation of a large group of patients seriously handicapped in a number of occupations where color vision is a prerequisite.

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 numberous clinical diagnostic problems.

Proposed Course of Project: To extend the method of spectral electroretinography and other tools for functional investigation to a study of acquired color defects, various congenital anomalies, glaucoma and optic neuritis.

Part B included Yes [X] No [ ]

Serial No. NINDB-58(a)

PHS - NIH
Individual Project Report
Calendar Year 1958

Part B. Honors, Awards and Publications

Publications other than abstracts from this project:

Dodt. E., Copenhaver, R.M., and Gunkel, R.D.: Photopischer Dominator und Farbkomponenten in menschlichen elektroretinogramm, Pflügers Arch., 267:497-507, 1958.

Goodman, G. and Gunkel, R.D.: Familial Electroretinographic and Adaptometic Studies in Retinitis Pigmentosa, Am. J. Ophth., 46:142-178 (Sept. Pt. II), 1958.

Honors and Awards relating to this project: None

Serial No. NINDB-59 (c)
1. Ophthalmology Branch
2. Physiology Section
3. Bethesda, Maryland
4. Same as NINDB-62 (c)

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: ERG Spectral Sensitivity Curves on Caucasians, Negroes, and Albinos

Principal Investigator: Eberhard Dodt, M.D.

Other Investigators: Ralph D. Gunkel, O.D. and Richard M. Copenhaver, M.D.

Cooperating Units: None

Man Years (calendar year 1958): Patient Days:
Total: .10
Professional: .05
Other: .05

Project Description:

Objectives: To study the relative spectral sensitivity curve in deeply pigmented and albinotic human eyes by means of electroretinography.

Methods Employed: Colored light stimuli are produced by using double interference filters in conjunction with a Xenon high pressure lamp. To measure the photopic sensitivity curve of albinos and negroes flickering light flashes at a rate of 32 per second are delivered by a rotating disc.

Patient Material: Negro patients exhibiting a low level of baseline activity and albinotic human eyes will be selected.

Major Findings: The relative spectral sensitivities for wavelengths longer than 583 were high in albinos and low in negroes, while dark caucasians and subjects with "blond" fundi showed intermediate sensitivities. The maximum sensitivity in albinos occurred at 610 mμ as compared with a peak sensitivity at 558 mμ for caucasians and negroes. The difference in spectral sensitivity in albinos and negroes was due to the reflection of light by blood in the former.

By trans-scleral illumination it was found that the only selective absorption of light in the tissue coats of the eye is due to blood. It was also determined that the blood volume in the sclera and choroid cannot be ascertained with this method. This work demonstrates the important effect which the density of the pigment epithelium has on the electro-retinal spectral sensitivity. With small area, intense light stimulation in individuals with a thin pigment epithelial layer of the retina and red sensitivity is markedly increased due to blood reflection.

Significance to Program of Institute: The data obtained in this project contribute materially to the present knowledge regarding the factors affecting the spectral sensitivity curve of the human eye as measured by electroretinography. Recent experiments in animals by Dodt have shown that the spectral reflectivity of the blood may cause distortion of the ERG spectral sensitivity curve. By the comparison of psychophysical data and ERG spectral sensitivity curves in patients, it is possible to evaluate the importance of the spectral reflectivity of blood in humans.

Proposed Course of Project: Since the investigation is completed the project is now terminated.

Part B included: Yes [XX] No [ ]

Serial No. NINDB-59 (c)

PHS - NIH
Individual Project Report
Calendar Year 1958

Part B. Honors, Awards and Publications

Publications other than abstracts from this project:

Dodt, E.; Copenhaver, R. M.; and Gunkel, R. D.: Electro-retinographic measurements of the spectral sensitivity in albinos, Negroes and whites, A.M.A. Arch. Ophth., in press.

Honors and awards relating to this project: None

Serial No. NINDB-60 (c)
1. Ophthalmology Branch
2. Physiology Section
3. Bethesda, Maryland
4. Same as NINDB-64 (c)

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Design and Construction of Ophthalmic Instruments

Principal Investigator: Ralph D. Gunkel, O.D.

Other Investigator: None

Cooperating Units:

Man Years (calendar year 1958): Patient Days: None
Total: .10
Professional: .05
Other: .05

Project Description:

Objectives: To make improvements of instruments used in clinical and laboratory ophthalmologic research.

Specifically: To further modify the Goldmann-Weekers Adaptometer to facilitate perimetric measurements of light sense thresholds, and in particular to measure pure cone function as separated from rod function in the retina.

To design and construct a combined head-holder and electrode-holder for use in electroretinography.

To design and/or construct such other devices as are required or suggested by current projects.

Methods Employed: Extensive testing with the double interference filters, elaborated on by Dr. Dodt for another project, has shown the need for using red light of a longer wavelength than that formerly provided in both the fixation

light and the test spot of the adaptometer. Apparently, even at threshold levels any wavelength shorter than about 672 mμ is perceived by the rods as well as by the cones, although in varying proportions by each. Hence, the para-macular retina, containing rods, sees the impure red light better than does the rod-free macula, which would obviously discourage good central fixation. This situation has been remedied and the test spots have been improved with Wratten filters, which appear to be adequate.

The size of the test spot was found to have unexpected importance in that a beam subtending about 10 minutes of arc gave what appears to be a characteristic cone curve, while the commonly-used one degree spot did not.

Small electric motors have now been mounted so as to move the fixation light an angular distance of 45° in either the vertical or the horizontal meridian in 15 minutes of time. This permits an essentially continuous profile instead of the plotting of five or six points as was formerly done.

Since it was found advantageous in spectral electro-retinography that the subject be seated in a chair instead of lying on his back, it was necessary to devise some means of preventing movement of the head, which would introduce various artifacts in the record. This was achieved with a double yoke arrangement of plastic material attached to a standard chin-rest. Adjustable pads restrict movement adequately, and built-in electrodes touch the forehead and bridge of the nose, thereby simplifying the procedure.

Patient Material: Examinations of dark adaptation and retinal profiles are done routinely on subjects referred here for electroretinographic differentiation of retinitis pigmentosa, night blindness, defective color vision and certain degenerative retinal conditions.

Major Findings: Normal values are being established for retinal profiles using the new color filters and smaller test spots. Certain losses of sensitivity at and around the macula of color blind persons have been observed. These and tentative data from patients with other eye diseases indicate the potential usefulness of the testing procedure for a variety of disease entities. Dark adaptation curves obtained with the modified instrument have been found quite satisfactory.

Significance to Program of Institute: It is believed that the modifications of the Goldmann-Weekers adaptometer and the device of immobilizing the patient's head contribute either to the information based on previously used techniques or to the accuracy of the testing procedure.

The study of retinal profiles has already been shown to be helpful in evaluating and corroborating photopic and scotopic electroretinographic data. It is possible that it may prove to be a sensitive method for detecting early retinal and optic nerve damage.

Proposed Course of Project: It seems to be of benefit that the present freedom and flexibility of the instrument project be maintained. This would serve the development of promising ideas.

Further studies should be made of perimetric light sense thresholds in diseases other than those studied. By this technique information might be gained supplementary to that obtained by the conventional visual field measurements.

Part B included Yes [ ] No. [X]

SUMMARY

During the period of this report, 211 patients have been studied on the wards, while in the Outpatient Department, 214 patients were examined in a total of 324 scheduled visits. The neurosurgical operating room saw the completion of 106 procedures. Of these, 35 were concerned with the surgical treatment and investigation of epileptic mechanisms, while 32 were performed for space-occupying lesions of the nervous system. The patients who came to ward and clinic investigation, as well as those who came to operative treatment, form the basis for reports and relevant laboratory work. There were 27 reports prepared for publication during this year.

As in the past, the majority of these patients were epileptics, and of the epileptic population, the greater proportion owed their epilepsy to disease of the temporal lobe. There were also cases of centrencephalic seizures and problems involving diffuse or multiple lesions.

From recent studies of temporal lobe epilepsy, it is now apparent that one of the significant causes is vascular malformation. Such malformations cannot be diagnosed by ordinary tests using contrast media, nor is there any particular clinical sign of their existence. Twelve of these lesions have been disclosed at operation. In each case, there was some alteration of the surface vessel pattern and some change in the size, color, and consistency of the surface gyri. These alterations were not remarkable and in some cases appeared only as subtle changes--changes so subtle that their significance was missed in the initial exposure. The surface changes are related to a lesion in depth. This is a small vascular tumor characterized by racemose vessels, and a fine network of these vessels is surrounded by gliotic tissue which is usually peri-incisural in location. The study of patients afflicted with such lesions has provided further information concerning epileptic mechanisms of temporal lobe seizures. These are unilateral lesions, yet their expression is often bilateral. This bilaterality disappears after excision of the lesion and thus is relevant to a primary epileptic focus, which in turn has activated distant structures.

The ward study of patients was concerned with epileptic mechanisms, pain problems, space-occupying lesions, transmission of infection in the ward environment, observation of abnormal individual and social behavior patterns, the effects of urea on intracranial pressure, the effects of hormones on seizure processes, the relationship of hormonal activity to spontaneous seizures, the autonomic concomitants of temporal lobe epilepsy, the clinical characteristics of automatisms, language studies, as well as the effects of hypophysectomy and depth stimulation responses. The observation of seizures has provided new information concerning the motor phenomena of automatic states and other sequels of temporal lobe discharge. Miss Pirnie has successfully photographed

1721 seizure signs. These photographs have been correlated so as to provide a panoramic survey of the motor and autonomic phenomena of temporal lobe epilepsy. In addition, the vocalization patterns and various verbal expressions which accompany seizures have been recorded. The interictal behavior of patients has occupied a considerable time in the ward studies. The language characteristics, the characteristics of hierarchy and social interaction, as well as the capabilities for perceptual function have been studied. There have been certain technical developments on the ward, as well. Miss Pirnie has worked out a prototype for a new neurosurgical bed, and Miss Pirnie and Dr. Edgar are studying the characteristics of air-born infection in the ward environment.

There have been certain developments in the neurosurgical operating room as well. Dr. Pritchard has developed a new dural scissors and reports on the history of this form of cutting instrument in intracranial and other neurological surgery. In conjunction with Mr. Riggle, Miss Lewis has developed a new communicating system for the operating room. Also, Miss Lewis has designed a new workroom plan which will be incorporated in the new surgical suite. This provides for greater aseptic precautions and increases the work capabilities of the nursing staff. In addition, Miss Lewis has begun the first phase of our training course in neurosurgical technique. This course is designed to provide the house officer in neurosurgery with a background in basic and specialized surgical principles. She is training Doctors Bucknam and Lewis in the nursing techniques of neurosurgery. In implementation of this training, Dr. Laskowski is teaching tissue handling technique and hemostatic methods in the primate surgery. In the future, all house officers in neurosurgery will be trained as neurosurgical nurses and will receive technical training in the operating room of Primate Neurology. Miss Lewis continues her work in neurosurgical technique and is gradually accumulating an extensive photographic record of the techniques used here, many of which she has developed herself. In the past year she has spent long hours working on the design problems for the new surgical suite.

The laboratory studies continue. In Neurosurgical Physiology, Dr. Li has continued the application of microelectrode techniques in tissue culture, and has studied neurological, as well as muscle elements. It is hoped that this combination of culture and electronic techniques may be transplanted to the new operating facility so that tissue freshly excised in the operating room can be cultured and studied in whole or part in a readily accessible laboratory. Dr. Li's studies during the past year include studies of cortical neurones, and in combination with Dr. Ortiz, study of neurological transmission characteristics and neurological regeneration. Dr. Ortiz has joined the Branch as a Visiting Scientist on loan from the faculty of the University of Mexico where he is Professor of Neurosurgery and Neuropathology.

In Neurosurgical Anatomy, Dr. Van Buren has reported on the anatomical effects of temporal lobectomy in the human. This work, carried out in conjunction with Dr. Yakovlev, establishes the first structural studies of the effects of temporal lobectomy in the human. He has continued his studies of the visual pathways in various

histological techniques. Also, he is continuing his studies on the anatomical effects of space-occupying lesions and is gradually accumulating clinical material for this purpose. He has been able to do some work in the postmortem room with his new stereotaxic device which is, as yet, untried in the clinical operating room. In addition, he has completed some of the cell counts of pituitary specimens derived at hypophysectomy and correlated this material with clinical and biochemical findings in the patients subjected to this procedure.

In Developmental Anatomy, Dr. Dekaban continues his studies of brain lesions in cases of so-called cerebral palsy. In collaboration with various extramural investigators, he is gradually accumulating a considerable store of pathological material, the study of which may throw some light on various and complicated lesions which are now camouflaged by the label, cerebral palsy. He has begun a study of the embryology of the human temporal lobe and this study, in turn, has been a natural outgrowth of some embryological investigations of the human brain. These first investigations concern reconstruction of early somite development in coronal sections. The temporal lobe studies will be undertaken in sagittal sections.

In the Laboratory of Neuropathology, Dr. Klatzo has pursued a variety of interests. With the use of tissue culture techniques, he has studied intracellular characteristics of astrocytes, and with the use of pathological specimens referred to him, has outlined the characteristics of Kuru disease. In this laboratory, Dr. Miguel has developed a new method for the quantitative study of precipitin reactions, and Dr. Laskowski studied the relationship of cerebral edema to experimental brain injury. He has also studied the effects of hypothermia on injured and normal brain tissue.

The Laboratory on Pain and Neuro-anesthesiology lost its chief this year through the resignation of Dr. Kenneth Hall. Dr. Hall left the Branch to accept appointment as Associate Professor of Anesthesiology in Charge of Research in Duke University Medical School. After Dr. Hall's departure, Dr. Pritchard has continued his project on fluothane, and is comparing this new anesthetic agent to the various accepted inhalation agents such as chloroform, ether, and cyclopropane. In addition, Dr. Pritchard is extending the heart-lung pump technique so as to provide an experimental design for the study of anesthesia effects on the cerebral circulation. In addition, he is making observations on patients under general anesthesia and hypothermia, as well as on chimpanzees in various anesthetic states. These observations are made with polygraphic techniques.

The Laboratory of Clinical Psychology saw the arrival of a new chief, Dr. Herbert Lansdell. Under his direction, this group has reviewed the previous work of the laboratory and has begun an energetic campaign for the development of successful testing of temporal lobe patients. In addition, they have organized a project for the further study of the effects of ablation and/or stimulation of Heschl's convolution. Dr. Lansdell himself is developing a prototype for the testing of chimpanzees whose temporal lobes have been ablated.

The Laboratory of Primate Neurology continues its study on hallucinogenic drugs, the effects of temporal and frontal ablation in the chimpanzee, and more recently, the effects of specific cortical excision on communication capabilities of these animals. Dr. Norris is beginning the study of depth electrode effects in the mesial temporal region, and Miss Lewis, Dr. Norris and Dr. Baldwin have begun the bio-assay of catechol amine production after temporal lobe stimulation. The excitable cortex of the chimpanzee has been investigated and the effects of precentral excision on stimulation patterns have been observed. Penicillin seizure patterns have been studied. Through a new technique, these studies have been made on both hemispheres simultaneously. It is now possible to study at surgical operation the entire convexity and polar surfaces of both hemispheres. With this technique, the effect of passive movement on the properties of the excitable cortex has been studied, as have the characteristics of spread of temporal lobe seizures. The social hierarchy and communication studies in the chimpanzee colony are being continued by Miss Lewis.

Mr. Meiller has developed a photographic technique which will permit color analysis of the surface vascular patterns of the brains of these animals as exposed at operation. Such a technique may permit further study of vascular concomitants of cortical seizures. Dr. Ortiz has joined the Laboratory in the study of the effects of the Mexican mushroom on the behavior of the chimpanzee, as well as its effects on the otherwise normal electrogram, blood pressure, EKG, and other vital signs in this animal. The first phase of the lysergic acid study was completed during this year and the Mexican mushroom project marks the beginning of a second phase which will incorporate other hallucinogenic substances. The apparent similarity between the clinical effects of these chemicals and the clinical expression of temporal lobe epilepsy remains an intriguing and stimulating challenge to further investigation.

National Institutes of Neurological

Diseases and Blindness

Clinical Research

Surgical Neurology Branch

Serial Numbers of Projects:

NINDB-61(c), NINDB-62(c), NINDB-63(c), NINDB-64(c), NINDB-65(c), NINDB-66(c), NINDB-67(c), NINDB-68(c), NINDB-69(c), NINDB-70(c), NINDB-71(c), NINDB-72(c), and NINDB-73(c).

Estimated Obligations for FY 1959

Total: $778,000

Direct: $271,000

Reimbursement: $507,000

Serial No. NINDB-61(c)

1. Surgical Neurology Branch
   EEG Branch
2. Neuropathological Section
   Primate Neurology Section
3. Bethesda, Maryland
4. NINDB-25(c)

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Epileptogenic Mechanisms in the Brain of Man.

Principal Investigator: Maitland Baldwin, M. D.

Other Investigators: C. Ajmone Marsan, M.D., D. B. Tower, M.D. J. M. Van Buren, M.D., I. Klatzo, M.D., R. Edgar, M.D., S. A. Lewis, R.N. and S. A. Bach, M.D.

Man Years (Calendar Year 1958):		Patient Days (Calendar Year 1958)
Total:	.25	3749
Professional:	.25	
Other:	.25	

Project Description:

Objectives:

a. To study causal mechanisms of epileptic seizures in man.

b. To study the electrographic characteristics of epileptogenic activity in the brain of man.

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 extravagant 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: This is a report of observation on 119 patients. By selection, the majority of these patients are afflicted with temporal lobe seizures. From the most recent study of these cases of temporal lobe epilepsy, it is apparent that cryptic angioma is a significant cause of this form of seizure. This deeply lying vascular lesion which seems to involve mesial temporal structures close to the junction between the circulations provided by middle cerebral and anterior choroidal branches now appears as an active agent in the production of epileptic activity. Perhaps it so alters the metabolic requirements of peri-insular tissue in which it lies as to produce an epileptogenic lesion. From a study of this year's cases and a review of previous material, it is evident that this racemose vascular lesion is a significant cause in the development of epileptogenic activity. It cannot be outlined by ordinary contrast studies, and as yet there is no obvious clinical correlation. Apparently, the lesion can be related to unilateral or bilateral electrographic abnormality, and it may or may not be related to ictal signs which have lateralizing significance. However, in the cases which have been studied recently, unilateral excision of this lesion produces a good result insofar as seizure frequency is concerned. In one such case, the lesion could not be totally eradicated because it involved the neighboring brain stem. After operation, this patient did not have any further clinical seizures, but he came to complain of a persisting and annoying perceptual aberration. The objects and people around him seemed more distinct, better lighted, and more vivid. Their vivid and distinct appearance seemed harsh to him, and from time to time he felt as if the objects in front of him were too brightly lighted. His post-operative electrographic studies showed residual epileptiform activity over the mid-temporal leads. Presumably this was relevant to the peri-incisural tissue left at operation. It may be that this remaining tissue has, through its epileptogenic characteristics, activated perceptual processes in the opposite intact temporal lobe and this activation is responsible for the continuing perceptual aberration.

During the past year, a group of patients with seizures were admitted for study under the primary diagnosis of temporal lobe epilepsy. After further study, it was apparent that these patients did not owe their seizures to an epileptiform process in either or both temporal lobes. However, their clinical seizure pattern was characterized by epigastric aurae, altered affect, altered awareness, posturing and adversive movements, as well as autonomic changes. These characteristics are usually related to temporal lobe epilepsy. Yet the significant lesions in these patients were frontal and close to the anterior cingulate gyrus. These cases are being analyzed in conjunction with a similar series under the supervision of Dr. David Daly at the Mayo Clinic. It is hoped that this analysis will serve to illustrate the characteristics of cingulate seizures and provide

Major Findings (cont'd):
a means for differentiating these seizures from those of temporal lobe epilepsy. Obviously, this differentiation has not been clear in the past and the lack of clarity has provided confusion and unnecessary errors in diagnosis.

The motor phenomena of temporal lobe epilepsy have been studied in some detail. It now seems that there are certain hand and other upper extremity postures which are characteristic of epileptic activity in one or both temporal lobes. Usually these hand and upper extremity movements have a lateralizing significance. They occur on the side opposite to the most active temporal lobe. The movements of head and neck in a temporal lobe seizure are usually such that there is turning to one or the other side. The chin points down and the movement is smooth and relatively slow. It is thus different from the adversive movement which is pathognomonic of supplementary motor or other posterior frontal foci. In these movements the chin points up and the movement is jerky and rapid. In ictal automatism, the patient usually looks down and, as is well known, frequently searches his person or immediate surroundings. These searching movements can be interrupted by placing an object in the person's hand, by voice or other sound, and by pain. They can be influenced in character by the type of the object placed in the hand, but neither this interruption nor any of the others serve to sever the chain of movements. In automatism, the patient uses his hand as if it were a flipper. He seems to "finger" an object, but he does not do so with precise movements. At least in the cases under observation, the hand is used with the fingers en bloc, as if the four fingers opposed the thumb as a unit.

During the past year, Miss Pirnie and her observation team have photographed 1721 such phenomena. These ictal movements and their autonomic concomitants are being successfully illustrated through the use of a trained team of nurse observers. This is the first time photography has recorded spontaneous seizure patterns in the natural sequence of temporal lobe epilepsy.

The autonomic concomitants of these temporal lobe seizures are being investigated by Dr. Van Buren. During this year, he has studied Metrazol seizures in seventeen patients. These activated responses were similar to those which he observed previously in spontaneous temporal lobe attacks. He noted a hypertension, tachycardia, respiratory apnea, fall in skin resistance and skin temperature, as well as swallowing movements and inhibition of gastric motility. Apparently the electrographic tracing is indirectly relevant to the autonomic or clinical features of these activated seizures. Thus, autonomic changes may appear coincident with bursts on the EEG or may appear independent of these electrographic abnormalities. He emphasizes the striking independence of electrographic, autonomic,

Major Findings (cont'd):

and clinical findings in time. He interprets this independence as significant of the fact that areas around the third ventricle may carry on their activity without influencing the electrographic manifestations of cortical activity.

Intermittent and paroxysmal perceptual aberration is a clinical characteristic of temporal lobe epilepsy. Such aberration does not always coincide with a clinically recognizable seizure pattern. It may occur without the other stigma of temporal lobe ictus. It appears that perceptual disorders of space and color perception are most frequent. Such patients do not assess the spatial characteristics of their surroundings with particular accuracy. Moreover, their surroundings often seem lighter, more distinct, more brightly colored than usual. The patients are aware of the unreality of these spaces and yet paradoxically describe them as being "more real than real". In temporal lobe epilepsy, perceptual aberration is never separate from disturbance of affect. In fact, in disturbance of the temporal lobe by epileptic process, the most frequent combination of signs are those of fear and perceptual aberration. It now appears that the physical basis of fear may be one of the most significant sources of clinical characteristics in temporal lobe epilepsy. This prompts the speculation that catechol amine or other adrenaline-like substances may be increased in amount as a result of mesial temporal discharge. Recently, Hoff has reported such an increase following stimulation of the temporal lobe in cats. Certainly the dilatation of the pupil, pallor of the skin, change in the vital signs, and other autonomic concomitants observed by Dr. Van Buren are all characteristic of adrenaline-like responses in the human. During this year, we have begun the study of effects of catechol amine on patients with temporal lobe seizures, and conversely, a search for the presence of unusual amounts of these chemical mediators in these patients.

Perceptual aberration and hallucinations may be induced in these patients with temporal lobe seizures by suggestion. Investigation of these intangibles was begun in the year previous and has been continued during the period of this report. Not all patients are affected by suggestion, but approximately fifty per cent respond to it. When the patients complain of a psychic aura or perceptual aberration, this is accompanied by a feeling of fear or nervousness. The feeling of fear or nervousness can be promoted and seems to serve as a wedge for suggestion on the development of the perceptual aberration. If the patient is asked if he is nervous, he may become nervous and may in turn develop his hallucination or illusion. Paradoxically, these patients do not respond well to hypnosis, which has been tried. In two cases, patients have complained of continuing illusions or hallucinations without other evidence of ictal process. These illusions or hallucinations had been previously noted as an aura or beginning of the habitual pattern. In these cases it was impossible to arrest the perceptual aberration by suggestion, but suggestion seemed to enhance the aberration.

Major Findings (cont'd):

In temporal lobe epilepsy, there is an ill-defined disturbance of body image. The patient does not have a usual appreciation of his own form. In the ictus, he often examines it or its appendages. In an attempt to define some of the characteristics of this aspect of perceptual aberration, patients have been asked to sketch or outline their own forms and faces. It appears that there is an unusual distortion of body image, if such a sample test is in any way reliable.

Most patients with temporal lobe epilepsy are said to have "memory difficulty". In the patients here, some time has been spent in further attempts to elucidate this difficulty. At present, it does not appear as a difficulty of memory. These patients have difficulty in relating in space and in time. This difficulty of relationship is evident in their language which does not use a normal quantity of substantives, and at its worst is a disconnected series of illogical relationships. One of these patients finds it extremely difficult to describe the form of an object. If he can achieve this description, it is often very difficult for him to relate the form of the object to its surroundings. Since these patients cannot achieve spatial and time relationships, they find it extremely difficult to record experience in coherent sequences readily available for recollection. Conversely, if they are provided with a clearly relevant sequence of events and objects and the relationship between the events and the objects is made quite clear to them, they can "memorize" the series with accuracy and often with excellence. Later they will recollect the sequence. However, if the relationship is not made clear to them, they cannot "remember" the sequence and they will complain of a "memory" difficulty.

Temporal lobe seizures often seem to occur in cycles. Thus a patient may be seizure free for thirty days and then suffer four or five seizures in a day. This cyclic characteristic may be related to some endocrine change. During this year we have begun to test the effects of estrogens and testosterone on seizure frequency. One patient who was receiving testosterone went into status during administration of the hormonal preparation. This effect may or may not be related to the administration. It is too early for any conclusions, but it seems valuable to pursue this aspect of the general investigation.

Patients undergoing spontaneous attacks of centrencephalic epilepsy are studied by simultaneous six-channel EEG recording and recording of blood pressure, skin temperature, heart rate, skin resistance, plethysmogram, esophogeal and gastric motility and respiration. They are subjected to continuous response testing which is also simultaneously recorded in order to define as closely as possible the periods of loss of consciousness. (This study is carried out with Dr. Allan Mirsky, NIMH.)

Major Findings (cont'd):

To date only five patients have been subject to these studies. The finding found typical of the petit mal "absence" consists of expiratory apnea which may or may not be associated with tachycardia and fall of skin resistance. In general, the findings were much less striking than those attending spontaneous automatisms of temporal lobe origin which were studied in the previous year (see Calendar Year 1957 "Epileptogenic Mechanisms in the Brain of Man"). Bursts of 3/sec. spike and wave activity or irregular polyspike and wave activity bilaterally synchronous and symmetrical in the frontal regions may appear without alteration in the patient's motor response to visual stimuli or autonomic change. Changes in both the latter features tend to appear with longer epileptic bursts, but still the degree of interference cannot be predicted from the appearance of the electrographic trace alone. For example, a 3/sec. spike and wave burst of comparable length and voltage may produce prolonged expiratory apnea and later produce very little change in the respiratory rhythm. Motor responses have been observed during spike and wave discharges though they are usually absent when the patient is apneic. In one instance when the patient firmly claimed to have recall during his spike and wave seizures, although he "could not move", he was never able to recall letters or colors shown to him. During the latter part of the spike and wave episode, however, without obvious electrographic change, he on occasion was able to respond correctly to visual stimuli.

Results to date indicate that this is a complex problem for correlation and will require considerable clinical material.

In the laboratory, the spread of penicillin-induced seizures has been observed as it occurred over the surface of both hemispheres. Thus, after simultaneous exposure of both cerebral hemispheres in the chimpanzee, a penicillin lesion was created in one or the other temporal cortex. Electrocorticographic recording over the surface of the cortex of both hemispheres seemed to indicate that once the seizure process spread outside the temporal lobe, it was first evident in the parasagittal region on the side opposite to the involved temporal lobe. It is probable that this spread is through subcortical structures and is mediated by transcortical connections. As the seizure discharge spreads across the cortex, it is preceded by discernible vascular change. The cortex darkens, the veins become injected, and the arteries constrict. This observation raises the old question of the significance of vascular change in the development and spread of an epileptic seizure. If such a vascular change occurs in both hemispheres as it does following widespread epileptiform activity, both hemispheres are subject to severe, and occasionally critical, edema. In one instance, this edema could not be relieved by injection of hypertonic solutions and postural

Major Findings (cont'd):
drainage. The dura could not be closed until one frontal lobe was amputated. This provides some demonstration of the potential severity of the postictal phenomena in the production of brain damage and subsequent neurological sequels.

Significance to Neurological Research: These observations may contribute towards further understanding of epileptic mechanisms as they occur in temporal lobes of higher primates.

Proposed Course of the Project: The various clinical and experimental studies will be continued. Both the clinical and experimental investigations will be strengthened by studies of catechol amines.

Part B included: Yes [X] No [ ]

Serial No. NINDB-61(c)

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Baldwin, M., Bailey, P.: Temporal Lobe Epilepsy Springfield, Chas. C. Thomas, 1958, pp. 581.

Baldwin, M.: Notes on the history of American military neurosurgery. In Meirowsky, A. M. (ed.): Trauma of Central and Peripheral Nervous System. (in press)

Hall, K., Baldwin, M., Norris, F.: Succinylcholine in awake craniotomy. Anesthesiology (in press)

Serial No. NINDB-62(c)

1. Surgical Neurology Branch
2.
3. Bethesda, Maryland
4. NINDB-26(c)

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Functional Representation in the Temporal Lobe of Man and Higher Primates.

Principal Investigator: Maitland Baldwin, M. D.

Other Investigators: John Van Buren, M. D., Shirley Lewis, R. N., and Sven A. Bach, M. D.

Cooperating Units: None.

Man Years (Calendar Year 1958):

Total:	.25
Professional:	.25
Other:	.25

Patient Days (Calendar Year 1958): 645

Project Description:

Objectives: To further understanding of functional representations within the temporal lobe of man and higher primates.

Methods Employed:

1. Electrical stimulation and recording of the human, chimpanzee and monkey temporal lobes: (a) directly, after operative exposure; (b) indirectly by depth electrodes and scalp recordings.
2. Ablation of all or parts of the temporal lobes.
3. Anatomical studies of whole brain after temporal lobe excisions.

Major Findings: During the past year the electrical stimulation of human and other higher primate temporal lobes has continued. In the results obtained from the human operating room, interest has been focused on the so-called psychical responses. The majority of these responses in recent cases have come from depth stimulation, yet previously the majority seemed to come from stimulation of the cortical mantle. In the Montreal series, almost all the psychical responses are

Major Findings (cont'd):
derived from cortical stimulation, yet in Falconer's series all these responses come from stimulation of the depth. The experience here seems to indicate that the response may come from either the surface or the depth. Obviously these are complicated reactions. The patient is telling us of a complex perceptual aberration or in some of the more "fashionable" responses, the patient tells of a memory or dream. It is doubtful if such a complicated response should be entirely relevant to either the cortex or the deep structures. It is more likely that the functional relationships include cortex and subcortical structures of both sides in a functioning unit.

Approximately 200 positive responses have been obtained by stimulation of the chimpanzee cortex. The majority of these are relevant to what Sherrington called 'excitable cortex'. Thus they are movements. Among these responses are many which were obtained through similar stimulation of the cortex on both sides. When the cortex is stimulated simultaneously so that right and left motor areas are activated in concert, either right or left extremity, face, or other peripheral part may move. The brain seems to select the precedence of activity which follows bilateral simultaneous stimulation. Passive movement of an extremity influences the effect of this type of stimulation. When the hand is clenched, stimulation of representation for fingers usually results in extension, whereas when the fingers are extended, stimulation of cortical representation is usually followed by flexion. Perhaps passive movement of the extremities on the one side influences response of extremities on the other. There may be a contralateral inhibitory process. Such passive movement does not seem to alter the spread of epileptiform discharge from the temporal cortex even though this spread is not coincident with ictal movements. Electrical stimulation of the post-central cortex evokes movements. These movements are not abolished by excision of the ipsilateral motor cortex.

Perhaps electrical stimulation of the mesial temporal structures is followed by increased secretion of catechol amines. This seems a reasonable supposition when one reviews movies of a chimpanzee taken when he is undergoing such stimulation. The autonomic phenomena which are concomitant with such stimulation are strikingly similar to those which follow an injection of adrenalin. In order to test this supposition, various experimental designs are being constructed. The first of these followed the model of Cannon for bio-assay of "excitable blood". More recently, lactic acid has been used as an indicator of increased catechol amines. Dr. Norris is composing an elaborate experimental design which includes capability for mesial temporal stimulation, extradural recording, systematic polygraphic recording, and biochemical assay. This should provide some further information on the relationship between epileptiform discharge in mesial temporal structures and secretion of catechol amines.

Major Findings (cont'd):

The study of ablation preparations continues. This year marks the fourth in which observation of the effects of bilateral temporal ablation has been possible. Four years after bilateral temporal lobectomy, the chimpanzee is readjusting socially and does not show any of the acute or immediate stigma which were reported previously. The animal remains more placid than his contemporaries, but this placidity is slight and it is impossible for an untutored observer to differentiate between the operated and unoperated animals. Such an animal after bilateral temporal lobectomy regains his place in the social hierarchy and seems to continue a normal sexual, play, feeding and learning development. On the other hand, four years after bilateral frontal lobectomy, a similar animal does not regain his place in the hierarchy and his individual and social habit patterns remain remarkably abnormal. There is a rapid recovery from destruction of mesial temporal structures. This takes approximately two months. As a corollary to such preparations, a 'Weber' syndrome was created in one animal who was ready for sacrifice. This syndrome was a reasonable portrayal of the syndrome as described in human patients. It demonstrated one of the (surgical) risks of temporal lobectomy.

Mesial temporal lesions affect communication in the chimpanzee for approximately four weeks after their creation. Recently, lateral temporal and parasylvian lesions have been created in an effort to determine their relationship to communication patterns. At present two such animals are available for study. In these, excisions were made in the area comparable to Broca in man. One is on the left side; the other on the right side. The animals are ambidexterous. Such lesions do not interrupt or obviously change vocalization, hand, upper extremity and face communication patterns.

The hallucinogenic substances which are contained in the Mexican mushroom do not affect the chimpanzees whose temporal lobes have been removed, yet these substances affect the normal chimpanzee so as to make him tame, relatively unaware of his surroundings, and somewhat ataxic. As is the case following lysergic acid administration, the temporal-lobectomized chimpanzee fails to respond to the psylicibin compounds of the Mexican mushroom.

During this year it has been established that the temporal lobe of the chimpanzee is relevant to the syndrome reaction which occurs when he receives lysergic acid. Moreover, it is apparent that the lateral temporal cortex is the significant element in the neurological chain which forms the background of this reaction. For the chimpanzee whose mesial temporal structures are removed reacts to the drug. However, his contemporary whose lateral temporal cortices have been removed fails to respond to the lysergic acid. As has been previously stated, removal of the frontal cortex does not affect this reaction.

Major Findings (cont'd):

Three brains of chimpanzees who have undergone temporal lobectomies are being studied in neurosurgical anatomy by Dr. Van Buren. In addition, he reports on the reconstruction of two human temporal lobe defects carried out at the Harvard Anatomical Museum with Dr. Paul I. Yakovlev. One case consisted in anterior temporal lobectomy for epilepsy. The contrasting case consisted of an infarction which involved the most posterior extremity of the sylvian fissure centering upon the temporo-parieto-occipital junction. With the anterior temporal lesion, nuclear degeneration appeared in the inferior and lateral portion of the pulvinar, the posterior portion of the medial geniculate body and the lateral portion of the lateral geniculate body. With the posterior temporal lesion the degeneration appeared in the middle and posterior portions of the pulvinar, the anterior portion of the medial geniculate body and the medial portion of the lateral geniculate body. There was also thinning out of cells of the posterior portion of the nucleus medialis dorsalis. Thus, the antero-posterior reversal of the auditory representation from auditory cortex to medial geniculate body has been noted and there is suggestion of dorsal ventral orientation of the projection of the pulvinar to the temporal cortex. The pars oralis of the pulvinar showed no degeneration in either case. Tract degeneration studies showed several points of interest. The stria terminalis in man appears to arise from the cortical and medial accessory basal nuclei of the amygdala since it remained intact when the lateral portions of the amygdala were destroyed by surgery. The anterior commissure was nearly entirely degenerated suggesting that the retained medial portions of the amygdala and region of the uncus received very little projection from the anterior commissure. A pathway between the amygdala and the brainstem which has received very little notice was found which passed mesially below the caudate nucleus through the substantia inominata, over the optic tract and downward in the lateral-most one-fifth of the cerebral peduncle. It could be followed as low as the upper pontine region. This degeneration could be followed easily on the Nissl sections by gliosis but only with difficulty on the myelin preparation. This apparently indicates an intermingling of normal fibers although there seems no doubt that the anterior temporal region provides fibers to Türck's bundle. Myelin degeneration in Türck's bundle of the posterior temporal region was sharp and well defined. In the anterior temporal region, a well-defined gliosis could be followed into the brachium of the inferior colliculus but neither of the brachia of the colliculi appeared degenerated with the posterior temporal lesion.

Significance to Neurological Research: These observations contribute to the further understanding of functional representation of the primate temporal lobe. Such understanding is in its way a contribution to knowledge of the structural basis of such abstract functions as perception, memory, hallucination, as well as the more discernible functions of the autonomic system which find correlates in the temporal lobe.

Proposed Course of the Project: This project is developing because of information derived from electrical, surgical, anatomical, and biochemical studies. The relationship of the mesial temporal structures to biochemistry of catechol amines must be clarified and further steps in the study of hallucinogenic substances may provide some clue as to this intricate chemical and physiological relationship, since the majority of these chemicals are related to the adrenalin compounds.

Part B included: Yes [X] No [ ]

Serial No. NINDB-62(c)

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Frost, L. L., Baldwin, M., and Wood, C. D.: Investigation of the primate amygdala: Movements of the face and jaws. Neurology, 8, No. 7; 543-546, 1958.

Serial No. NINDB 63 (c)
1. Surgical Neurology Branch
2.
3. Bethesda, Maryland
4. Same as NINDB 1957 26(c)

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project title: Effect of Tumors upon the Central Nervous System Function and Structure

Principal Investigator: J. M. Van Buren, M. D.

Other Investigators: Maitland Baldwin, M. D.

Cooperating Units:

Man Years (Calendar Year 1958):		Patient Days (Calendar Year 1958):
Total:	.25	603
Professional:	.25	
Other:	.25	

Project Description:

Objective: This study has a dual aim: (1) to carry out physiological-anatomical correlations in man, (2) to evaluate the effects of newer methods of treatment for 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:

In conjunction with the Branch of Endocrinology, NCI under the direction of Dr. Delbert Bergenstal, a study has been made of the quantitative anatomical and endocrinological evaluation of graded hypophysectomy in man. Thirteen cases formed the basis of this study. From the serial sections of the sella volumetric

estimation was made of the size of the retained pituitary fragment and differential cell counts were made in this fragment post mortem. This finding was correlated in each case with the patient's clinical course and the response of the thyroid and adrenal function, the level of gonadatrophins and the presence or absence of diabetes insipidus.

Between 160 mm.^{3} and 0.3 mm.^{3} of pituitary tissue were left in the sella in the 13 cases. Initially after surgery and for a period extending up to 3 months, there was profound depression of thyroid and adrenal activity, and the gonadatrophin levels fell to negligible figures. It was during this time that tumor remission might occur and this was seen in about 50% of the cases. The most striking feature was that the presence of tumor remission and evidence of severe hypopituitarism was present in all cases without regard to the amount of pituitary tissue remaining in the sella. Thereafter, in the case retaining 160 mm.^{3} of pituitary (which incidentally had a very definite objective tumor remission) thyroid and adrenal function returned to normal, although throughout the patient's 16 month postoperative course the gonadatrophin levels remained near the vanishing point. Thus, there is strong suggestion that depression of the individual trophic pituitary hormones is not the same for all the trophic hormones. The one feature common to all cases was surgical section to the pituitary stalk and this may indeed be the essential feature.

The need for posterior pituitary extract to control diabetes insipidus might or might not be present but this could not be correlated in any way with the amount of pituitary tissue remaining in the sella.

In 5 cases where the cholesterol values were followed in the post-hypophysectomy period the maximum rise of the cholesterol values (presumably an index of decreased thyroid function) were seen between one and three months following surgery, then all the values began to fall toward normal limits. Curiously enough, this initial rise did not seem related to the volume of pituitary remaining in the sella nor did the eventual fall appear to be so related since quantities of pituitary under 3 cu. mm. might be associated with such a fall. Interpretation of this finding is somewhat difficult since in liver disease from which most of these patients suffered, a spontaneous fall in cholesterol may appear.

Histological features of interest showed a uniformly slow rate of chromophile cells both of the alpha and beta types (10,000 cells counted per case). This finding was interpreted as degranulation of the chromophile cells to increased demand for pituitary hormones. The pharyngeal pituitary gland was examined

in 6 cases of the 13. The measurements of the pharyngeal pituitary all lay within the lower limits of normal and not the slightest evidence of secondary hypertrophy was seen despite the claims of some European investigators.

This study is now in manuscript form and will be submitted to the Journal of Neurosurgery.

During the present calendar year tumor cases have provided the post mortem material for studies of the visual system.

Significance to Neurological Research:

The present study on hypophysectomized patients has provided some basic knowledge regarding the reason for effectiveness of hypophysectomy in the treatment of metastatic tumors of the breast. The curious finding that the clinical results of incomplete hypophysectomy were apparently as good as those of complete hypophysectomy seems explained by the profound depression of pituitary hormonal output which seems nearly independent of the amount of pituitary tissue removed. The differential response of the various trophic hormones to pituitary injury had not been previously confirmed in man with anatomical control.

The importance of this project in providing valuable anatomical material for further study of the human visual system should be emphasized.

Proposed course of project:

Using the lead provided by the above study, the operation has now been changed to a simple section of the pituitary stalk without removing any pituitary tissue. The effect upon the individual's endocrine status and tumor will be evaluated in another dozen cases. At present 4 such cases have been carried out. It is possible that later in the course of these patients the area will be re-exposed and the pituitary removed. This will provide an additional facet for investigation.

The hypothalami in 7 of the hypophysectomized patients are being prepared in serial section for study of cell structure and neurosecretion. Whether this study will prove practical and fruitful is yet to be determined.

Part B included Yes [ ] No [X]

Serial No. NINDB 64 (c)
1. Surgical Neurology Branch
2.
3. Bethesda, Maryland
4. Same as NINDB 1957 38(c)

PHS-NIH
Individual Project Report
Calendar Year 1958

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 1958):

Total: .25
Professional: .25
Other: .25

Patient Days (Calendar Year 1958):

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.

Method Employed:

The methods employed have been given in detail in report for Calendar Year 1957.

Major Findings:

The preliminary findings have been given in the report for Calendar Year 1957 and will not be repeated. Since it has appeared desirable to confirm these findings in a greater quantity of material before publication, our efforts in the present year have been concerned primarily with the collection of more material. Specifically, material collected has been as follows:

(A) Retina: 8 cases, (2 chiasmal lesions, 3 papilledema, 2 normal, 1 amblyopia due to life long strabismus).

(B) Lateral geniculate body: 4 cases, (1 temporo-parietal infarct, 2 chiasmal lesions, and 1 enucleation of long duration).

(C) Study of the visual fields following temporal lobe defects. This study is now terminated. See reference given below.

Significance to Neurological Research:

The general aims have been previously given in the 1957 Calendar report. In brief, it has been considered desirable to re-examine the visual system in manby anatomical reconstruction studies which may be correlated with the clinical examinations of the field of vision.

Proposed course of project:

It is planned to use a projector (which has been under construction for the past fourteen months) for two dimensional reconstruction of the ganglion cell pattern in the retina. These reconstructions will be in terms of ganglion cell thickness and will be plotted with retinal distances equated to degrees of visual arc. In this way they will be readily comparable with the patient's visual fields, which in most cases, were obtained prior to death. The hypophysectomy material has provided a goodly quantity of normal material which is important for establishing a baseline.

The lateral geniculate study still suffers from insufficent cases so that collection will be continued.

Part B included [X] Yes [ ] No

Serial No. NINDB-64(c)

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B. Honors, Awards and Publications

Publications other than abstracts from this project:

J. M. Van Buren, M. D. and M. Baldwin, M. D. The Architecture of the Optic Radiation in the Temporal Lobe of Man. Brain, 81: 15-40, 1958.

Honors and Awards relating to this project: None

Serial No. NINDB 65 (c)
1. Surgical Neurology
2.
3. Bethesda, Maryland
4. Same as NINDB 1957 [illegible]

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project title: Studies of Involuntary Movements

Principal Investigator: J. M. Van Buren, M. D.

Other Investigators: Maitland Baldwin, M. D.

Cooperating Units:

Man Years (Calendar Year 1958):

Total: .25
Professional: .25
Other: .25

Patient Days (Calendar Year 1958):

8

Project Description:

Objective: At the present time there is no adequate explanation of the cause or 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 movement is being investigated. Whether this will prove to be a practical recording technique remains to be determined.

Major Findings:

The initial considerations were given in the calendar year report of 1957.

The actual testing of the stereotaxic instrument on cadaver material has been severely handicapped by the great difficulty in obtaining cadaver material at the NIH. After much negotiation we were permitted to carry out our first stereotaxic placement on a cadaver in May 1958 and since this time have been able to carry out our studies with only 5 cadavers. On the whole, the results have been encouraging, in that they have shown that the principle of the arcuate electrode carrier (please see explanation in previous annual report) is a sound one under practical operating circumstances and that the apparatus is mechanically accurate. The problem of obtaining good pneumography in the cadaver was eventually solved simultaneous ventricular and cisternal punctures then clearing the fluid from the ventricle by introducing air in the cisterna magna. The foramen of Monro has proved to be a useful zero point for the stereotaxic apparatus and initial localization errors have been corrected in later stereotaxic placements. Due to difficulties in aligning the present base-line of the stereotaxic instrument with the horizontal plane defined by the anterior and posterior commissure an increase in the antero-posterior tilting mechanism will have to be made by a small mechanical change. This failure of adequate tilting caused a number of the posterior lesions to be erroneously high.

The method described for preparation of our own brain atlas has proved economical in time and effort and appears more accurate than the use of paraffin embedding and myelin sections. Its limitation lies in the failure of finer details, particularly in the thalamus to be as evident. This, however, is not considered of major importance since gross estimation of the position of the lesion in the thalamus is easily achieved.

The use of various fixatives has been investigated. It was initially thought important to provide a fixative which would support the brain (in order that string suspension would not distort it) and which would not cause changes in weight or size of the brain. Consequently, all brains were measured both for weight and displacement at the time they were removed from the skull and at one and two week intervals thereafter. A solution made up of glycerin and formalin and water provided adequate support of the brain but caused excessive shrinking (over 3%). Thereupon mixtures of formalin, water and mercuric chloride were used which again supported the brain in an adequate fashion but it was found that the mercuric chloride

bleached the gray-white differentiation in such a degree that anatomical structures became difficult to distinguish on the photograph. We have finally returned to fixation of the brain in formalin by suspension from the vertebral artery and find that the degree of shrinkage reaches negligible figures in two weeks' time (1-2%) which is about the limit of accuracy of our method of estimation).

The effect of carotid profusion was investigated since it was thought that injection of formalin into the carotid system might produce swelling of the basal ganglia and thalamus since a complete wash through could not be achieved in the cadaver material, and therefore introduce error. In order to evaluate this, ventriculograms were carried out, the 100 cc. of formalin injected in one internal carotid artery, then ventriculography repeated. Small but definite distortions of the side injected appeared from this study so that we have abandoned the practice of carotid injection.

Significance to Neurological Research:

The present work has served simply to acquaint the principal investigator with the mechanical proficiency of his stereotaxic instrument and thus has dealt simply 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.).

Continued use of the stereotaxic instrument on cadaver material is planned and it is hoped that the quantity of material may increase in the future. When the investigator is satisfied with his proficiency in the use of this instrument, patients suffering from basal ganglia disease will be admitted for treatment by destruction of various portions of the pallidum or thalamus. During the course of this therapy, studies will be made of the areas to be destroyed by depth electrode recording and stimulation.

Part B included Yes [ ] No [X]

1. Surgical Neurology Branch
2.
3. Bethesda, Maryland
4. Same as NINDB 1957

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project title: Pain Mechanisms

Principal Investigator: J. M. Van Buren, M. D.

Other Investigators: Mildred Blevins

Cooperating Units:

Man Years (Calendar Year 1958):		Patient Days (Calendar Year 1958):
Total:	.10	211
Professional:	.05	
Other:	.05	

Project Description:

Objective: The essential interest of this study is centered upon methods for evaluation of pain with its ultimate goal being a quantitative evaluation of the pain from which the individual is suffering. Depending upon the degree to which the primary aim of the study is achieved the following studies can be undertaken: (1) evaluation of the standard surgical procedures for relief of pain, (2) evaluation of standard medical procedures for relief of pain, (3) the surgical approach to pain pathways may be expected to provide an opportunity for study. Surgical lesions of the central nervous system will be exploited as far as possible as the opportunity arises.

Method Employed:

(A) Psychometric Methods: The patient is subjected to Rohrsach test and the Minneapolis Multiphasic Personality Inventory. In addition, he is evaluated during a formal psychiatric interview.

(B) Autonomic function: Simultaneous records of blood pressure, skin temperature, electrocardiogram, skin resistance, respiratory rate pattern, finger plethysmogram and esophogeal and gastric pressures are made.

Major Findings:

In report for the previous calendar year of 1957 the findings have suggested that those patients complaining of pain which appeared more functional than organic in origin had unusually had unstable autonomic responses.

In the present year examinations have been continued in an attempt to correlate the degree of autonomic responsiveness with other features of the patient's clinical picture and the picture defined by psychometric testing. In sum, the results have been of practically no value. Autonomic responses to apparently the same pain stimulus varied from examination to examination on the whole tending to decrease as the patient becomes more used to the examiner and the testing situation. In sum, our failure in achieving any sound information on this study lies in our failure to achieve a stable response baseline which can be satisfactorily compared with a postoperative baseline (thus eliminating the factors of adaption), and of even more importance the failure to establish a baseline which may be compared from patient to patient in a group.

Significance to Neurological Research:

Any method which will quantitate a patient's pain in an objective fashion is obviously of the greatest importance in many spheres of research. Our use of autonomic recording seemed a possible lead but it has not proved fruitful.

Proposed course of project:

The formal study of this subject has been terminated.

Part B included Yes [ ] No [X]

Serial No. NINDB - 6 (c)
1. Surgical Neurology Branch
2. Primate Neurology
3. Bethesda 14, Maryland
4. NINDB-43 (c)

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Study of Cortical Intracellular Potentials.

Principal Investigator: Choh-luh Li, M. D.

Other Investigator: None

Cooperating Unit: None

Man Years (calendar year 1958):
Total: .25
Professional: .25
Other: .25

Patient Days (calendar year 1958)

NONE

Project Description:

Objective: Recently the dendrites were believed to generate electrical activity largely responsible for the potentials recorded from the surface of the cortex. There wasalso evidence from histochemical studies and tissue cultures that the glia element and the nerve cell should be considered as a functional unit. Thus the understanding of the activity of the different components or elements in the cerebral cortex based on physiological studies may throw some light on the function of the cortex.

Method Employed: Cats under light anesthesia were used. The intracellular potentials were recorded with glass micropipette electrodes. The response of potential were tested by local application of strychnine.

Major Findings: The intracellular potentials recorded from the cortex were found to be five in types. (1) Steady potentials of -62±9 mV unresponsive to afferent stimulation and local application of strychnine presumably originating from glia elements. (2) Large slow potentials presumably originating from dendrites. (3) Small potentials presumably from synaptic regions. (4) Brief spikes with an inflexion in the rising phase presumably recorded from cell bodies. (5) Simple brief spikes

from axons. These potentials differed not only in their size and time course, but also in their responses to strychnine. Strychnine showed no effect on the glia cells and axons but either depolarized or hyperpolarized the membrane of the cell body. It also appeared to enhance the activity of the small potential and suppressed the large slow potential. The results of this study also suggest that the mechanism of synaptic transmission in the central nervous system may be similar to that across the neuromuscular junctions. Further they also suggest that the importance of dendrites in the production of electrical activity of the cerebral cortex may be over publicized.

Significance to Neurological Research: This study identified different forms of intracellular potentials ascribed to different elements in the cortex and suggested that the spontaneous behavior and responses to stimulation are different from these different elements. It was also in these studies that depolarization and hyperpolarization of the cell membrane of the cortical neurones by strychnine were first reported. This observation suggests that there may be difference in metabolism of different nerve cells in the cerebral cortex. It was also in this study that small potentials similar to miniature end plate potentials were described indicating that the mechanism of synaptic transmission in the central nervous system and in the neuromuscular junction may well by the same.

Proposed Course of Project: The small potentials recorded intracellularly from the cortex will be further investigated. This may yield to some understanding of the action of the anesthetic agents which are known to block either monosynaptic or polysynaptic transmissions. The study of the large slow potentials presumably recorded from the dendrites and the spontaneous oscillations of potentials recorded from cell membranes will be continued. Finally the action of convulsive drugs and acticonvulsive drugs will be tested with the simple method described above.

Part B included Yes [X] No [ ]

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Li, Choh-luh: Cortical Intracellular Potentials and their Responses to Strychnine. J. Neurophysiol. (in press). 1958

Honors and Awards relating to this project:

1. [illegible]
2. [illegible]
3. [illegible]
4.

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A:

Project Title: Factors Determining the Discharge of a Motor Neuron in Cerebral Cortex.

Principal Investigator: Choh-luh Li, M. D.

Other Investigator: None

Cooperating Unit: None

Man Years (calendar year 1958)
Total: .25
Professional: .25
Other: .25

Patient Days (calendar year 1958)
None

Project Description:

Objective: To study the activity of nerve cells in cerebral cortex [illegible] to changes of the external and internal environment.

Methods Employed: Cats either under light anesthesia or d-tubocurarine [illegible] used. The activity of nerve cells in motor cortex was recorded with [illegible] pipette electrodes while electrical stimulation was applied to various [illegible] cortical structures and peripheral sensory nerves. The cells which are intimately related to motor function have descending axons to the medullary pyramid and were identified by their responses to antidromic stimulation, and those in the motor cortex which do not have descending axons were identified as internuncial cells.

Major Findings: As previously reported stimulation of the nucleus ventralis lateralis of the thalamus activates the cells with descending axons and suppresses the activity of the internuncial cells, suggesting that this thalamic nucleus may have some control over the motor activity of the experimental animal. It was also found that the internuncial cells in the motor cortex could be influenced by the sensory volley set up at the periphery. The sensory volley, at times was also capable of exciting a motor neurone in the cerebrum. Furthermore, it was not infrequent to observe that the sensory volley may inhibit or facilitate the discharge of a cortical motor cell. This study also demonstrated that the refractory periods of the pyramidal fibers varied from 1.5 to 2.5 milliseconds and conduction velocity from 8 meters to 95 meters per second.

Significance to Neurological Research: The above observations [illegible] emphasized the role of subcortical structures and external stimuli in the function of motor activity. It may be said that while the motor cortex is immediately concerned in the initiation of movement, influences from internal or external sources on the activity of motor cells cannot be overlooked. This study provides direct evidence that these influences indeed exist.

Proposed Course of Project: Further studies of the relationships between the activity of other subcortical structures such as the corpus striatum, the red nucleus, the vestibular, reticular and subthalamic nuclei as well as the cerebellum and of the motor neurones in the cerebral cortex and in the spinal cord may yield valuable information about the mechanism of motor function.

Part B included Yes [x] No [ ]

Serial No. [illegible]

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards and Publications

Publications other than abstracts from this project:

Activity of Interneurons in the Motor Cortex
International Symposium. Recticular Formation
Henry Ford Hospital, Detriot. Little, Brown
and Co., 459-272, 1958.

Li, Choh-luh. Some Properties of Pyramidal Neurones
in the Motor Cortex with Particular Reference to Sensory
Stimulation. J. Neurophysiol. (in press). 1958

Honors and Awards relating to this project:

Serial No. NIMDB - 59
1. Surgical Neurology Branch
2. Primate Neurology
3. Bethesda 14, Maryland
4.

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: The Problem of Synchronous Activity of Nerve Cells in Cerebral Cortex

Principle Investigator: Choh-luh Li, M. D.

Other Investigators: None

Cooperating Unit: None

Man Years (calendar year 1958):
Total: .25
Professional: .25
Other: .25

Patient Days (calendar year 1958): NONE

Project Description:

Objective: Since the statement made by Adrian in 1935 it has been generally accepted that the activity of nerve cells in the cerebral cortex were synchronous when the subject was at rest or when the cortex was synchronously activated. And if the subject was alert the discharge of cortical cells was said to be "dys-synchronized". There has been, however, no direct evidence in support. The present study is attempt to test this hypothesis.

Method Employed: The activity of a cortical cell was recorded with a micropipette electrode while that of the others was studied with another microelectrode.

Major Findings: In general the notion proposed by Adrian was given support by direct evidence with the following reservations: (1) Only very few nerve cells in a sphere of 1 mm in the cerebral cortex would discharge precisely at the same instant. (2) A synchronous volley evoked discharges of nerve cells with a temporal descrepancies varying from 2 milliseconds to 20 milliseconds. (3) Application of strychnine activate about 85% but not all of the nerve cells. (4) A temporal relationship between neuronal activity still exist in "aroused" cortex.

Significance: It has been said that the neurons in an epileptogenic cortex tend to fire in unison and neurons in normal cortex of an alert subject randomly discharge. The present study demonstrated that this is a generalization with certain degree of truth, based on logical thinking but not on facts. The methods of simultaneous investigation of the activity of different nerve cells may provide additional information about the integration function of the central nervous system.

Proposed Course of Study: Multiple recording with microelectrodes from single nerve cells will be used in the study of epileptic activity of the cerebral cortex as well as factors determining the discharge of motor cells in cortex.

Part B included Yes [X] No [ ]

Serial No. NINDB-[illegible]

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards and Publications

Publications other than abstracts from this project:

Li, Choh-luh Synchronization of Neuronal Activity in Cerebral Cortex. Science. (in press).

Honors and Awards relating to this project:

Serial No. NINDB-50 (c)
1. Surgical Neurology Branch
2. Primate Neurology
3. Bethesda 14, Maryland
4. NINDB-41 (c)

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Neuromuscular Transmission in Hypothermia

Principal Investigator: Choh-luh Li, M. D.

Other Investigators: None

Cooperating Unit: None

Man Years (calendar year 1958):
Total: .25
Professional: .25
Other: .25

Patient Days (calendar year 1958): None

Project Description:

Objective: To study the performance of neuromuscular junction in mammals at low temperatures as compared to that in the amphibia. The latter was reported last year with the collaboration of Dr. Peter Gouras.

Method Employed: The anterior gracilis muscle and the obturator nerve of the rat were exposed and the miniature endplate potentials, endplate potentials, action potentials and resting potentials were recorded while the animal was subjected to various temperatures between body temperature and -4°C. At these temperatures electrocardiograms of the animal ware also taken.

Major Findings: As in the frog there were also a critical body temperature below which action potentials of the muscle in response to obturator nerve stimulation became less frequent. This was 15°C. At about 4-5°C action potential failed and there were only endplate potentials elicitable by nerve stimulation. The miniature endplate potentials could be recorded by body temperature as low as 4°C but not below. The resting membrane potentials showed no significant change at temperatures between body temperature and 10°C, below which they began to fall, and at body temperature of 0°C no resting potential was recorded. Furthermore, during the process of cooling some muscle fibers became spontaneously active with discharges of fibrillation potentials.

Significance of Neurological Research: This study indicates that there is a critical body temperature in mammals below which the transmission of impulses across the neuromuscular junction become impeded and if the temperature is further lowered to 4°C transmission is blocked. This observation may be of some use in processes involving hypothermia which are to be carried out in the laboratory or in the operating room for human patients.

Proposed course of Project: In the future similar experiments will be conducted with inquiries into the action of some neuromuscular drugs. The preparation described above is found to be most suitable for this type of investigation with intracellular microelectrodes; since at low temperatures twitch movement of the muscle was reduced and anesthesia was not required, yet miniature endplate potentials, action potentials and resting potentials could be readily recorded.

Part B included Yes ☒ No ☐

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Li, Choh-luh: Effect of Cooling on Neuromuscular Transmission in the Rat. Amer. J. Physiol. 194: 200-206, 1958.

Honors and Awards relating to this project:

Serial No. NINDB - 71
1. Surgical Neurology Branch
2. Primate Neurology
3. Bethesda 14, Maryland
4.

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Effect of Cooling on Conduction of Impulses in Cranial and Peripheral Nerves.

Principal Investigator: Dr. A. Ortiz, M. D.

Other Investigator: Dr. Choh-luh Li, M. D.

Cooperating Units: None.

Man Years (calendar year 1958):
Total: .10
Professional: .05
Other: .05

Patient Days (calendar year 1958): None

Project Description:

Objective: Experiments and surgical procedures designed to abolish functions of the nervous tissue have been primarily performed either by ablation or electrolysis. It is thought that extreme low temperature locally applied to the tissue may have similar results without other undesirable complication

Method Employed: A small segment of the optic nerve and the sciatic nerve were subjected to -150°C for 30 seconds. The animals were than kept for 1 day - 4 months and the impulse conduction was tested at various intervals.

Major Findings: This set of 7 experiments was initiated only 3 weeks when this report was submitted. Results obtained should be considered inconclusive and will be reported at a later date.

Significance to Neurological Research: When all the dates are assembled information about functional interruption and functional recovery subsequent to cold may be of some significance in further improvement of the operative techniques presently employed.

Proposed Course of Project: This study will be continued and may be extended from nerve fibers to nerve substance.

Part B included Yes [X] No [ ]

Serial No. NINDB - 72
1. Surgical Neurology Branch
2. Primate Neurology
3. Bethesda 14, Maryland
4.

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Study of Pharmaceutic Agents Acting on Various Cortical and Subcortical Structures of the Brain.

Principle Investigator: A. Ortiz, M. D.

Other Investigators: M. Baldwin, M. D. and Choh-luh Li, M. D.

Cooperating Unit: None

Man Years (calendar year 1958):
Total: .10
Professional: .05
Other: .05

Patient Days (calendar year 1958):
None

Project Description:

Objective: Since the introduction of 5-Ht, LSD-25, LSD interest in research of mental disorders has been greatly enchanced, yet little has been known about the underlying neurological mechanisms of their effects. Recently a certain specis of mushrooms found in Mexico was found also to cause similar results. This study is designed to investigate which cerebral structure is most effected and how these agents would alter the electrical activity and responses of the nervous tissue.

Method Employed: Cats and monkeys were used. Multiple electrodes, which were also capable of injecting minute quantities of the testing chemicle agents, are inserted into the various deep structures of the brain. Recording of electrical activity and responses to stimulation from these structures and from cortical surface were made.

Major Findings: The results, though interesting, were still inconclusive for a conclusive statement to be made.

Significance to Neurological Research: to be seen

Proposed Course of Project: This study will be continued.

Part B included Yes ☐ No ☒

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.
Project Title: Properties of Cultured Nerve and Muscle Cells

Principal Investigators: Choh-Luh Li, Igor Klatzo and [illegible]

Other Investigators: King Engel

Cooperating Unit: None

Man Years (calendar year 1958):
Total: .10
Professional: .05
Other: .05

Patient Days: (calendar year) None

Project Description:

Objective: Spontaneous electrical potentials and potentials in response to electrical stimulation recorded from single elements in the central nervous system has been carried out in the past without the advantage of direct vision. The previous experiments also were subjected to various uncontrollable factors, e.g., presynaptic random bombardment and anesthesia. With the establishment of a tissue culture laboratory, it seemed to offer an opportunity to study the unit property of the nerve elements in isolated form under controlled external environment. It was also desirable to study various epileptic and anti-epileptic agents acting on the nerve cell membrane. Furthermore agents presumably effecting the neuromuscular junctions may be tested on culture muscle cells without endplate organs. Finally the presence of an electrically excitable membrane of brain tumor cells may be disclosed. It was also planned that with a similar method study the epileptogenic tissue removed from patients could be investigated. This project is therefore a long term proposition and the results will have to depend upon the techniques to be developed.

Methods Employed: In order to test the methods intracellular recording from heart muscles, skeletal muscles and spinal ganglia of 2-3 week old chick embryo were carried out. The results were comparable to those obtained from adult rats. With this assurance experiments were performed in skeletal muscle cells and spinal ganglion cells after growing 7-21 days in tissue culture. The culture technique was essentially the same as that described by Murray, Bornstein and P merat and the recording stimulating methods were similar to those used by Li and McIlwain.

Major Results: The observations obtained from the spinal ganglion cells resembled those from the nerve cells of the cerebral cortex and spinal cord. The results of cultured chick muscles could be summarized as follows: (1) Cells with slow responses might remain inactive after excitation for as long as 4.2 seconds and take no part in the initiation of spontaneous rhythmic spike discharges. (2) Cells with twitch responses had a refractory period ranging from 25 to 35 milliseconds and were responsible for the spontaneous rhythmic spike discharges. The spikes generated from these cells might be as large as 100 mV and 2.0 msec. (3) Resting membrane potentials (66 ± 5 mV) showed no significant difference in cells with twitch and slow responses, nor was there any change with age of the cells from 7 to 21 days. (4) Spontaneous rhythmic oscillation of potential could occur in the absence of spike discharges; but having attained a critical level of depolarization, they initiated spike discharges. The spike discharges did not interfere with the rhythm of the oscillating potentials.

Significance to Neurological Research: In the experiments with spinal ganglion cells it appears that the results may also be applicable to nerve cells of mammals. The results from chick cultured muscles are similar to those found in denervated mammalian skeletal muscles and suggest that the mechanisms of the fibrillation potentials in both cases are similar. Furthermore, the two types of responses suggested a differentiation of function being present in embryonic muscle cells.

Proposed Course of Project: A co-relation of the development change and psychological function of the muscle will be studied. Investigations on the action of acetylcholine, ClO, curarine, etc., on cultured mammalian muscles will be carried out. Study of the spinal ganglion cells and other nerve cells, tumor cells and epileptogenic cells are planned.

Part B included Yes [X] No [ ]

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Li, Choh-lun, Klatzo, I., Baldwin, M., and Engel, K.
Properties of Cultured Nerve and Muscle Cells.
J. Comp. Neurol. (in press).

Honors and Awards relating to this project:

National Institute of Neurological

Diseases and Blindness

Clinical Research

Surgical Neurology Branch

Section on Clinical Neuropathology

Serial Numbers of Projects:

NINDB-74(c), NINDB-75(c), NINDB-76(c), NINDB-77(c), NINDB-78(c), NINDB-79(c), NINDB-80(c), and NINDB-81(c).

Estimated Obligations for FY 1959

Total: $119,500

Direct: $55,200

Reimbursement: $64,300

Serial No. NINDB - 74 (C)

1. Surgical Neurology Branch
2. Clinical Neuropathology Section
3. Bethesda, Maryland
4. New

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Pinocytosis of Labelled Proteins in Tissue Culture.

Principal Investigator: Igor Klatzo, M. D.

Other Investigators: W. K. Engel, M. D. and J. Miquel, Ph. D.

Man Years (calendar year 1958):
Total: .25
Professional: .25
Other: .25

Patient Days (calendar years 1958): 0

Project Description:

Objectives: Pinocytosis or "drinking by the cells" is a phenomenon which has been reported by a number of workers in tissue culture. Its intrinsic role in cell metabolism has been suspected; however, these assumptions are based only on phase-contrast observations of intracytoplasmic vacuole formation. By labelling the proteins with fluorescent component and feeding cultures with these labelled proteins it should be possible to demonstrate the uptake of various proteins by living cells and follow their metabolic fate. The differences between individual cell types could be demonstrated in this respect. By changing environment of the cultures influence of various factors (pH, temperature, chemical substances, etc.) on the cellular protein metabolism could be studied.

Methods Employed: New-born kitten and rat cerebellum was grown in vitro. Cat serum albumin and rabbit serum globulin were labelled with fluorescein isothiocyanate. Cultures were "starved" for three hours receiving only balanced salt solution and consequently fed with labelled proteins in concentrations corresponding to their usual content in the medium. After washing for different periods of time, in balanced salt solution the cultures were observed under the fluorescence microscope.

Major Findings: Our preliminary findings indicate that it is possible to demonstrate protein uptake by living cells grown in vitro. A significant difference in metabolism of proteins by various cellular elements has been observed. Cultures washed for a brief period of time after feeding showed abundant labelled proteins in the macrophages and only few fluorescent droplets in the glial elements. Cultures washed for several hours in balanced salt revealed abundant green fluorescent droplets in glial cells, whereas, the macrophages showed mostly autofluorescence of various lipid substances. Also, some differences between behaviour of albumins and globulins have been noted.

Proposed course of the project: It is proposed to continue this investigation in order to accumulate more information along the lines mentioned in the statement about the objective of this project.

Part B included: Yes [ ] No [x]

Serial No. NINDB - 75 (C)
1. Surgical Neurology Branch
2. Clinical Neuropathology Section
3. Bethesda, Maryland
4. NINDB 35 (C)

PHS-NIH
Individual Project Report
Calendar Year 1958

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 E. W. Emmart, M. D.

Cooperating Units: NIAMD-E. W. Emmart, M. D. Project No. NIAMD (62303)31.

Man Years (calendar year 1958):
Total: .25
Professional: .25
Other: .25

Patient Days (calendar year 1958): 762

Project Description:

Objectives: The morphological localization of myosin in striated muscle was studied using fluorescent antibody technique. Information derived from the study of the normal muscle was used as a base-line for the observations on the behaviour of myosin in muscle affected by various neuromuscular disorders. Supplementary information was derived from study of the experimental muscle lesions in the rabbit and of the embryonic chick muscle grown in tissue culture.

Methods Employed: Coons' fluorescent antibody technique was applied for this study. The rabbits were immunized with human myosin and the obtained globulin fraction of antisera was labelled with fluorescein isothiocyanate. Muscle biopsies

Methods Employed (continued):
from the patients and experimental animals were stained with fluorescent antibody and examined in the fluorescence microscope. The embryonic chick muscle was grown in the Maximow slides and studied on consecutive days in the phase-contrast polarized light and by staining with fluorescent antibody.

Major Findings: In the normal muscle the specific staining for myosin was observed in A band, I and M bands appearing unstained and Z band showing occasionally non-specific autofluorescence. Study of various pathological processes in human muscle revealed a striking persistence of antigenic reactivity of myosin in the fibers with far advanced degeneration. Regenerating fibers observed in cases of polymyositis and experimental muscle injury showed similar features to those muscle fibers grown from the chick embryo. In acute muscle injury and in a few cases of polymyositis occasionally few macrophages contained green-fluorescent inclusions in their cytoplasm. This observation may be of importance for the interpretation of the possible mechanism of hypersensitivity due to release of muscle proteins.

Proposed course of the project: This project is completed.

Part B included: Yes [x] No [ ]

Serial No. NINDB 75 (C)

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards and Publication

Publication other than abstracts from this project:

"Demonstration of Myosin in Human Striated Muscle by Fluorescent Antibody".

Igor Klatzo, M. D., Beni Horvath, M. D. and E. W. Emmart, M. D.

Published in the Proceedings of the Society for Experimental Biology and Medicine, 1958, Vol. 97, 135-140.

Honors and Awards relating to this project:

Serial No. NINDB - 76 (C)

1. Surgical Neurology Branch
2. Clinical Neuropathology Section
3. Bethesda, Maryland
4. NINDB 37 (C)

PHS-NIH
Individual Project Report
Calendar Year 1958

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.

Man Years (calendar year 1958):
Total: .25
Professional: .25
Other: .25

Patient Days (calendar year 1958): 0

Project Description:

Objectives: Investigation of pathological changes in Kuru Disease affecting the Fore people of New Guinea was undertaken in 14 cases in which brains and other tissues were available for study.

Methods Employed: Established histological and histochemical techniques were employed for this study.

Major Findings: The main pathological findings in Kuru were confined to the central nervous system and they consisted of: (1) Widespread neuronal degeneration. (2) Myelin degeneration affecting predominantly cortico-spinal and spinocerebellar tracts. (3) Intense and widespread astroglial and microglial proliferation. (4) Perivascular cuffings with mononuclear elements. (5) Presence of peculiar plaque-like bodies in half of the cases studied.

Proposed course of the project: This project is completed.

Part B included: Yes [x] No [ ]

Serial No. NINDB - 76 (C)

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards and Publication

Publication other than abstracts from this project:

"Pathology of Kuru", Igor Klatzo, M. D., D. C. Gajdusek, M. D. and V. Zigas, M. D.

Accepted for Publication in "Laboratory Investigation".

Honors and Awards relating to this project:

Serial No. NINDB - 77 (C)

1. Surgical Neurology Branch
2. Clinical Neuropathology Section
3. Bethesda, Maryland
4. New

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Study of Regeneration in the Central Nervous System.

Principal Investigator: Armando Ortiz-Galvan, M.D.

Other Investigators: Edward J. Laskowski, M.D. and Igor Klatzo, M.D.

Man Years (calendar year 1958):
Total: .25
Professional: .25
Other: .25

Patient Days (calendar year 1958): 0

Project Description:

Objectives: The problem of regeneration of nervous elements within the central nervous system is of an obvious importance. The numerous investigations in this field indicate that the main obstacle for successful regeneration of the nervous fibers is encountered in the reaction of the connective tissue which blocks the pathways. By application of the metal plate at a low temperature to the optic nerve it is hoped that the connective tissue reaction will be reduced to a minimum. This assumption is based on the study of cold lesion produced in the cortex of the cat. In addition, the intra-cysternal injection of the prednisolone compound, which is one of the most powerful adreno-cortical steroids, may further reduce mesodermal reaction and thus provide conditions for effective regeneration of the optic nerve fibers.

Methods Employed: A series of cats are being operated and experimental lesions are produced in the optic nerve by application of a metal plate at low temperature. In addition, one

Method Employed(continued):
group of animals is being injected intra-cysternally with prednisolone. The animals will be sacrificed at various time intervals ranging from one week up to four months.

The progress of regeneration would also be followed electrophysiologically by photic stimulation and recordings from various parts of the central nervous system.

Proposed course of the project: It is proposed to continue this project to obtain complete data based on histological, electrophysiological observations from the groups of studied animals.

Part B included: Yes ☐ No ☒

Serial No. NINDB - 78 (C)

1. Surgical Neurology Branch
2. Clinical Neuropathology Section
3. Bethesda, Maryland
4. Formerly NINDB 33 (C)

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Histochemical and Electrophysiological Observations on the Muscle Fibers Grown in Vitro.

Principal Investigator: W. K. Engel, M. D.

Other Investigators: Choh Lu Li, M.D. and Igor Klatzo, M. D.

Man Years (calendar year 1958):
Total: .10
Professional: .05
Other: .05

Patient Days (calendar year 1958): 0

Project Description:

Objectives: Muscle tissue of chick embryo or new-born rat grown in tissue culture presents an exceptionally suitable object for correlations between the appearance of various chemical substances, demonstrated by histochemical methods, and electrical activity of the muscle fibers. Specifically, it is planned to correlate the observations on the nucleic acids, polysaccharides and contractile muscle proteins with the electrical activity of the corresponding living cells by intracellular microelectrode technic.

Methods Employed: Muscle tissue obtained from 14 day old chick embryos or new-born rat is grown in vitro. The cultures are studied on consecutive days with the following methods: (1) Nucleic acids, with methyl green-pyronin, gallocyanin with controls by digestion with ribonuclease. (2) Polysaccharides, with PAS,Toluidine blue, etc. (3) Contractile

Methods Employed (continued):
muscle proteins, with specific fluorescent antibodies. Before undergoing the histochemical procedures the muscle fibers are observed and photographed in phase contrast and in polarized light. For correlation, the corresponding cultures are subjected to study of electrical activity with intracellular microelectrodes.

Major Findings: The dynamic changes in RNA content has been demonstrated with gallocyanin and Toluidine blue methods. The first appearance and localization of myosin in myofibrills has been followed with specific fluorescent antibody. Data on the electrical activity have been obtained from the cultures several weeks old.

Proposed course of the project: It is proposed to continue this investigation to complete the lacking observations for correlative interpretation of the findings.

Part B included: Yes ☐ No ☒

Serial No. NINDB - 79 (C)

1. Surgical Neurology Branch
2. Clinical Neuropathology Section
3. Bethesda, Maryland
4. New

PHS-NIH
Individual Project Report
Calendar Year 1958

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 1958):
Total: .25
Professional: .25
Other: .25

Patient Days (calendar year 1958): 0

Project Description:

Objectives: A simple and quantitative method for estimation of precipitin reaction is of obvious value. By application of antigen-antibody mixtures to the chromatographic paper with consecutive separation of soluble proteins in the paper the insoluble antigen-antibody precipitate can be quantitatively evaluated by simple calorimetric methods. By using fluorescent antibody instead of serum in the test, the ratio between the amount of antibody to antigen in the precipitate can be quantitatively estimated.

Methods Employed: Serial dilutions of the mixture of antigen-antibody are applied to the chromatographic paper and run with buffer. The insoluble antigen-antibody precipitate remains at the starting line, whereas, the soluble proteins move away through the paper. The paper strips are stained for proteins with bromphenol blue. The dye bound to the precipitate is eluted and estimated quantitatively in the calorimeter. Similarly the fluorescein isothiocyanate bound to the antibody in the precipitate is eluted and quantitatively analysed in ultra-violet spectrophotometer.

Major Findings: This method has been applied to the precipitin reaction between antigens of contractile muscle proteins and their respective antibodies. The quantitative data obtained with this method were in agreement with much more complicated and cumbersome Kjehldal nitrogen determinations. The sensitivity of the method was estimated to be as low as 1 gamma of nitrogen.

Proposed course of the project: It is proposed to evaluate further this method in application to various immuno-chemical systems.

Part B included: Yes [ ] No [x]

Serial No. NINDB - 80 (C)

1. Surgical Neurology Branch
2. Clinical Neuropathology Section
3. Bethesda, Maryland
4. NINDB 29 (C) & 30 (C)

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: The Relationship between Edema, Blood-Brain-Barrier and Tissue Elements in Experimental Brain Injury.

Principal Investigator: Igor Klatzo, M. D.

Other Investigators: A. Piraux, M. D. and Edward J. Laskowski, M. D.

Man Years (calendar year 1958):.

Total:	.25
Professional:	.25
Other:	.25

Patient Days (calendar year 1958): 0

Project Description:

Objectives: The objective is to study the interrelationship between edema, blood-brain-barrier and behaviour of brain tissue elements.

Methods Employed: In order to allow conclusions about the chronological sequences of the changes observed, the brain lesion associated with edema should be reproducible with great uniformity. This requirement was satisfied by the application of low temperature to the exposed cortex under constant conditions of time and temperature. Groups of cats were sacrificed following this procedure at various time intervals.

Sodium fluorescein was used for study of blood-brain-barrier. Following fluorescence photography the brain tissue was subjected to a variety of histological and histochemical procedures. A group of animals was sacrificed for electrophoretic study of protein patterns in the edematous and normal white matter.

Major Findings: The development of edema was observed within 6 hours in the white matter underlying the site of cold application. The area of edema exhibited strong PAS-positive staining of astrocytes and less intense PAS staining of interstitial spaces. Histochemical analysis of PAS-positive staining in the edematous white matter suggested glycoprotein nature of the substances involved. The break-down of blood-brain-barrier in the edematous white matter as tested with sodium fluorescein followed after approximately 18 hours. Electrophoretic studies performed at the time of maximal intensity of edema and break-down of blood-brain-barrier indicated an appreciable increase of total proteins with striking elevation of albumins in the area of edema.

Fluorescence in the superficial layers of the cortex persisted one month after injury and was associated with the presence of small astrocytes, lacking well-formed vascular foot-plates.

Proposed course of the Project: This project is completed.

Part B included: Yes [x] No [ ]

Serial No. NINDB 80 (C)

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards and Publications

Publication other than abstracts from this project:

"The Relationship between Edema, Blood-Brain-Barrier and Tissue Elements in a Local Brain Injury".

Igor Klatzo, M. D., Andre Piraux, M. D. and Edward J. Laskowski, M. D.

Publication: Journal of Neuropathology and Experimental Neurology Vol. XVII, No. 4, October, 1958.

Honors and Awards relating to this project:

Serial No. NINDB 81 (C)

1. Surgical Neurology Branch
2. Clinical Neuropathology Section
3. Bethesda, Maryland
4. NINDB 31 (C)

PHS-NIH
Individual Project Report
Calendar Year 1958

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 1958):		Patient Days (calendar year 1958): 0
Total:	.25	
Professional:	.25	
Other:	.25	

Project Description:

Objectives: In view of the recent interest in the use of hypothermia in neurosurgical procedures, the objective of this investigation is to assess the effects of the lowered body temperature on the various aspects of brain injury such as edema, permeability of blood-brain-barrier, etc. Also, an elucidation of tolerance of normal brain tissue to various degrees of hypothermia is imperative.

Methods Employed: The assessment of the effects of hypothermia is based on a comparative study of our standard cold lesion, as described in Project NINDB 29 (C), in normothermic and hypothermic animals. Groups of cats were submitted to lowered body temperature and were operated on in a similar manner when the rectal temperature reached 26° C. The animals were maintained at a rectal temperature of 24-28° C for periods of 4-6 hours after application of the cold plate. The cats were sacrificed at various time intervals and the brain tissue was submitted to procedures, similar to those used in normothermic animals.

Major Findings: The most striking difference between hypothermic and normothermic animals were revealed in the behaviour of the blood-brain-barrier. Twenty-four hours after cold application in normothermic animals there was intense fluorescence of the white matter extending into the adjacent gyri. In contrast, all hypothermic animals sacrificed after 24 hours showed the fluorescence limited to a peripheral margin surrounding the non-fluorescent superficial necrotic lesion. At 48 hours there was, however, an increase in the area of fluorescence in these hypothermic animals but this was still less than seen at maximal edema at 24 hours in the normothermic group.

Histological preparations reveal a lesser astroglial reaction in hypothermic preparations at comparable periods of sacrifice. The PAS-positive staining of the astrocytes and the interstitial substance is similarly diminished in the area of edema in the hypothermic animal.

Proposed course of the project: These observations are now based on sufficient numbers of animals to be conclusive and this phase of the project is complete. It is planned, however, to continue the study of this lesion followed by the induction of hypothermia in an effort to evaluate the use of lowered body temperatures in the treatment of brain trauma.

Part B included: Yes [x] No [ ]

Serial No. NINDB 81 (C)

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards and Publication

Publication other than abstracts from this project:

"Observations on the Effects of Hypothermia on Experimental Brain Lesions", Edward J. Laskowski, M. D.

Accepted for Publication in The American College of Surgeons Surgical Forum, Volume IX.

Honors and Awards relating to this project:

National Institute of Neurological

Diseases and Blindness

Clinical Research

Surgical Neurology Branch

Section on Developmental Neurology

Serial Numbers of Projects:

NINDB-82(c), NINDB-83(c), NINDB-84(c), NINDB-85(c), NINDB-86(c), and NINDB-87(c).

Estimated Obligations for FY 1959

Total: $128,000

Direct: $36,800

Reimbursement: $91,200

Serial No. NINDB-82 (c)
1. Surgical Neurology
2. Developmental Neurology
3. Bethesda, Maryland
4. Same as NINDB-45 (c)

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: The Investigation of the Site, Type and Extent of Lesions Involving the CNS in Cerebral Palsy and Allied Conditions.

Principal Investigator: Anatole S. Dekaban, M.D.

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1958):
Total: .35
Professional: .35
Other: .35

Patient Days (calendar year 1958)
1,215

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	28	22.5
	Children Female	28	20.5

Clinical Project

Major Findings:

During 1958 a total of 56 patients were studied in great detail as in-patients and 28 on the out-patient basis. Analysis of results of clinical investigations revealed that in 62 percent of cases the site of lesion was determined, in 27 percent the abnormality was of diffuse character and in 11 percent the localization was not possible. In 29 percent of cases the pathological lesion was compatible with destructive process, in 12 percent with congenital malformation or hydrocephalus, in 21 percent it was of diffuse character and in the remainder of 38 it could not be estimated with confidence.

Special tests as complement fixing antibodies, estimation of lipid contents in the cerebrospinal fluid, special retinal studies, estimation of amino acids in urine, phenylalanine in blood, genetic assey and a very detailed neurological assessment including interpretation of pneumoencephalogram allowed us to make etiological diagnosis in 43 percent of all patients. In 34 percent of the cases the etiological diagnosis was presumptive and in the remaining 23 percent only symptomatic diagnosis could be made.

Final analysis of the material has to await accumulation of more patients. Results of studies of prenatal factors in the etiology, pathology and clinical manifestations are reported in 5 publications during the year of 1958 and two more are in press.

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: At the present time we have detailed data on the total of 141 patients but further accumulation of the material is needed before global analysis can be attempted. Nevertheless various

important aspects arising from this study have been already evaluated and this resulted in 7 publications.

Part B included Yes [X] No [ ]

Part B: Honors, Awards and Publications

Publications other than abstracts from this project:

Dekaban, Anatole, O'Rourke, James and Cornman, Tillye: Abnormalities in offspring related to maternal rubella during pregnancy. Neurology 8: 387-392, 1958.

Dekaban, Anatole and Magee, Kenneth: Occurrence of neurological abnormalities in infants born to diabetic mothers. Neurology 8: 193-200, 1958.

Baldwin, Maitland and Dekaban, Anatole: The surgical separation of siamese twins conjoined by the heads (cephalopagus frontalis) followed by normal development. J. Neurol. Neurosurg. Psychiat. 21: 195-202, 1958.

Dekaban, Anatole: Mental deficiency: recessive transmission to all children by parents similarly affected. Arch. Neurol. & Psychiat. 79: 123-131, 1958.

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. A.M.A. Archives of Ophthalmology. In press.

Dekaban, Anatole: Arhinencephaly. Amer. J. Mental Defic. In press.

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.

1. Surgical Neurology
2. Developmental Neurology
3. Bethesda Maryland
4. Same as NINDB-48 (c)

PHS-NIH
Individual Project Report
Calendar Year 1958

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; Carolyn May Smith, R.N., NINDB.

Cooperating Units: National Naval Medical Center and Walter Reed Army Hospital.

Man Years (calendar year 1958):
Total: .25
Professional: .25
Other: .25

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: There are 4,480 products of pregnancy under study. Up to date we have completed follow-up examinations on 68 percent of the infants. Great efforts are being made to increase follow-up studies to pass the mark of 80 percent. When this is accomplished analysis and evaluation of the entire material will be begun. This will be a task consuming much of our time during the 1959 year, however, it is anticipated that a number of important observations will be obtained.

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 be able to reveal the relative importance of various 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 project and preparation of the material for publications is likely to take two more years of work.

Part B included Yes [ ] No [X]

1. Surgical Neurology
2. Developmental Neurology
3. Bethesda, Maryland
4. Same as NINDB-46 (c)

PHS-NIH
Individual Project Report
Calendar Year 1958

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 Roring

Cooperating Units: None

Man Years (calendar year 1958):
Total: .20
Professional: .20
Other: .20

Project Description:

Objectives: The causation and pathology of the majority of mental defects and cerebral palsy patients are largely unknown. Detailed examination of the brains of children who suffered from such disorders and the correlation of these findings with the clinical data is expected to provide valuable information for elucidation of etiology of these conditions and so to 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 brains from children with organic brain lesions were processed and studied.

Neuropathological project

Major Findings: The analysis of pathological findings revealed that in nine children the abnormality in the central nervous system was of prenatal origin, in five it was compatible with birth injury, in four it was a result of intracranial infection, in one cerebral neoplasm was present and in one no significant CNS abnormality was detected.

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 of these specimen up to date resulted in 3 publications.

Proposed Course of the Project: Further accumulation of data is needed before final evaluation will be attempted.

Part B included Yes [X] No [ ]

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Dekaban, Anatole and Norman, Ronald: Hemiplegia in early life associated with thrombosis of the sagittal sinus and its tributary veins in one hemisphere. J. of Neuropath. and Exper. Neurology 17: 461-470, 1958.

Dekaban, Anatole: Is needle puncture of the brain entirely harmless. Neurology 8: 556-557, 1958.

Dekaban, Anatole: Arhinencephaly in an infant born to a diabetic mother. J. Neuropath. and Exper. Neurol. In press.

Honors and Awards Relating to this Project: None

1. Surgical Neurology
2. Developmental Neurology
3. Bethesda, Maryland
4. Same as NINDB-49 (c)

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: The Incidence and the Type of the Central Nervous System Abnormalities Encountered in Offspring Born to Diabetic Mothers.

Principal Investigator: Anatole S. Dekaban, M.D.
Robert L. Baird, M.D.

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1958):
Total: .10
Professional: .10
Other: .10

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: 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

Correlation of the severity of the diabetes with the result of pregnancy:

Severity of Diabetes	Abortions	Stillbirths	Neonatal Deaths	Abnormal Surviving	Normal Surviving	Total
Mild 9(W1;N8)	7(W0;N7)	3(W1;N2)	1(W0;N1)	1(W1;N0)	13(W1;N12)	25(W3;N22)
Moderate 22(W11;N11)	23(W10;N13)	8(W3;N5)	9(W3;N6)	7(W1;N6)	34(W17;N17)	81(W34;N47)
Severe 17(W5;N12)	17(W6;N11)	7(W3;N4)	3(W1;N2)	1(W0;N1)	23(W6;N17)	51(W16;N35)
Total No. of Pregnancies 234(W57;N177)	47	18	13	9	70	157

The final outcome of pregnancy in 48 diabetic mothers before the diagnosis and in 48 normal controls.

	Abortions	Stillbirths	Neonatal Deaths	Abnormal Surviving	Normal Surviving	Total
Before Diagnosis of Diabetes 78(W5;N73)	16(W2;N14)	4(W1;N3)	1(W0;N1)	2(W0;N2)	55(W2;N53)	78(W5;N73)
Normal Controls 249(W66;N183)	31(W8;N23)	3(W0;N3)	12(W0;N12)	1(W0;N1)	202(W58;N144)	249(W66;N183)

These data are critically analyzed in regard to various modifying factors and the results are being prepared for publications.

Major Findings: The overall fetal wastage of pregnancies in the diabetic mothers was 43.4 percent as compared to 17.6 percent in the normal control. Of the surviving offspring born to the diabetic mothers 6.7 percent showed congenital malformations or various neurological abnormalities; this compares with only 0.48 percent of the abnormal children in the non-diabetic control group

Significance to Neurological Research: To further our knowledge of the cause of cerebral palsy and allied conditions.

Proposed Course of the Project: Final evaluation and description of the data will be completed within ten months.

Part B included Yes [ ] No [x]

Serial No. NINDB-86 (c)
1. Surgical Neurology
2. Developmental Neurology
3. Bethesda, Maryland
4. Same as NINDB-50 (c)

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Measurements of External and Internal Orbital Distance in Males and Females from Birth to Adulthood.

Principal Investigator: Anatole S. Dekaban, M.D.

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1958):
Total: .05
Professional: .05
Other: .05

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: This material is being currently validated and subsequently it will be subjected to the statistical analysis.

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 data obtained from the measurements are currently analyzed. Subsequently the material will be prepared for publication.

Part B included Yes / / No /X/

Serial No. NINDB-81 (c)
1. Surgical Neurology
2. Developmental Neurology
3. Bethesda, Maryland
4. Same as NINDB-47 (c)

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Preparation of the Horizons of the Normal Development of the CNS in Mice and Experimental Production of Congenital Malformations of the CNS.

Principal Investigator: Anatole S. Dekaban, M.D.

Other Investigators: Marie J. Kendall, B.A.

Cooperating Units: None

Man Years (calendar year 1958):
Total: .05
Professional: .05
Other: .05

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 progressive 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 microscopic 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 during the past 8 months. Beginning in January this experimental project will be resumed.

Part B included Yes [ ] No [x]

National Institute of Neurological

Diseases and Blindness

Clinical Research

Surgical Neurology Branch

Section on Clinical Psychology

Serial Numbers of Projects:

NINDB-88(c), NINDB-89(c), NINDB-90(c).

Estimated Obligations for FY 1959

Total: $24,500

Direct: $24,000

Reimbursement: $500

Serial No. NINDB-88 (c)

1. Surgical Neurology Branch
2. Section on Clinical Psychology
3. Bethesda, Maryland
4. New

PHS-NIH
Individual Project Report
Calendar Year 1958

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 1958):
Total: .5
Professional: .5
Other: .5

Patient Days (calendar year 1958):
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.

Methods Employed: A Wisconsin General Test Apparatus for primates will be used, with the discrimination tray modified so that there is a plastic box (for the disrupting objects) between the discrimination cups.

Major Findings: None. The apparatus has been built; laboratory space is being arranged; some preliminary training of monkeys has begun.

Significance of the program to the Institute: The method should yield a means of more precisely describing the nature of temporal lobe function in emotion; the "elaborating" function in relation to perception in primates could be clarified.

Proposed course of the project: The first efforts to establish the utility of this method will use monkeys rather than the more expensive chimpanzees.

Part B included Yes [ ] No [X]

Serial No. NINDB-89 (c)
1. Surgical Neurology Branch
2. Section on Clinical Psychology
3. Bethesda, Maryland
4. Same as NINDB-55 (c)

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Psychological Evaluation of Temporal Lobe Disease

Principal Investigator: H. Lansdell

Other Investigators: M. Baldwin, M. Blevins, J. Weissbach and A. Mirsky

Cooperating Units: NIMH Section on Animal Behavior

Man Years (calendar year 1958):
Total: .5
Professional: .5
Other: .5

Patient Days (calendar year 1958):
None

(about 40 surgery cases; more than double for non-surgery cases--per year)

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: Mrs. J. Weissbach (formerly Olhoeft) and Miss M. Blevins (in cooperation with Dr. L. Frost and Mr. R. Savard) have reported a tendency for patients with left temporal lobe removals to be "Poor communicators," these patients do not appear to differ in other standard respects from other patients.

No other significant new findings have been established in these areas. A variety of new tests have recently been added to the standard battery given to the patients.

Significance of the program to the Institute: Future analysis of follow-up data and diagnostic efficiency of the tests may help in the efficacy of temporal lobe surgery. A careful survey of the test data on previous and contemporary cases should enable more objective description of the nature of dysfunctions with temporal lobe disorders. Such material will elucidate and help distinguish between contemporary conceptions of temporal lobe function.

Proposed course of the project: Publication of the study on the "poor communicators." Observations are yet to be obtained on auditory function during neurosurgery. Sufficient objective observations are yet to be obtained on cases with most clearly-established loci of epileptic disorders. Adequate control data are to be obtained.

Part B included Yes ☐ No ☒

Serial No. NINDB-90 (c)
1. Surgical Neurology Branch
2. Section on Clinical Psychology
3. Bethesda, Maryland
4. New

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Body Temperature in Chimpanzees with Bilateral Temporal Lobe Damage.

Principal Investigator: Mildred L. Blevins

Other Investigators: A. Rowe, S. Lewis, N. Mills and F. Smith

Cooperating Units: None

Man Years (calendar year 1958):
Total: .5
Professional: .5
Other: .5

Patient Days (calendar year 1958):
None

Project Description:

Objective: To record the rectal temperature of normal chimpanzees and chimpanzees with damage to both temporal lobes.

Methods Employed: Rectal temperatures were obtained from chimpanzees, both in the runs and in individual cages, over at least/20-day period.

Major Findings: The mean temperatures of three normal chimpanzees were found to be 37.4, 37.8, and 37.8°C (one male and two females respectively). The mean temperatures of the temporal lobe damaged animals at least four months postoperative were 36.5, 36.7 and 37.1°C (male and two females). The preoperative values on the first two animals were 37.9 and 37.7°C. A survey of the literature indicates there are no established normative values.

Significance of the program to the Institute: Normative temperature values have been obtained for this colony. The values will prove useful in checking the course of ill-health, or the effects of brain operations, in chimpanzees adapted to the procedure.

Proposed course of the project: None further, except possible publication of the established drop in temperature with brain operations of this type.

Part B included Yes [ ] No [X]

National Institute of Neurological Diseases and Blindness

Clinical Research

Surgical Neurology Branch

Section on Pain

Serial Numbers of Projects:

NINDB-91(c), NINDB-92(c), NINDB-93(c), and NINDB-94(c).

Estimated Obligations for FY 1959

Total: $26,000

Direct: $25,500

Reimbursement: $500

Serial No. NINDB-91 (c)
1. Surgical Neurology Branch
2. Section on Pain
3. Bethesda, Maryland
4. New

PHS-NIH
Individual Project Report
Calendar Year 1958

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.
William Lee Pritchard, M. D.

Cooperating Units: None

Man Years (calendar year 1958):
Other: .33
Professional: .33
Total: .33

Project Description:

Objectives: To continue clinical and experimental observations on the properties of volatile anaesthetics which promise to be particularly adaptable to use in neurosurgery. Properties to be determined include potency, rate of recovery, physiological side-effects, combustibility, and latent toxicity.

Methods Employed: Fluothane was submitted to extensive experimental and clinical study. Acute experiments using subject dogs were performed to determine physiological effects of fluothane, administered in concentrations equivalent to 1 - clinical usage and 2 - lethal doses. Pathological studies were performed on these dogs to correlate morphological effects with the physiological observations.

Clinico-pathological observations were also performed in the primate laboratory where fluothane is used as the primary anaesthetic agent in neurosurgical procedures on subject chimpanzees.

Clinical studies were performed in the operating room where fluothane was administered to patients undergoing surgery for a variety of neurological disorders. Clinical observations and electronic polygraphic recording of significant physiological parameters were carried out.

"Flu-ether", an azotropic mixture of fluothane was employed in experimental studies similar to those described above. The physico-chemical properties and physiological effects in acute dog experiments were determined.

Major Findings: Fluothane has proven to be a potent, non-combustible, non-toxic anaesthetic agent from which the patient recovers rapidly following withdrawal. It is a safe agent if administered with respect for its potency. This is most satisfactory done by use of the "Fluothane" vaporizer.

"Flu-ether" was found to be non-combustible and a relatively stable agent by various chemico-physical criteria. Physiological effects in acute dog experiments generally paralleled those of fluothane, the latter being the more potent of the two.

Significance to neurological research: Fluothane is particularly adaptable to the problem of anaesthesiology as applied to neurosurgery.

Proposed Course: Clinical use of this agent will be continued in the human operating room.

Clinico-pathological studies will be continued in the laboratory, on subject chimpanzees and dogs.

Part B included Yes [X] No [ ]

Serial No. _____

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards and Publications.

1. Hall, Kenneth D. and Norris, Forbes H., Jr. Respiratory and cardiovascular effects of fluothane in dogs. Anesthesiology, Vol. 19, No. 3, May-June, 1958. pp 339-352.

2. Hall, Kenneth D. and Norris, Forbes H., Jr. Fluothane sensitization of dog heart to action of epinethrine. Anesthesiology, Vol. 19, Sept-Oct., 1958. pp 631-641.

Serial No. NINDB-92 (c)
1. Surgical Neurology Branch
2. Section on Pain
3. Bethesda, Maryland
4. New

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Hypothermia in Neuroanesthesiology.

Principal Investigator: Kenneth D. Hall, M. D.

Other Investigators: Edward Laskowski, M. D.
Forbes H. Norris, M. D.
William Lee Pritchard, M. D.

Cooperating Units: None

Man Years (calendar year 1958):
Total: .33
Professional: .33
Other: .33

Project Description:

Objectives: The current widespread interest in the use of hypothermia during intracranial surgery and in certain acute situations encountered in the treatment of neurological disorders has prompted clinical and experimental studies of patients to determine and evaluate the role of hypothermia.

Methods Employed: Clinical studies involve patients submitted to total body immersion to achieve a hypothermic state. Physiological parameters are recorded; observations are made regarding the effects of cooling on the brain and their relation to technical aspects of the surgical procedure. Fourteen patients underwent this type of procedure during the past year.

Laboratory investigations are underway to achieve differential cooling of the brain while maintaining normal temperature in the rest of the body. The means employed involves the extracorporeal shunting of the

cerebral blood supply through a cooling chamber, after which the blood is returned to its normal cerebral pathway by means of a modified pump or "mechanical heart." Numerous technical and physiological problems present themselves for solution.

Major Findings: Clinical experience with hypothermia is as yet too limited in number of patients, too brief in long-term follow-up, and too poorly documented by pathological studies to reach final evaluations. Preliminary observations are based on objective findings during surgical procedures, such as the consistency of brain tissue, degree of swelling or bleeding, technical ease of manipulating structures. Physiological studies have outlined the tolerances involved in subjecting a patient to body cooling. The considerable increase in total anaesthesia time to which the patient is submitted probably does not sufficiently increase the anesthesia risk to deny the patient and surgeon whatever technical advantages may accrue thereby.

The experimental work is performed on subject dogs, and the development of this project is insufficient to present results.

Significance to neurological research: The role of hypothermia in neuroanesthesiology will be determined by such studies as outlined herein.

The experimental procedure described above represents the development of a laboratory preparation whereby innumerable aspects of brain physiology, chemistry, metabolism and pathology may be studied in addition to our immediate interest, the effects of hypothermia.

Proposed Course of the Project: It is intended to continue the course outlined above to evaluate results, and thereby determine the role of hypothermia in clinical application.

Part B included Yes [ ] No [X]

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Succinyl Choline in Awake Craniotomy.

Principal Investigator: Kenneth D. Hall, M. D.

Other Investigators: Maitland Baldwin, M. D.
Forbes H. Norris, Jr., M. D.
William Lee Pritchard, M. D.

Cooperating Units: None

Man Years (calendar year 1958):
Total: .33
Professional: .33
Other: .33

Project Description:

Objectives: In the surgery of epilepsy on humans and in electroencephalographic and cortical stimulation studies performed on chimpanzees, it is desirable to have the subject awake, cooperative and able to tolerate the procedure. The development of such a technique as controlled paralysis by the administration of succinyl choline has been a major interest of this section.

Methods Employed: Succinyl choline is administered to the subject by intravenous drip therapy, the subject having previously been induced and intubated by conventional anaesthetic technique. The subject is allowed to awake from his anaesthetic. Hyperventilation is performed by the anaesthetist. He is paralyzed, but comfortable, as he is being mechanically respirated and local anaesthesia is used during the surgical procedure. The absence of spontaneous muscle activity and artefacts due to anaesthetic agents, renders more successful the electrocortical studies as performed in human patients. By sign language, the human patient can communicate his reactions to cortical stimulation.

By titration of the level of paralysis the experimental animal dog be allowed sufficient muscular activity to detect body movement in response to cortical stimulation.

Major Findings: This technique has been developed to the degree of proficiency that it represents an important adjunct to the surgery of epilepsy.

Proposed Course of the Project: This technique will be used as indicated during operations on human patients. Further experience will be derived from its use in the primate laboratory in physiological and pharmacological experiments in progress.

Part B included Yes [X] No [ ]

Serial No. _____

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B. Honors, Awards and Publications.

Hall, Kenneth D., Baldwin, Maitland, and Norris, Forbes H., Jr.: Succinyl choline in awake craniotomy. Anesthesiology. In Press.

Serial No. NINDB-94 (C)
1. Surgical Neurology Branch
2. Section on Pain
3. Bethesda, Maryland
4. New

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: The Effect of Hypertonic Urea Solution on Intracranial Pressure.

Principal Investigator: William Lee Pritchard, M. D.

Other Investigators: Robert Edgar, M. D.

Cooperating Units: None

Man Years (calendar year 1958):
Other: 1
Professional: .5
Total: .5

Project Description:

Objectives: There is current widespread interest in the administration of intravenous hypertonic urea solution for the reduction of intracranial pressure and cerebral edema. This technique promises to be of considerable value as an adjunct to routine intracranial surgery, as well as in acute situations often encountered in neurosurgery. Possible side effects of this regimen await evaluation.

Methods Employed: Patients undergoing surgery for suspected brain tumor and ward patients with clinical evidence of increased intracranial pressure receive intravenous urea solution. Observations on gross appearance of the brain at time of surgery, and on vital signs and neurological status of bed patients are made. Blood chemistry and urinalysis studies are also performed. Only a limited number of patients have been subjected to this regimen.

Major Findings: That brain volume and intracranial pressure may be reduced is apparent in this study. Thus far, no serious side-effects have been noted, although they may be expected in certain situations.

Significance to Neurological Research: If this technique should fulfill its promise, it will prove an invaluable adjunct to the management of neurosurgical patients.

Proposed Course of the Project: Clinical Trial of urea will be continued. In addition, arrangements are being made to perform tonometry on patients receiving urea, to determine the effect on intraocular pressure, and the possible relationship of the latter to intracranial pressure.

Part B included Yes [ ] No [X]

Annual Report of the Basic Research Program

[illegible]

January 1, to December 31, 1958

INTRODUCTION

The important role of the nervous system in the pursuit and achievement of anything in science tends too often to be neglected, or even denied. We live in a Logical Positivist Society which overlooks the psychological processes of science in much the same way that a Puritanical Society fails to admit to public accounting certain fundamental biological processes!

Obstacles in our path as experimental scientists are by no means exclusively technical difficulties: in large measure, rather, they are conceptual obstacles imposed according to the historical moment and philosophical context of our work. Although we tend to deprecate consideration of history and philosophy as not directly contributing to the task at hand, we cannot escape in our actions as experimentalists from certain initial assumptions which may be crucial and yet which we usually have accepted without careful examination.

It is my purpose, first, to illustrate how dependent all branches of science are upon an insight into the operations of the nervous system. The ultimate grasp of any branch of knowledge depends upon conceptual achievements in the mental and neurological fields. Already there is evidence that ignorance in these fields is holding back progress in some others. Second, I wish to invite reflection as to the validity of certain pervading assumptions which affect research, teaching and practice in the mental and neurological fields. What I mean will, I hope, become clear in the course of consideration of three questions: What is our conception of reality? How do we consider mind and brain? How do answers to these questions affect undertakings of physicians and experimentalists in the biomedical sciences?

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* As in the previous Annual Report, the Laboratory Chiefs provided comprehensive statements of research progress throughout the year. In the following paragraphs I have attempted to continue, as last year, the exploration of more general questions. 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.

WHAT IS OUR CONCEPTION OF REALITY?

Many different concepts of reality have appeared through the long span of history. Even with our present degree of technical sophistication there is no consensus as to what constitutes the "real" world. Contemporary theories of reality can be distinguished as belonging to a rather extended spectrum which is subjectivistic, almost solipsistic at one end, and materialistic determinism at the other. The experimentalist asks different questions of nature and treats the materials of his research, including his instrumentation, quite differently depending upon the point of departure he assumes.

Science is first of all a collective, social activity. Scientists operate not only with instruments and language of common understanding but also with certain abstractions such as charge, field, electron, atom, molecule, and so on---things which do not belong to the everyday world but are derived indirectly out of scientific experiments. In experiments, the scientist must deal not only with things which can be seized by the finger and thumb, so to speak, but also with things which can be appreciated only by means of elaborate extensions of the sense organs. We have to get accustomed to extrapolations into dimensions where our sense organs fail us, and where some of the space-time and causality relations of everyday experience seem no longer to hold true. Moreover, and especially in this extended range, we are already familiar with the necessity to remain tentative about our conceptions of "reality."

There seems to be no definitive boundary separating the finger and thumb world from the microworld of electron-microscope and X-ray diffraction. Yet the relation between what is revealed by the experimentalist's instruments and what is implied conceptually to him through these revelations is by no means obvious. When we extend ourselves still further to deal with atomic systems, we encounter the indeterminism of Heisenberg. We become dependent upon probability even with supposedly objectively occurring phenomena. For example, if we wish to determine the location of an electron in orbit around an atom by bombarding it with a small particle whereby a photon will be given off which we can then observe, the finite birth time of the photon takes so long in relation to the speed of the electron that the electron will have made a million passes

around its orbit during that birth time; and no amount of obstetrical scrutiny will reduce ambiguity as to where the electron was when it was struck by the bombarding particle. Thus the concept of absolute precision in physical measurements at this scale becomes absurd. Determinism is out of the question in the original sense of the word.

It is necessary in any treatment concerning concepts of reality to consider in some further detail the means by which such concepts are achieved. This is especially important because ordinary contemplation fails us when we try to explain "reality" entirely in accordance with our experience and familiarity with everyday sensory perceptions. As Max Born has said recently, "Matter as given by our senses appears as a secondary phenomenon, created by the interaction of our sense organs with processes whose nature can be discovered only indirectly through theoretical interpretations of experimentally observed relationships; in other words, through a mental effort. To designate the result of this operation by the old word 'matter' seems to me wrong."

The whole realm that science reveals finds its rational order and meaning through mental activity. It needs to be underlined that mental processes enter into the definition of reality at several stages:

First, with respect to the sensory perceptions necessary to observation.

Second, in the mental processes essential to the establishment of a theoretical notion. Here it must be observed that, whereas a theory can be tested by experience, there is no logical way to proceed from experience to the setting up of a theory. This must be a mental leap.

Third, since science is a collective social enterprise, mental processes invade the acts of communication through symbols and language to other competent individuals and, thereafter, although suffering considerable reduction, to the culture of a given era. The culture in turn, as a further and frequently overriding influence, has already put its own mental strictures on any conception of reality by virtue of the philosophical limitations it has imposed upon the scientist prior to the beginning of his experimentation.

It is rare for the proponent of a new concept in any branch of science to attempt to communicate all of the steps which led him to the final theory. It is impossible for him to analyze all of the assumptions which went into its establishment. The assumptions which he accepts on least reflection are those common to his intellectual community; moreover, they may not even be recognized as assumptions. On top of this are the commonplace limitations imposed by lack of time, insufficient intellectual rigor, over-optimism, or by too great a pride or self-justification.

We conclude that in order to understand the nature of the universe, in order to improve our conception of reality, certain definite obstacles in our path relate to the incompleteness of our understanding of the nervous system. This in itself constitutes a worthwhile or even a compelling reason, to add to all the more obvious ones, why it is important that we increase our efforts in the pursuit of research basic to neurology and psychiatry.

We need to learn how signals enter the nervous system, how they are distorted by ongoing activity in the brain, and how this in turn is affected by previous experience; how incoming, central and outgoing signals are related to mechanisms of reward and punishment and emotional experience and expression; how learning occurs; what are the nimble processes of creativity; and what are the limitations of our mnemonic, conceptual and linguistic response systems. These unsolved problems cannot be settled according to some simple recipe. There may be shortcuts, but few are revealed. These problems call instead for the most imaginatively resourceful exploitation of the anatomical, physiological, chemical, psychological and sociological channels of science.

Thus, it is evident that the solution of problems in all branches of science, not only in the biomedical fields, depends upon the furtherance and the success of basic research in the mental and neurological disciplines.

HOW DO WE CONSIDER MIND AND BRAIN?

Mankind has asked questions about himself since the beginning of reflective thinking. But it remained for the Greeks to bring up the idea of "mind" for the first time. It was around 400 B.C. that this concept first came into existence. No suggestion of what we conceive to be mind and mental, nor of the idea of the separateness of mind from matter, is found in earlier writings throughout Egypt, Babylonia and Greece. In fact this conception is singularly unique to Western civilization. Although Plato didn't invent the idea of mind, it is largely from his writings and influence that we have inherited the fundamental assumption that the world possesses two inevitably separate and incommensurable aspects: relating on the one hand to mind, and on the other hand to matter.

Throughout the writings of both Plato and Aristotle, the peculiar attributes of living things are accounted for by a very broad conception of universal pneuma or world-soul. This, in its loftiest form, accounts for what we identify by the term "mental life." Cruder aspects of the soul, responsible for vegetative life, growth and reproduction, inhabit all living creatures including plants. Animals are endowed with an additional, qualitatively higher-order aspect of soul which "animates" them, i.e., which gives them a capacity for movement. Man alone possesses the third, highest form of soul, the rational soul, which concerns mental processes.

Plato and Aristotle freed us from certain otherwise foreboding metaphysical considerations that attach to the material forms of life. With respect to dissecting the dead, they relieved us by authoritatively reenforcing the conception that on death "the soul flies away with joy" leaving the carcass a "disjected membrane" no longer pertaining to life, no longer containing a soul.

The particular flavor of Western civilization which was contributed by Greek culture played a conspicuous role in the development of scientific inquiry through its impact on the first humanists: the scientists and natural philosophers of the Renaissance. During a later period of especially rapid development of science, during the first half of the 17th Century, Descartes went a further step beyond Plato and Aristotle. He took the rational soul

which had been projected throughout the ventricular system of the brain, and deposited it in the pineal gland. Descartes revealed the remainder of the nervous system and body of man as being an instrumentality, capable of acquiring suitable relations with the rational soul in the pineal, but otherwise being of an automatic sort---like the brains and bodies of animals which Descartes still considered to lack the rational aspects of soul. This enabling gesture did in physiology what Plato and Aristotle had done earlier in anatomy; it left the brain (except for the pineal gland) available for scientific investigation as a "mechanism." From this moment onward, scientists could be concerned with what nervous mechanisms are involved in consciousness, perception, appetite, reward and punishment, and freedom of will, questions that had been raised earlier by the Greeks

During the next 200 years, great advances were made in knowledge of the gross anatomy of the nervous system. Its mechanisms were successively interpreted, by analogy, as if the brain and the nerves were operated by a mechanical hydraulic system, such as the complex water fountain machines invented at that time. Later, they were thought of as accounted for by the universal aether of Sir Isaac Newton, through which gravity was posited to act instantaneously at a distance upon the planets. Still later, the brain was interpreted on the basis of the actions of animal electricity Every age seems to make use of the most complex analogy conceivable in that era. Our age analogizes the brain to an electronic computer.

Seventy-five years ago, our intellectual great-grandfathers supposed that the mechanisms of the brain would become clear as soon as the microscopic detail of brain anatomy could be worked out, using the newly acquired techniques for making thin slices and for staining nervous tissue This optimism was succeeded by a pessimism characteristic of the succeeding generation, that anatomy alone would not suffice: one needed to understand the <u>living</u> brain and it was presupposed that there never could be satisfactory techniques for this purpose.

Within the last twenty to thirty years, however, more has been accomplished toward achieving an understanding of the great question originally raised by the Greeks than during the previous two millennia. We should, therefore, be more optimistic than our intellectual grandfathers although perhaps less optimistic than our intellectual great-grandfathers. To our enormous advantage now, a cluster of

technical achievements has made possible the examination of the living, waking, behaving organism in terms of internal brain mechanisms. There is presently flowering a tremendous renaissance of all of the sciences relating to the nervous system. All that is being discovered in neuroanatomy, neurochemistry, neuropharmacology, neurophysiology, psychology and sociology has suddenly become far more meaningful to each of the other complementary disciplines. This rapid expansion and increase of conceptual penetration into these important fields has occurred at a time when the world--more than ever before--needs desperately to understand and to be able to deal with basic human capabilities and limitations.

In the last five to ten years, scientists have learned many crucial features concerning the basis of consciousness, the mechanisms of appetite, the way experience forms and distorts perception, the limitations of our mechanisms for memory, emotion, communication, the identification of internal reward and punishment systems, and have come a great way toward understanding the unity of mind and brain.

For example, it is now demonstrated experimentally that the cortex is not the first step in sensation nor does it appear to be the last step either. Instead, something fairly continuous and dynamic takes place all along the ascending ("sensory") pathways during wakefulness. There is an erosion of impulses that originally started into the nervous system and an intrusion of additional impulses into the pattern; these alterations being conditioned by the previous experience of the individual. This is exactly what one might introspectively suppose would take place within the brain: a reduction and distortion from the actual nature of the stimulating world, of information relating to perception. Not only does the brainstem reticular formation affect consciousness, as Magoun pointed out some ten years ago, but in this way also it alters the content of consciousness. This effect proceeds from sense receptors to whatever end point you wish to choose. It continues right up to the final motoneuron discharge, if you will. These mechanisms, which shape perception and performance are built into the value systems of the organism into the central reward and punishment mechanisms, and are inextricably bound up with the circuits essential to emotional experience and expression. The brain thus operates teleologically, that is, it is affected by internal value systems which in turn are based upon previous evolutionary and individual experience.

Some of the great questions raised by the Greeks have now been proven approachable by experimentation. By means of resourceful theoretical and experimental approach which implicates all of the complementary disciplines of biophysics, anatomy, physiology, chemistry, psychology, genetics and sociology, scientists are beginning to derive principles that will define the ranges of invariance and of indeterminacy in the operation of the nervous system.

From this brief description, it is, I hope, evident that much is being learned through basic research on the brain and behavior, and that the findings from such research are important in relation to our better understanding the very basis and limitations of human knowledge. Measurable outside experience is not the whole of experience. Because of indeterminacy, mechanism is not the whole explanation of a given reality. Reason and feeling are not at war from their natures: they are fused elements which we separate in our minds only by reflection. Does man have freedom to manipulate the channels between his feeling and his ideas? Can he choose his purposes? I am inclined to believe that mental and neurological research now supports the surest hopes in this regard that have ever been put before mankind.

HOW DO ANSWERS TO THESE QUESTIONS AFFECT UNDERTAKINGS OF PHYSICIANS AND EXPERIMENTALISTS IN THE BIOMEDICAL SCIENCES?

We have long been accustomed (intellectually) to doubt the capacities of our common senses in the appreciation of nature. We have been obliged to do this wherever there has been sufficient scientific consensus in favor of some other than a common sense view. Yet we seldom reflect on the lack of foundation for most of our other prior assumptions and how much they may affect our quest into the unknown. As regards the scientific frontier under investigation, we still tend to cling to a common sense view and to the prevailing assumptions of our environment.

Traditionally, as biomedical scientists, we think we know something about mind, and something about matter, and that these are incommensurably different from each other

First of all, it should be recognized that this whole schema is based upon an assumption we inherited from Plato. Second, the old definition of matter is now known to be operationally incomplete: it fails to include the mental processes inevitably involved in any conception of matter. It follows, therefore, that until the basis and limitations of these mental processes are completely understood, any definition of matter must be accordingly limited and tentative. Altogether this may seem hard to accept. Yet the alternatives, over which so many philosophers have struggled, are not particularly alluring. Let us consider them briefly: Some believe that thoughts, ideas, wishes and other mental phenomena are essentially epiphenomena, that they grow out of, or run parallel to, certain particular material events; others consider that such mental events are essentially incorporeal, perhaps occupying only "virtual space;" still others consider that mind is a property of matter, that there may be a certain amount of "mindness" extended in small degree throughout all forms of matter. You may have your choice. In each case, it is still the old word "matter" that is being considered. Bertrand Russell on the other hand holds "that whatever we know without inference is mental, and that the physical world is only known as regards certain abstract features of its space-time structure---features which, because of their abstractness, do not suffice to show whether the physical world is or is not different in intrinsic character from the world of mind." This dismisses the prior assumption and gives encouragement to further scientific study of the whole issue.

I believe that the assumption of Plato, even though it has a great hold on our imaginations, is unnecessary, and is unnecessarily distracting in relation to our quest for further knowledge about the nervous system. Its admission as an original premise seems to me to be unhelpful to our everyday performance in the hospital, classroom and research laboratory. I feel this is true mainly because it fosters professional, intellectual and conceptual isolation among scientists who are trying to understand the whole man.

When people say, in common parlance, that they can know or recognize matter independently of mind, they are really confessing a further assumption that is unnecessary and may indeed be entirely wrong. What they really mean is that they consider a percept to lie in some other category, presumably not so unreliably mental, as compared with a

thought, memory or wish. The latter, admittedly mental functions, are supposedly less tractible, less invarient, and perhaps less substantial (in the literal sense of the word) than is a percept. The fact is, we don't yet know enough to make such a comparative evaluation.

We do know, however, that perception is definitely a mental act. We know also that in vast ways it is subject to error in the sense that Descartes meant this, and upon which he based his whole philosophy of universal doubt. Furthermore, we know that the sensory messages upon which perception must depend are themselves also subject to direct interference through action of the central nervous system. A central control is exerted even out to the peripheral sense organs, and acting throughout the entire trajectory of the ascending "sensory" pathways. This control, which was unknown until only a few years ago, appears to be exercised in accordance with some kind of internal "value system" which itself is reacting to previous as well as concurrent experiences; thus, the modulation of incoming sensory impulses seems to be based upon "expectation," "relative significance to self," and so forth. The value system, moreover, is accessible to the central mechanisms involved in "reward" and "punishment," emotional experience and expression, and the presumably more objective and depersonalized systems of neocortex. This complex set of systems is built into the chassis, so to speak, and cannot be divorced from either the ascending signals coming from the outside world, or from the outgoing sensory control impulses which modulate the incoming messages. Evidently the nervous system is continually practicing its control over sensory pathways just as it has long been known to do in relation to motor performance. Presumably the brain can shape our perceptions more or less like it shapes our comportment: in both cases teleological mechanisms are at work.

At the very least, then, perception is a mental act; and the data upon which this mental act is dependent are themselves acted upon according to the cumulative patterns of previous mental acts. The psychologist, from an external view of behavior, has long known this to be true, and has been trying to communicate that to other scientists. But many scientists, not being directly involved in examining the processes of perception, have continued to preserve

a distinction between what they have assumed can be directly, clearly and unequivocally apprehended through the senses and what might thereafter be subject to mental operations. This can now be thrown out on other than psychological grounds.

Biomedical science is not alone in being affected by erroneous prior assumptions. For example, similar difficulties existed in classical physics during the last century when that discipline was considered to be concerned only with "inanimate matter." Many physicists wondered then whether concepts of force, energy, and so on, had to do with "physically real" problems, or whether they were only a kind of logical instrument, needed only for the time being. In the course of much theoretical and experimental work, such primitive definitions gave way before more subtle conceptions which incorporated both matter and energy and now flirt with a further incorporation of field forces. Thus, formerly isolated aspects of physics, some of which appeared to lie completely outside the proper province of that discipline, have come to be viewed much more holistically. Yet, even in a field as advanced as physics, there still exist great bogs of ambiguities in atomic theory, cosmology and other areas. The one universally respected conviction is that we must be tentative, willing to tolerate ambiguities, and prepared to participate in quite revolutionary non-common-sense ways of conceiving of nature. The major advances in physics have been associated with greater unification of theory. I do not pretend that brain and mind will follow a similar history, but only that the speed of progress in any discipline and the dimension of contribution of individual scientists are dependent upon the tentativeness with which fundamental assumptions are accepted.

Now, taking the common sense view of the separateness of mind and matter, a view which seems to pervade most of our culture, does that operate to our disadvantage——as patients, physicians, professors or experimentalists? Does it retard our advancement toward a more complete understanding of life? If we saw that it were disadvantageous, would we abandon it?

First, let me be explicit. I am not attempting to make an exclusive abstraction from experience, either of brain or of mind. I am acknowledging, however, that it is only by conceptual artifice and cultural habit that we consider these separately: in effect, the expression mind-brain or brain-mind is more adequate than either mind or brain alone. A further practical handicap of Plato's

assumption besides its interference with conceptual advancement lies in the professional antagonisms it cultivates. The extreme views are readily characterized: Those who believe too zealously in matter are confident that biological phenomena can be "explained" entirely on the basis of laws of physics and chemistry. Mind is an illusion. Since mind is the most anthropomorphic thing in man, it should be dispensed with. "Teleology, also," they say, "should have no place, excepting, of course, for homeostasis and a few other selected forms of purposive biology." It comes as a shock to these persons to learn that modern physics is becoming more anthropomorphic, even to admitting mind. Those who believe too zealously in mind, on the other hand, can show that matter is inferred. They mistakenly conclude from this that matter is therefore an illusion. Think of the impact of this conceptual isolation on a patient with a disorder of his "brain-mind!" This will inevitably interfere with the recognition of illness, finding professional help, participating in the therapeutic regimen, seeking rehabilitation and explaining his disorders to himself and to society! My own experience leads me to believe that there is no intellectual satisfaction to be derived from considering either of these "opposites" to be an illusion.

Ideas, not things, rule mankind. When we use some concept for purposes of guidance in our daily lives, we must avoid confusing the concept with experience and believing the one is a sufficient explanation of the other. Where can we divide the nervous system to hedge off the limits of mind? Until we know enough to be able to answer that question, how can we be satisfied with practically complete isolation in training, research and practice between those who examine and treat the mind, and those who examine and treat the brain? We cannot immediately dispense with such isolation as exists, but how long should we be satisfied with this as an adequate intellectual frame of reference? Of course, until it was possible to determine some of the brain mechanisms responsible for certain mental processes, such isolation was perhaps inevitable, even though it were acknowledged that both professional lines of activity ultimately relate to the same organ system. But already there are several brain mechanisms known to be responsible for mental processes. These have been discovered through the efforts of various mental and neurological

disciplines, often paired up with each other. How much more effective the collaboration where the intellectual and conceptual force of two or more disciplines can be combined within single individuals.

It has now been found possible experimentally and intellectually to cross boundaries that had so long remained inviolate that their irresistability was generally conceded. Some of these accomplishments are still hardly within our grasp. At a time of such rapid technical achievement, it is more than usually necessary to be highly selective of our investment of time and action into those things which will lead most directly to more fundamental understanding. It is always easy to imagine something worth doing, but what is most worthwhile is very difficult to determine: yet, that more critical determination is principally what separates us from great achievement. Although we are often preoccupied with dollar budgets, dollars are seldom our most precious commodity. We cannot dispense with budgets, but we can perhaps attend more conspicuously to more important things. We can perhaps give more consideration to what should we do instead of simply feeling compelled to do something. Perhaps a wastage most to be regretted occurs as a result of pursuing perfectly justifiable research when a modest investment of creative thinking might have suggested something far better.

In every discipline relating to the nervous system, there has occurred during the last decade considerable discarding, or at least drastic compromising, of some of the most fundamental principles embraced by that discipline. There is a swiftly flowing stream of intellectual movement in progress. This is evident all around us, although the attitude we often reveal in describing such progress (particularly if it is our own work) would suggest that we are at last coming to some sort of leveling off; there is tranquility ahead, still waters. Rather, I believe the future of this movement will be more swift and compelling than in the past. I think I can hear rapids ahead, and am looking forward to them, even though they may give all of us a particularly severe drubbing. What are we here for? To conquer our ignorance.

Perhaps the most reasonable way to liberate ourselves from the many kinds of psychological stricture to which we often seem so committed is not to take ourselves too seriously. This leavening is difficult to achieve without risking real or imagined deterioration, especially in a self-conscious environment.

GENERAL COMMENTARY

Much has been said about "collaborative" and "interdisciplinary" endeavors in contemporary research. Last summer Dr. [redacted] Clemente and I thought it might be of interest to examine data prepared for the previous Annual Report of each of the two Institutes to see whether these data reflected much or little collaboration. One of the prime arguments used in the establishment of the National Institutes of Health emphasized the supposed advantages of bringing together scientists in complementary disciplines who would presumably work together toward the solution of problems relating to certain disease categories. In the case of the Basic Research Program, NINDB-NIMH, approximately a dozen disciplines were considered especially pertinent.

The Annual Report does not by any means reflect the entire amount of collaborative research in being. Instead it reflects only those projects which yielded sufficient experimental results to go well beyond the "pilot" stage. Although a given project might be abandoned later without publication, usually one or more and sometimes several papers are published as a consequence of a single project. Some projects continue for years; most of them involve more than half a man's entire scientific efforts for the given year.

In assembling the data we made no attempt to judge the value of collaborative or interdisciplinary research; the facts simply reflected the scientists' estimations of how they had expended their research efforts. More than half of the projects reflected collaboration extending beyond the limits of the laboratory group. This usually means interdisciplinary research. About one fourth of the projects reflected inter-institute collaboration throughout NIH. Nearly 40 per cent of the projects were reported by solitary investigators. This Annual Report reflects approximately the same proportions. Inasmuch as collaboration is not encouraged for its own sake, and there are inevitably some handicaps to its prosecution, the remarkably high proportion of collaborative activity probably justifies the original arguments in favor of bringing the complementary disciplines together.

Other forms of communication besides collaboration in research involve research publication and communication at scientific meetings. This year has seen an excellent harvest of outstanding research papers from the Program. The entire enterprise can readily be justified on the basis of a few of the really creative ideas. The status of the Program is further measured by the large number of invitations that come to our scientists to provide papers or lectures and to join outstanding university faculties. Considering the number of highly qualified scientists in the Program and the number of important posts available, we are bound to lose some good men every year. But we also enjoy quite favorable recruitment currents which bring scientists to the Program. We are still increasing in strength as an intellectual and experimental resource for training and experience of scientists at all levels. Nearly every major university in this country and some fifteen universities in foreign countries are represented in our Program this year. A few of our own people were enabled to visit laboratories or attend meetings in a dozen foreign countries this year. There is now beginning some exchange with even Russia and Poland. We were hosts for three weeks to Professor Jerzy Konorski, Head of Neurophysiology in the Nencki Institute in Warsaw, and for a briefer time to a number of Russians, including Professor Bykov of Leningrad, Professors Sarkisov and Propper-Grashchenkov of Moscow, and others. Within the year, there have been distinguished visitors from more than 22 different countries. The Program is well proven as an intellectual and research resource of high international regard.

A new style of educational experience at the NIH was initiated last year by Dr. Cantoni. He invited Professor Martin Pollock, an enzyme chemist from England, to spend a period of time at the National Institutes of Health. Dr. Pollock and a number of leading scientists at the NIH and nearby laboratories made themselves available for an extended program of informal seminars and discussions. This program was open to interested scientists from all the Institutes and nearby laboratories. Most of the participants engaged in preliminary reading and study prior to the beginning of these meetings and many of them engaged full-time in this sort of "intellectual workshop" with Dr. Pollock and each other. The outcome seems to have been very favorable and has already had an important influence on biochemical research locally.

Two major Symposia were sponsored during the year by the Basic Research Program. One traced the development of concepts throughout the first century of neurochemistry and

paid especial tribute to J.L.W. Thudichum, the founder of neurochemistry. Projections were made by scientists representing disciplines allied to neurochemistry as to the nature of the need for an understanding of the neurochemical bases of neurological and psychological processes, and appreciation was expressed for the research potentialities and opportunities of this field for the future. The other Symposium brought together for the first time, for considered discussion, experts on the medical, legal and social problems relating to narcotic addiction. Both Symposia were well attended and lively. Each led to a more widespread and intensive interest in the intellectual content of the issues involved, especially as this leads to new research directions.

One of the traditional ways of improving the creative power of an organization--through the use of expert consultants--has been even more actively exploited by the Program this year than in the past. The National Institutes of Health established a pattern for advice to the Institutes through Boards of Scientific Counselors. Boards were established for each Institute and reviewed the independent and combined Programs. The Boards gave encouragement and intellectual stimulation, as well as much-appreciated advice. A number of other experts continue to advise and participate in more limited aspects of the Program.

One of the Consultants, who spent a considerable time working in the Basic Research Program, is Professor Leo Szilard. During the course of his period as Consultant, he devised a new theory for the nature of the aging process. This is a lusty theory which "explains" a vast amount of adventitious facts in addition to those directly concerned with the genetic factors in the aging process. Coming as it does into a field that needs a broader theoretical basis, and being a theory that can be tested experimentally at a number of points, Dr. Szilard's ideas will provide much stimulation to the several disciplines engaged in studying aging and will tend to focus attention upon central unifying concepts. Dr. Szilard's theoretical study has already been completed and discussed among scientists at the NIH and elsewhere and is to appear in the January, 1959, issue of the Proceedings of the National Academy of Science.

On the fringe of our more central research mission, the Program participated in a nationwide television program, sponsored by the American Association for the Advancement of

Science and the National Academy of Science. This program, CONQUEST, produced by CBS, emphasized some of the research possibilities being exploited in laboratories all around the world to determine brain organization and the relationship of this to neurological and psychological functions. The Brain Story drew a larger audience than any previous CONQUEST show and won the Thomas Alva Edison Award for the "best science show for youth in 1958." All twenty or so of the newspaper reviews were very favorable and even superlative and the mail response, many from students in high schools and universities, indicated that the program conveyed a good deal of intellectual content as well as appeal. Official ratings reported that somewhere between 15 and 20 million persons viewed the program. For the first time, a science program beat "The Lone Ranger."

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Our profoundest concern as scientists is associated with the intellectual content of our disciplines, and especially with the creative processes necessary to progress in these fields. All administrative considerations ought to be directed to encourage, develop and exercise these essentially intellectual faculties. What can be done to make ourselves more creative? One suggestion is that hypotheses should be treated opposite to casual tradition: that is, be more welcomed in their most initial stages and more skeptically held when they are better established.

Our guide for progress, individually and collectively (to paraphrase Jacques Barzun) grows out of our interests, viewed objectively, in the long-range and in the largest possible sense; our interests to improve ourselves and our work; our interests made as self-aware as possible. It is unlikely that mankind can attain what it does not wish to strive for and impossible to seek what is conceived as unattainable.

Robert B. Livingston

Robert B. Livingston, M.D.

Laboratory of Neuroanatomical Sciences

William F. Windle

The Laboratory of Neuroanatomical Sciences is organized as four Sections and a Field Station. Research in each of these units has a distinctive character, although there is a healthy amount of overlapping and integration of interests within the whole. As implied by the name, the laboratory's investigative programs emphasize structure, but few projects are limited to morphology, and none lacks a strong functional basis. Various disciplines are represented among the twenty-one full time scientists and four active consultants now in residence; zoology, cytology, embryology, physiology, biochemistry, neurosurgery, medical neurology, neuropathology, psychology, and even obstetrics are fields of primary interest of various members of the staff. This wide diversity imparts a quality to the laboratory, found in few if any other neuroanatomical departments in this country.

Preparation of a brief summary of annual research progress is complicated by this diversity of interest. Each Section Chief has prepared a summarizing report and these have been combined.

Section on Neurocytology
S. L. Palay, Chief

The work of this section has been considerably advanced by the exchange of our electron microscope for a new model in June, 1958. The new microscope permits us to carry on the work which we intended to do during the previous year with the previous model.

A study of the fine structure of axons in the central nervous system of fishes shows that axoplasm contains three longitudinally arranged elements: mitochondria, canaliculi, and neurofilaments. The most prominent of

These are 10 neurofilaments, 60-100 Å in diameter which nearly fill the volume of the large axons. That these filaments are not artifacts is demonstrated by positive birefringence of the axons in fresh preparations. This study was carried on in collaboration with Dr. Bairati, University of Milan; Dr. Gordon, Research Associate, participated.

Dr. Brightman has completed a study of the effects of large doses of irradiation to the base of the brain. This investigation indicated that alterations in the vascular supply of nervous tissue are probably primary events in the destruction of nervous tissue by X-rays.

Dr. Brightman in collaboration with Dr. Albers has also studied the distribution of butyrylcholinesterase activity in the CNS of several common animals. He found that in the rat, goldfish, and toad, this enzyme occurs in the endothelium of vessels, whereas in the cat and fowl, the enzyme resides in the neuroglia. Since inhibitors of this enzyme have been reported to produce demyelinization in the fowl, this study suggests a relationship between glial butyrylcholinesterase and myelin formation by neuroglia cells.

Dr. Albers, partly in collaboration with Dr. Brady, has studied two enzymes in the central nervous system that are involved in the metabolism of γ-aminobutyric acid. Both of these, glutamic decarboxylase and γ-aminobutyrate transaminase, are found in relatively high concentrations in gray matter and are virtually absent from white matter. The role of these enzymes in the metabolism or in the specific function of tissues of the central nervous system is not clear.

The Section was host to Dr. Angelo Bairati (University of Milan), Dr. E. E. Manuelidis (Yale University), and Dr. Sheila Donahue (Columbia University) for periods of 6 to 9 months.

Section on Experimental Neuropathology
J. Cammermeyer, Chief

The research of this Section has continued within the framework of a central theme and long range plan. To state this briefly, the aims are to reveal extra- and

intraspinal factors involved in the normal maintenance of spinal cord structure and function, and to ascertain the degrees of deviation from normality which will cause myelopathies.

Experiments have been devised to disclose relationships of extraspinal factors to spinal cord malfunction. These have been held in abeyance for lack of systematic study of the contents of the epidural space which surrounds the spinal cord. To provide some of the much needed information, a morphological study of the epidural fat, its relations, variation with age and species differences has been carried out by Dr. Helen Ramsey and is now ready for publication. The importance of epidural fat is that it permits changes to occur along the vertebral column without tearing the contents. However, the deposition of fat is found to be of a complex nature in the growing and adult cats and other species and varies individually to some extent.

Initiation of an experimental approach to the myelopathies has been hampered, furthermore, by lack of information about the volume of spinal cord. Exact knowledge has been unavailable regarding alterations in normal spinal cord histology and cytology occurring in consequence of removing the spinal cord. Finally, the adequacies of commonly used histological techniques had to be studied prior to going forward with a well-controlled investigation of experimentally produced myelopathies.

Size of the normal spinal cord and of its several regions has been established in several species. The size of the spinal cord and of its regions can be estimated from the correlation which exists between spinal cord volume and size of other anatomical structures more accessible for measurement.

Effects of chemical and physical agents used for preparing histological material have been investigated. Every step in fixation, removal and subsequent histological treatment of the spinal cord has been surveyed. On the basis of the information obtained through measurement of the spinal cord and of its component cell structures, a procedure has been developed whereby errors in the histological preparations are now reduced to a minimum.

and artifactual changes avoided. We are now prepared to investigate experimentally produced volumetric and morphological changes of the spinal cord.

A preliminary study has been carried out on the size of neuroglial nuclei. These structures increase in diameter significantly with the size of the spinal cord. Thus, when it is desired to compare an experimental animal with a normal one for the purpose of determining whether or not an experimental agent has had an effect on the nervous system, we now know that the question can be answered only with animals of identical size.

Effects on spinal cord volume and nerve cell structure following administration of urea in various dosages and for various lengths of time are being investigated in collaboration with Dr. Ziemnowicz. This study is one of a series on dehydrating agents. Other studies on the spinal cord are being conducted jointly with Dr. Mignon Malm, using isotopes in an attempt to visualize the routes of transportation of tagged metabolites. Finally, the responses of neurons and neuroglia cells to motor activity and to pharmacological agents are projected Experiments of simple design, it is hoped, will provide a better understanding of the mechanism in spinal cord reactions and an explanation for differences in reaction between spinal cord regions and between the spinal cord and brain.

The Section has been host to Dr. and Mrs. Stanislaw Ziemnowicz (Jagellon University, Poland) and Dr. Mignon Malm (University of Stockholm, Sweden) during 1958.

Section on Functional Neuroanatomy
G. L. Rasmussen, Chief

At present the work of this Section is concerned primarily with nervous pathways and connections of the brain and spinal cord, with emphasis on the neural mechanisms of auditory and vestibular function. Practically all the studies have engaged the joint attention of the Section Chief and Dr. Gacek, Research Associate.

An efferent nervous component of the vestibular nerve has been revealed which is comparable in many anatomical respects to the efferent cochlear bundle. The vestibular efferents have been traced from their origin in the lateral vestibular nucleus throughout the vestibular nerve and its branches as far as the receptor organs of the vestibular labyrinth. The possible relationship of these efferents to the hair cell receptors themselves is currently under study.

In order to better understand the neural mechanisms 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 projections from the latter to higher auditory nuclear groups are being restudied in more detail than heretofore by the experimental anatomical approach.

Mr. Boord, a University of Maryland student, completed a study of the innervation of the vestibular and auditory apparatus of the chinchilla. This work served as thesis material for his M.S. degree. He is continuing studies of the auditory system here under a PHS Research Fellowship. The project concerns the establishment of an efferent component of the cochlea in submammalian vertebrates possessing a less developed hearing mechanism than present in mammals. The results of this investigation will be incorporated into a thesis for the Ph.D. degree from the University of Maryland.

Mr. Morest, senior medical student of Yale University, carried out an independent research study on the fiber connections of the area postrema of the medulla oblongata during the summer under the COSTEP program. He was able to establish certain anatomical connections with midbrain structures and to correlate the results with previous electrophysiological observations at Yale.

Dr. J. B. Walther of the Max-Planck Gesellschaft, Bad Nauheim, Germany, is expected to join this Section before the end of this year. He has been studying physiology of sense receptors and central sensory mechanisms for several years and will turn his attention to the anatomy and physiology of the efferent auditory and visual

cooperation. Dr. Kallman's [illegible] is being [illegible] by the National Academy of Sciences under the Visiting Research Program.

Dr. Leo Massopust left the Section during the current year to accept an academic appointment elsewhere.

Section on Development and Regeneration
W. F. Windle, Chief

The research activities of this Section fall into four principal categories, (a) neurogenesis, (b) regenerative potentialities of central and peripheral neurons (c) experimentally induced structural alterations in the central nervous system, and (d) technical development.

(a) Drs. Sidman and Miale have examined the behavior of cells in the neural tube of mouse embryos with techniques of tissue culture and autoradiography, using tritium-labeled thymidine. This nucleotide is incorporated into DNA of cells about to divide and remains as a permanent marker. Whole embryos cultured in vitro survived for a week or more and mitotic activity was vigorous. Nuclei of cells lying in the mantle layer some distance away from the lining of the neural tube synthesized new DNA and then migrated to the ependymal layer. Subsequently they migrated laterad and prepared for differentiation. As growth continued, subsidiary patterns of mitosis and migration appeared. Whole organ (or embryo) culture has provided access to a time in development that has resisted investigative efforts in the past. Permanent labeling of cellular components and tracing them differentially by autoradiography have opened an entirely new avenue of research in developmental processes.

Histogenesis has been studied in the retinas of normal mice and in those of a genetic strain with dystrophy of this organ by Drs. Sidman and Feder. No histological differences appeared until the ninth day of gestation, both normal and dystrophic eyes developing equally until that time. Cone cells appeared a little earlier than rods and the rod-bipolar synapses were seen on the eighth day. After the ninth day the rods and cones degenerated and scarcely a trace of the photoreceptor layer remained at

the fifteenth day. The dystrophic condition resembles retinitis pigmentosa, a human disorder of genetic origin with similar histopathology.

(b) Investigation of the phenomenon of central nervous system regeneration has been continued, Dr. Campbell of Columbia University collaborating. The monkey's spinal cord was severed surgically, leaving a gap of 2 to 6 mm. The cord stumps and this gap were then enclosed in a small sheet of Millipore filter sterilized by radiation. Without the enclosing filter, the cord stumps became capped by neuroglia and connective tissue and a random scar formed in the gap. With Millipore, the tissues in the gap were organized longitudinally; the gap was bridged by spindle-shaped cells and blood vessels, and a few regenerating intraspinal neurons followed this oriented tissue in crossing the gap. Small fascicles of regenerated neurons were present there 3 to 4 months after operation. They resembled peripheral nerve roots by virtue of the presence among their fibers of Schwannlike cells. No functional restitution was obtained.

Dr. Guth has continued investigations of heterogenous nerve regeneration and functional restitution. In collaboration with Dr. Frank, it was found that function could be restored to the paralyzed hemidiaphragm of the rat after phrenic neurotomy by directing central vagus nerve fibers to regenerate into the peripheral phrenic stump. The recurrent laryngeal branch of the vagus nerve displays bursts of activity which are synchronized with those of the phrenic to the diaphragm, which accounts for their ability to take over phrenic function after regeneration. These experiments have been extended to the monkey, Drs. Campbell of Columbia and Soutter of Boston University collaborating. If successful in the monkey, the latter investigators plan to apply the knowledge to human patients with diaphragmatic paralyses.

Other studies by Drs. Guth and C. J. Bailey pertain to pupillary function after degeneration of preganglionic pupillodilator fibers in cats. These experiments are expected to shed light on the question of plasticity of central nervous connections and have theoretical implications of considerable interest.

Drs. Tobias of the University of California and van Wagenen of Yale provided the Section with perfused-fixed brains of four adult monkeys in which the pituitary stalk and gland had been irradiated in the California betatron early in life. The neuropathological material was prepared here, and Dr. Cammermeyer reported the observations. The path of the horizontally directed beam was seen in the temporal lobes of one animal only. The other three showed no damage there, and remarkably little effect was encountered even in the infundibular region adjacent to the pituitary stalk. This technique was used very effectively to produce a small circumscribed lesion in the hypophysis alone.

Our project on anatomical correlates of the functional changes resembling human paralysis agitans that appeared in chronically reserpinized monkeys and cats was terminated with publication of the results (Windle and Cammermeyer '58). After daily administration of this drug (0.2 - 0.6 mg/kg) for as long as 18 months, no gross abnormalities were found in the brain. Microscopically there were no hemorrhages, infarcts, softening, demyelination, neuroglial reactions nor phagocytosis. However, conspicuous cytological changes appeared in the cerebral cortex, basal ganglia and brain stem. Cell nuclei and nucleoli appeared pale and enlarged; the karyoplasm often showed a "hole" due to removal of some substance during the process of histological preparation of the tissues. These were interpreted as nonpathological, probably reversible changes. Distribution of altered cells did not conform to pathological foci of human paralysis agitans. This study is the first in which a specific morphological effect of administering reserpine has been reported.

The pattern of neuropathology of asphyxia neonatorum in the monkey is being investigated by Dr. Ranck, using material obtained from the Puerto Rico Field Station studies. The first thorough study of the effect at 10 days of life, after asphyxiation for 17 minutes during birth and subsequent resuscitation, has been completed.

Histology of the brain of a nonasphyxiated monkey of similar age was studied for control. Gross pathology was not seen. Microscopical changes were extensive, almost perfectly bilaterally symmetrical, and localized by cytoarchitecturally defined nuclei. Cerebral and cerebellar cortex showed very little damage. But in all other regions and in the spinal cord neuronal loss amounted to 20 to 100 per cent. An early macrophage reaction occurred and astrocytic hyperplasia was seen in most damaged regions. There were no hemorrhages nor thromboses. The inferior colliculus, some thalamic nuclei, the subthalamic nucleus, globus pallidus, reticular formation, eye-muscle nuclei, vestibular, trigeminal, cochlear nuclei, gracile and cuneate, cerebellar, and superior olivary nuclei and the gray matter of the spinal cord were most severely damaged. Among undamaged regions were the amygdala, olfactory nuclei, most of the hypothalamus, lateral geniculate bodies, pontile and inferior olivary nuclei, and most of the structures along the floor of the fourth ventricle. Primary motor and sensory elements were less affected than interneurons. The neuropathological picture in the infant monkey resembled that reported in human infants with "kernicterus", and was unlike that found in asphyxiated human beings. This is the first experimental study of the kind in any neonatal primate.

(d) Other studies under way in this Section were largely concerned with developing new techniques. Drs. Feder and Sidman designed and put to use a freeze-substitution method which has given significantly improved fixation of small tissues and preservation of a variety of chemical substances in them. They have investigated structure and function of photoreceptor cells and obtained the first convincing evidence that cone cells are present in the retinas of rats and mice.

Dr. Wolf, Research Associate, has explored the significance of acridine orange for staining neurons *in vitro* and *in vivo*, comparing the fluorescent image of living cells with that of fixed or injured elements. Acridine orange was found to be of remarkably low toxicity and to provide an excellent tool for studying chemical reactivity of living cells. The data suggest that healthy cells have no free polyanions that can bind the dye metachromatically and that DNA becomes metachromatic only after cellular injury.

Field Station of Perinatal Physiology
(Subsidiary of Laboratory of Neuroanatomical Sciences)
W. F. Windle, Chief

Experimental investigations during the first full year of activity at the laboratories in Puerto Rico have been concerned with adverse factors in the perinatal period of rhesus monkeys resulting in neurological and psychological deficits in the offspring. The first adverse factor tested was asphyxia neonatorum.

Inasmuch as little was known about neonatal monkeys and nothing at all about fetal physiology of any subhuman primate, it has been necessary to begin to collect data pertinent to experimental neurological work with monkey fetuses and infants. In doing so, opportunities have presented themselves to collect ancillary data in respect to gestation, behavior, growth and development. The free range colony of 300 rhesus monkeys on Cayo Santiago and a caged breeding colony now numbering 75 large female animals of the same species have served these purposes.

An observational study of behavior and social organization was started in June, 1956, by Mr. Altmann, to obtain information on normal subjects. Infants have been followed after birth and their growth and development recorded. There is an annual cycle of reproductive activity in this free range colony, births falling in the months from February to June. On the other hand, the caged monkeys under laboratory conditions in Puerto Rico conceive and give birth to infants in all months. This has not been the experience in northern latitudes. The meaning of the difference between free and caged animals is unknown. The two-year study of social behavior has made possible an estimation of the maximum population the island can support. This should be 1,000 to 1,200 animals, which will provide an important reservoir for experimental studies. Dr. Koford, from the University of California, will continue the investigations on Cayo Santiago, since Mr. Altmann has returned to graduate studies at Harvard.

Anthropometric studies on free ranging rhesus monkeys have been under way for two years; these were recently taken over by Dr. Gavan, of the Medical College of South Carolina, who is about to add the technique of radiography to more conventional measurements of skeletal growth. An attempt is

being made to establish standard physical measurements by which accurate estimates of ages of monkeys can be made. Since birth records are available for more than half the animals on the island, it should be possible to collect the necessary data in a short time. Cage-reared monkeys will be compared with those reared by their mothers in a natural habitat.

Establishment of criteria for neurological examination, especially for infants was needed. Although the monkey has been extensively used for neurological experiments in many institutions, no satisfactory standards for neurological examination have been published. Drs. Ranck and Marisa Ramirez de Arellano have made considerable progress with neurological examination of the infant monkey, and the program is being extended by Drs. Comba, McCroskey and Jacobson. A protocol has progressed through several editions and should be completed in another year.

Data on menstruation of rhesus monkeys under standard conditions are being collected. The menstrual cycles of individual monkeys are subject to wide variations which appear to be equally spread throughout the year. Dr. Jacobson found no relation between regularity of the cycles and fertility, but a change in the time of mating within the cycle (day 11 instead of day 14) tended to alter the time of "implantation bleeding". Dr. Hertz, of the National Cancer Institute, is collaborating with a study of the efficacy of an early pregnancy test. The caged breeding colony has been remarkably fertile, 50 pregnancies resulting from matings last year.

Dr. Jacobson collaborating with Dr. Pelegrina, Professor of Obstetrics at the University of Puerto Rico, is investigating the nerve supply of the endometrium in a number of species of animals including the monkey and man. The endometrium of the cat was found to be densely supplied by fine terminal nerves around the endometrial glands and under the surface epithelium as well as around blood vessels. The possibility of neuronal participation in menstruation is being considered.

Data are being collected on maturation in infant rhesus monkeys and on care required for rearing them. A nursery, resembling in many details those in use for care of human

infants, has been established. Records are kept of daily weight, food intake, body temperature, respiratory rate, dental eruption, heart rate (EKG), grasp reflex, etc. Infant monkeys have shown a regular pattern of growth and development, but with wide ranges. Little information for infant monkeys has been available, but the caged and free ranging colonies with a combined birth rate of 100 or more a year will soon provide it.

Neurological deficits of experimentally induced asphyxia have been investigated by all members of the Field Station with collaboration of several scientists on the faculty of the University of Puerto Rico Medical School. 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. Others were delivered at once to serve as controls. Asphyxiation times were varied; some infants were able to breathe spontaneously while others had to be resuscitated by inflating their lungs with oxygen. Asphyxiated and control infants were reared 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, and a great variety of physiological data was recorded for later study, review and comparison. Infants which seemed unlikely to survive, as well as some healthy infants, were killed by perfusion-fixation for histological studies.

Infants asphyxiated for 11.5 minutes or less and which breathed spontaneously seldom showed neurological deficits for more than a day or two. A few had deficits in sucking, righting and motor dexterity for longer periods --one of them for 10 days. Infants asphyxiated 7 to 17 minutes and requiring resuscitation, all had sucking deficits and abnormalities of voluntary motion for as long as 3 weeks. Other defects observed were retinal hemorrhages, abnormal postural reactions, failure to localize sounds, hypotonic or hypertonic musculature, a III-nerve palsy, a 3-per-second tremor, status epilepticus, papilledema, and

loss of temperature control. At present there are 3 infants (3 weeks to 3 months of age) which seem likely to survive with permanent neurological damage. All others now in the nursery are overtly normal.

Dr. C. J. Bailey has constructed a battery of psychological tests for surviving asphyxiated infant monkeys and their nonasphyxiated controls. Recently Miss Saxon, from Dr. Harlow's laboratory at the University of Wisconsin, has taken over this aspect of the project. Eight pairs of monkey infants have been started on these tests; these are the animals surviving the asphyxiation at birth and overtly normal in appearance at present. So far, no consistent difference between these asphyxiated and nonasphyxiated infants have been revealed by the tests. In other test situations the 3 infants retaining obvious neurological deficits have clearly failed in learning test situations.

The pilot study of neurological and psychological deficits of asphyxia neonatorum in guinea pigs, begun by Drs. C. J. Bailey and Marisa Ramirez de Arellano, is nearing completion. This supplements earlier studies in this species by Windle and Becker, extending it to older ages and examining effects of lesser degrees of asphyxia. Significant differences between controls and experimental animals in the Becker maze at 8 to 13 weeks of age were encountered in respect to running time. With a closed field water maze, asphyxiated guinea pigs 12 to 19 months old made significantly more errors than their controls, but there was no consistent difference in retention. All the animals were killed, the brains sectioned serially and prepared for histological study, which is being carried out at present in the Section on Development and Regeneration.

Drs. Combs and McCroskey have begun experiments in the cerebellum, implanting electrodes in nuclei and pathways to record, acutely and chronically, electrical activity during induced cerebellar seizures. Since defects of posture, integration and co-ordination are prevalent in infant monkeys after asphyxia neonatorum, it is important to explore the role of the cerebellum.

Other Activities,
Laboratory of Neuroanatomical Sciences

The senior scientists of the Laboratory of Neuroanatomical Sciences have been called upon to participate in a number of activities which are not directly related to conduction of laboratory experimentation. Several are serving on study sections, fellowship committees and research advisory panels.

Dr. Palay has served on the National Research Council Specialty Board; Anatomy and Physiology Review, DRG; and has been Secretary-Treasurer of the Washington Society of Electron Microscopy during the past year.

Dr. Rasmussen is a member of the Traineeship Review Board, NINDB; and the Committee on Hearing and Bioacoustics, National Academy of Sciences-National Research Council.

The Chief of the Laboratory is serving on the following: Human Embryology and Development Study Section, DRG; Foreign Fellowship Committee, DRG, Anatomical Sciences Training Committee, DGMS; Executive Committee, American Association of Anatomists; Membership Committee, American Academy of Neurology; Committee on International Collaboration, American Academy of Neurology; Research Advisory Panel, National Multiple Sclerosis Society; Research Advisory Board, United Cerebral Palsy; and Committee on Primates, National Academy of Sciences-National Research Council.

Editorial tasks have engaged some of the investigators' time during the year. Two monographs, "Neurological and Psychological Deficits of Asphyxia Neonatorum" and "The Process of Aging in the Nervous System", resulted from NINDB-supported conferences and are being published in 1958 by Charles C Thomas. Dr. Palay edited "Frontiers of Cytology", published by Yale University Press. A translation of Ramón y Cajal's little-known book on neurogenesis, by Dr. Guth, is in press. Organization of a conference on "Neural Mechanisms of Auditory and Vestibular Function" has been completed by Dr. Rasmussen. A new scientific journal, "Experimental Neurology", has been launched by Academic Press, Inc., under editorship of the Chief of this laboratory, Dr. Palay also serving on its editorial board.

William F. Windle

NATIONAL INSTITUTE OF NEUROLOGICAL DISEASES AND BLINDNESS

Basic Research Program

Laboratory of Neuroanatomical Sciences

and

Puerto Rico Project

Estimated Obligations for FY 1959

Total: $646,900
Direct: 531,500
Reimbursement: 115,400

Individual Projects DR 1 through 22

NC 1 through 7

EP 1 through 3

FN 1 through 6

1. Neuroanatomical [illegible]
2. Section on Development and Regeneration
3. Bethesda, Maryland
4. Same as NINDB-NA-1-1957

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Development of Intrinsic Structures of the Human Brain.

Principal Investigators: W. F. Windle and L. Guth

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1958):
Total: 0.7
Professional: 0.6
Other: 0.1

Project Description:

Objectives: To study genesis and subsequent embryological development of nerve fiber tracts and nerve cells in the human central nervous system.

Methods employed: Study with the light microscope of serial sections of human embryos stained by neurological silver methods to bring out the fine details of neuron growth. A collection of human embryos which has been assembled gradually over the last 20 years constitutes the material for the present study.

Major findings: Largely as a spare time and out-of-hours endeavor, Dr. Guth has translated the book: Etudes sur la Neurogenèse de Quelques Vertébres" by S. Ramón y Cajal. This relatively inaccessible monograph is of fundamental importance to all studies in neuroembryology. The manuscript of this translation has been accepted for publication.

Little progress has been made with the microscopical study of the human embryo series, because of priority of other projects. The brain stem and spinal cord of one specimen have been studied rather thoroughly. Literature has been reviewed and pertinent articles translated.

Part A Project Description (cont'd)

Significance. Knowledge of intrinsic development of the brain is scanty; there has been no systematic human study Most descriptions of brain development have been based on material not stained specifically for neural structures. Until we have detailed knowledge of intrinsic structural development of the human brain, we will not have an adequate basis for understanding the normal process of aging, advent of pathological conditions and significance of functional aberrations.

Proposed course of the project: To continue the project as time permits.

Part B included: Yes

Serial No. NINDB-NA-DR-1

PHS-NIH
Individual Project Report
Calendar Year 1958

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:

Serial No. NINDB-NA-DR-2
1. Neuroanatomical Sciences
2. Section on Development and Regeneration
3. Bethesda, Maryland
4. New

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Histogenesis of normal and dystrophic retinas in mice.

Principal Investigator: Richard L. Sidman

Other Investigators: Ned Feder

Cooperating Units: None

Man Years (calendar year 1958):
Total: 1.0
Professional: 0.7
Other: 0.3

Project Description:

Objectives: To find the primary defect leading to retinal dystrophy in mice, in the hope of clarifying the comparable human disease, retinitis pigmentosa.

Methods employed: A comparison was made of the histochemical properties of the developing retina in the normal "C" and "C x C_3H" strains of mice and the pure "C_3H" strain which has a retinal dystrophy. Carefully inbred colonies of these mice with timed pregnancies were maintained. Eyes were fixed by our modified freeze-substitution method, embedded in polyester wax and sectioned serially at 5 or 3μ. Specimens were taken daily from birth to 24 days of age in normal and dystrophic groups. A method was developed for maintaining the whole mouse eye in vitro in organ culture.

Major findings: Several new observations were made on the normal development of the retina. The interstitial substance was found to be present at birth and increased in staining intensity during the first week after birth. The outer segments of rods and cones appeared as slender fibrils on the eighth day of life and subsequently thickened and elongated.

Part A Project Description (cont d)

From their inception they were PAS positive and stained for lipid. The cone cells developed somewhat sooner than the rods. The rod-bipolar synapse was demonstrable on the eighth day, and the number increased over the ninth to eleventh days. Developmental events were the same in the dystrophic mice. After the ninth or tenth day the rods and cones failed to develop and, indeed, rapidly degenerated, so that by the fifteenth day hardly a trace remained of the entire photoreceptor layer. However, no histochemical distinctions between normal and dystrophic retinas were found prior to the ninth day.

The organ culture experiments likewise failed to define the disease, but did eliminate some further possible causes. The retina differentiated in vitro and formed its 3 layers of cells. Rod and cone outer segments did not differentiate, even though the retina survived for many days after reaching the stage where these outer segments should have formed. The normal and dystrophic retinas behaved alike--they differentiated to the same extent and showed no signs of degeneration of photoreceptor cells. The addition of excess vitamin A to the culture medium did not cause formation of outer segments. Thus the disease probably does not arise because of a systemic noxious influence appearing on the ninth day of life, or of an intrinsic defect which manifests itself on that day. Rather it appears that the retina must reach a given stage of differentiation before the degeneration sets in, but the nature of the stimulus to degeneration remains unknown.

Significance: The significance arises from the analogy with the human disease, retinitis pigmentosa, a disease of similar genetic origin and histopathology. The human disease is commonly considered an abiatrophy, a degenerative disease of unknown cause in a mature cell. The mouse disease is clearly not an abiatrophy; it is a developmental disease, involving degeneration of immature cells. In man the photoreceptor cells, especially the rods, may likewise fail to mature in the dystrophic subjects and indeed, there is some electroretinographic data to support this idea.

Proposed course of project: The work described is being prepared for publication and the project will be terminated.

Part B included: No

Serial No. NINDB-NA-DR-3
1. Neuroanatomical Sciences
2. Section on Development and Regeneration
3. Bethesda, Maryland
4. New

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Histogenesis in the embryonic mammalian nervous system.

Principal Investigator: Richard L. Sidman

Other Investigators: Irene Miale

Cooperating Units: None

Man Years (calendar year 1958):
Total: 1.1
Professional: 0.7
Other: 0.4

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: (1) The intact 8 or 9 day mouse embryo was cultured *in vitro*, to separate the developing embryo from its normal environment.

(2) Autoradiography using tritium labelled thymidine (thymidine-H^3) was employed. This labelled nucleotide is incorporated into deoxyribonucleic acid (DNA) of cells about to enter cell division and thereafter remains as a permanent marker in those cells. Labelled embryonic tissues were fixed in Bonin's fluid, embedded in wax, sectioned, and prepared for autoradiographic study by Lebland's dipped liquid emulsion technique.

Major findings: (1) A method was developed which allows culture of whole mouse embryos. Embryos explanted at stages during closure of the neural tube and formation of somites differentiated at a slower rate *in vitro* than *in vivo*, but formed a complete neuraxis and most somites. Limb buds did not form. The embryos survived up to a week *in vitro*, with maintenance of body form. The heart initiated its contractions *in vitro* and continued to beat for up to 3 weeks, even though

Part A. Project Description (cont'd)

other organs had become lysed. Mitotic activity in the brain remained vigorous for 4 to 7 days in vitro, but little differentiation occurred.

(2) By autoradiography with thymidine-H^3 the basic pattern of cell proliferation in the immature neural tube, composed of a pseudo-stratified columnar epithelium, could be studied. Nuclei lying at a distance from the ventricular surface synthesized new DNA and then migrated to the ventricular surface to divide. Subsequently these cells again migrated laterally and contributed to the thickening of the neural tube which accompanied differentiation of neural cells. Almost the entire population of cells in the young neural tube were either migrating to the ventricular surface in preparation for cell division or migrating away from it in preparation for differentiation. As the various regions of the brain assumed their specialized character, a number of subsidiary patterns of cell division and migration appeared.

Significance: (a) One major deterrent to the study of mammalian embryology is the inaccessibility of the embryo. The development of a method for culturing whole embryos allows experimentation on nutrition and oxygen requirements, and allows experimental surgical intervention. Such studies have proved most beneficial with amphibian and chick embryos, and should be extended to the mammal. On the other hand, these methods for culturing the mouse embryo are less refined than methods available for lower vertebrates, and the curled shape of the mammalian embryo is less favorable for experimental manipulation.

(b) Autoradiography with thymidine-H^3 is a powerful new tool not heretofore used for the study of embryological processes. It allows labelling 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.

Proposed course of project: The results described above are being prepared for publication. Analysis of regional development in the brain is in progress and will continue for at least the first 8 months of 1959.

Part B included: No

Serial No NINDB-NA-DR-4
1. Neuroanatomical Sciences
2. Section on Development and Regeneration
3. Bethesda, Maryland
4. Same as NINDB-NA-2-1957

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Regeneration in the central nervous system.

Principal Investigator: W. F. Windle

Other Investigators: James B. Campbell

Cooperating Units: Department of Neurosurgery
College of Physicians and Surgeons
Columbia University

Man Years (calendar year 1958):
Total: 0.8
Professional: 0.5
Other: 0.3

Project Description:

Objectives: To study factors regulating regeneration in the central nervous system. To learn the role played by the neuroglia cells in degeneration and regeneration. To attempt to alter the normal response of the central nervous system to injury in such a way that a milieu favorable to functional regeneration will develop. To learn why regeneration occurs so readily in the central nervous system of the lower vertebrates and is so difficult to achieve in mammals and man.

Methods employed: The spinal cord of cynomolgous monkeys was transected in the midthoracic region, leaving gaps of 2 to 6 mm. The cord ends and gap were enclosed in a wrapping of Millipore filter; in other monkeys the Millipore was omitted for control. Pudendal nerves were severed to facilitate bladder and bowel emptying. Neurological examinations were made at weekly intervals and recorded cinematographically. Monkeys were killed after 3 weeks to 4 months and histological preparations of the cord lesions studied and compared.

Major findings: Without Millipore, the cord stumps soon became capped by pial connective tissue and a randomly oriented scar formed in the gap, as was reported previously

Part A Project Description (cont'd)

in cats and monkeys. In the Millipore-wrapped lesions, tissues were oriented longitudinally and the gaps bridged by spindle-shaped cells and blood vessels. A few intraspinal neurons from the rostral stump regenerated into this oriented tissue and crossed the gap, but could not be followed down the caudal stump By 3 to 4 months the regenerated nerve fibers had formed small fascicles, resembling peripheral nerve rootlets by virtue of Schwannlike cells along their fibers. It seemed that regenerating central neurons, reaching the gap, had acquired characteristics of peripheral neurons. If they descended into the caudal stump, a point as yet not proved, they lost this characteristic below the lesion.

No functional restitution was observed by 4 months.

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: To continue this collaborative study, extending observations to longer times, and younger monkeys Reports emanating from USSR indicate that very young mammals show remarkable powers of regeneration and restitution of structures surgically severed or removed from the brain.

Part B included: Yes

Serial No. NINDB-NA-DR-4

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Windle, W. F.: Regeneration in Relation to the Process of Aging in the Nervous System. Chap. 4 in "The Process of Aging in the Nervous System," James Birren, Henry Imus and William Windle, Editors. Springfield, Illinois, Charles C Thomas, 1958.

Windle, W. F., J. O. Smart and Jane J. Beers: Residual Function after Subtotal Spinal Cord Transection in Adult Cats. Neurology, 8:518-521, 1958.

Honors and Awards relating to this project: None

Serial No. NINDB-NA-DR-5
1. Neuroanatomical Sciences
2. Section on Development and Regeneration
3. Bethesda, Maryland
4. Same as NINDB-NA-5-1957

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Functional and Structural Changes in Reserpinized Animals

Principal Investigator: William F. Windle

Other Investigators: Jan Cammermeyer

Cooperating Units: None

Man Years (calendar year 1958):
Total: 0.7
Professional: 0.4
Other: 0.3

Project Description:

Objectives: To determine possible anatomical correlates of the functional changes resembling paralysis agitans in animals under chronic administration of reserpine.

Methods employed: Monkeys and cats were given reserpine (0.2-0.6 mg/kg) daily for varying periods of time. After establishing altered functional states, they were killed, perfused-fixed, and the nervous system studied by histological methods.

Major findings: Monkeys killed after 2 years of chronic administration of reserpine, and cats, after a few days or weeks, showed no gross abnormalities of the brain. Microscopically there were no hemorrhages, infarcts, softening, demyelination, neuroglial reactions nor phagocytosis. However, conspicuous cytological changes were found in the cerebral cortex, basal ganglia and brain stem. These involved cell nuclei and nucleoli, which appeared pale and enlarged and often showed a "hole" in the karyoplasm due to removal of some substance during the process of histological preparation. These were interpreted as nonpathological, probably reversible changes, because there was no indication of cell loss.

Part A Project Description (cont'd)

The neuronal changes were distributed in a pattern quite unlike that of pathological changes in human paralysis agitans.

Significance: Present studies provide the first indication of possible neuronal changes resulting from prolonged administration of low doses of reserpine. It should give grounds for caution in respect to prolonged use of this "tranquilizer" drug.

Proposed course of project: The project is dormant at present because of pressure of other projects, lack of space and personnel.

Part B included: Yes

Serial No. MINDB-NA-DR-5

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Feringa, E. R. and W. F. Windle: Induction of Hypokinesia, Rigidity and Tremor in Primates with Reserpine. Proc. 1st Internat. Cong. Neur. Sc.; London. Pergamon Press, 1958 (In press).

Windle, W. F. and Jan Cammermeyer: Functional and Structural Observations on Chronically Reserpinized Monkeys. Science, 127: 1503-1504, 1958.

Honors and Awards relating to this project: None

Serial No. NINDB-NA-DR-6
1. Neuroanatomical Sciences
2. Section on Development and Regeneration
3. Bethesda, Maryland
4. New

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Neuronal specificity in the autonomic nervous system.

Principal Investigator: Lloyd Guth

Other Investigators: Clark J. Bailey

Cooperating Units: None

Man Years (calendar year 1958):
Total: 0.8
Professional: 0.6
Other: 0.2

Project Description:

Objectives: To determine whether autonomic nerve fibers can maintain the function of autonomic effector organs other than those which they normally innervate.

Methods employed: 1. Preganglionic vagosympathetic nerve anastomosis. 2. Transection of preganglionic white rami T1, T2, and T3. This procedure interrupts all pupil preganglionics to the superior cervical sympathetic ganglion and leaves intact only those fibers which subserve other functions (e.g. vasomotion, piloerection and nictitating membrane retraction). Pupil size as a function of light intensity will be measured to determine whether collaterals from T4 to T7 will restore pupillary function.

Major findings: None as yet.

Significance: This experiment may shed light on the mechanism by which functional recovery so often occurs after sympathectomy. It may also clarify the still-unsettled question of the role of the sympathetic nervous system in pupillary

Part A. Project Description (cont'd)

function. Finally it is also designed to determine whether alteration of specificity of autonomic neurons can occur.

Proposed course of project: The project is being actively pursued at the moment and will constitute the major portion of the coming year's work.

Part B included: No

Serial No. NIMDB-NA-DR-7
1. Neuroanatomical Sciences
2. Section on Development and Regeneration
3. Bethesda, Maryland
4. New

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Heterogeneous Reinnervation of the Diaphragm

Principal Investigator: Lloyd Guth

Other Investigators: Karl Frank
Lamar Soutter, Boston University School of Medicine
James B. Campbell, Columbia University College of Physicians and Surgeons

Cooperating Units: Laboratory of Neurophysiology

Man Years (calendar year 1958):
Total: 0.9
Professional: 0.7
Other: 0.2

Project Description:

Objectives: To determine whether diaphragmatic function can be maintained by nerves other than the phrenic.

Methods employed: The proximal vagus and distal phrenic segments were anastomosed in the rat and the proximal recurrent laryngeal and distal phrenic has been anastomosed in both rats and in monkeys. Arterial sleeves or Millipore tubes were used for the anastomoses. Conventional electrophysiological recording techniques have been employed.

Major findings: 1. Diaphragmatic function is restored in the rat within 6 months after vagophrenic anastomosis. 2. The normal vagus nerve transmits efferent respiratory bursts synchronous with those of the phrenic nerve. The efferent bursts are carried by the recurrent laryngeal fibers within the vagus nerve.

Part A. Project Description (cont'd)

Significance: If the human recurrent laryngeal nerve also transmits efferent respiratory volleys, there is a possibility that this nerve can substitute for the phrenic nerve. Such an operation might enable patients with bulbar poliomyelitis to survive without the assistance of an artificial respirator.

Proposed course of project: Anastomosis of recurrent laryngeal and phrenic nerves has been performed in the monkey and the rat. These animals will be examined for evidence of diaphragmatic function as soon as sufficient time has elapsed for the nerve regeneration to be completed. If diaphragmatic function is restored in these animals the operation will be performed on man.

Part B included: Yes

Serial No. NINDB-NA-DR-7

PHS-NIH
Individual Project Report
Calendar Year 1958

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. 1959 (in press).

Honors and Awards relating to this project:

Serial No. [illegible]-NX-8
1. Neurological and S[illegible]
2. Section on Developm[illegible]
and Regenerat[illegible]
3. Bethesda, Maryland
4. New

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Experimental analysis of the nerve fiber - taste bud relationship.

Principal Investigator: Lloyd Guth

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1958):
Total: 0.5
Professional: 0.3
Other: 0.2

Project Description:

Objectives: To study the so-called "trophic influence" of nerve fibers on taste buds.

Methods employed: Transection of the gustatory nerves and reinnervation of the circumvallate papilla with various nerves. X-irradiation of the circumvallate papilla.

Major findings: 1. Denervation of the circumvallate papilla results in loss of taste buds and thinning of the mucosal epithelium. 2. Reinnervation of the papilla with glossopharyngeal or vagus nerves results in redifferentiation of taste buds whereas reinnervation with the hypoglossal nerve fails to initiate taste bud formation. 3. Mitotic counts on colchicinized, denervated lingual epithelium has failed to reveal any effect of denervation on mitotic rate. However the variability of the method requires that a large volume of material be studied. 4. Study of a small number of x-irradiated tongues failed to reveal evidence of alteration in taste buds.

Significance: This is one of the few areas in which the problem of differentiation and induction may be studied in the adult mammal rather than in amphibia or embryos.

Page 2 Serial No. NINDB-NA-DR.-8

Part A. Project Description (cont'd)

Proposed course of project: Further work on the project is postponed until additional technical assistance becomes available to assist in the preparation and study of material.

Part B included: Yes

Serial No. NINDB-NA-DR-8

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Guth, L.: The effects of glossopharyngeal nerve transection on the circumvallate papilla of the rat. Anat. Rec. 128:715-732, 1957 (but appearing in spring, 1958).

Guth, L.: Taste buds on the cat's circumvallate papilla after reinnervation by glossopharyngeal, vagus, and hypoglossal nerves. Anat. Rec. 130:25-37, 1958.

Honors and Awards relating to this project:

Serial No. NINDB-NA-DR-9
1. Neuroanatomical Sciences
2. Section on Development and Regeneration
3. Bethesda, Maryland
4. New

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Nervous System Pathology in Macaca Mulatta after Asphyxia Neonatorum

Principal Investigators: J. B. Ranck and W. F. Windle

Other Investigators: J. Cammermeyer

Cooperative Units: University of Puerto Rico School of Medicine and U. S. Public Health Service Clinic, San Juan, Puerto Rico.

Man Years (calendar year 1958):
Total: 1.2
Professional: 0.9
Other: 0.3

Project Description:

Objectives: To determine the pattern of neuropathology 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 microscopic pathology. Cinematographic and other records of experiments were used for

Part A Project Description (cont'd)

reviewing functional observations. Two were selected for initial study.

Major findings: One experimental animal, which showed extensive neurological deficits during life was killed after nine days and paired with a control of similar age. The brain damage was almost perfectly symmetrical in the experimental animal and was principally in the grey matter. There were changes in white matter and destruction of myelin (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 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. In a few folia of the vermis of the cerebellum there was loss of Purkinje cells and a slight neuroglial reaction.

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. There was an intense "neuronophagia" in the thalamus.

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 grey matter of the cord (except in thoracic segments) and the roof nuclei of the cerebellum.

Among the undamaged regions were the amygdala, olfactory nuclei, most of the hypothalamus, the lateral geniculate, pontine nuclei, inferior olive, most of the nuclei on the floor of the fourth ventricle, and the stratum zonale and substantia gelatinosa of the spinal nucleus of the trigeminal nerve and the dorsal horn of the spinal cord.

Significance: This is the first experimental neuropathological study in an infrahuman neonatal primate. Histopatholog

Part A Project Description (cont'd)

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 are prominent. The picture in the monkey after asphyxia neonatorum resembles that reported in human infants with "kernicterus".

Proposed course of project: To continue as quickly as time and preparation of material permit.

Part B included: No

Serial No. NIMDB-NA-DR-10
1. Neuroanatomical Sciences
2. Section on Development and Regeneration
3. Bethesda, Maryland
4. Same as NIMDB-NA-10-195?

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: The significance of the acridine orange staining of neurons in vitro and in vivo.

Principal Investigator: K. M. Wolf

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1958):
Total: 0.8
Professional: 0.6
Other: 0.2

Project Description:

Objectives: a) To establish the fluorescent image which breathing neurons will display when stained with acridine orange (AO); to compare it with the image displayed by fixed neurons or by injured, teased ones stained supravitally; and to establish the histochemical significance of these images. b) To study the interactions of AO and the substrates to which it binds by spectroscopic methods.

Methods employed: a) Cultures of fibroblasts and pigment epithelial cells were grown in Paul perfusion chambers. Cultures of chick embryo spinal ganglia have been grown in plasma clots by the Maximow double coverslip method. These cultures, and supravitally traced materials, have been stained with AO and examined by fluorescence microscopy.

b) Solutions of AO in combination with various polynucleotides have been studied by absorption spectroscopy and the spectra have been mathematically analyzed.

Part A. Project Description (cont'd)

Major findings: a) Cell monolayer cultures grown continuously in the presence of one part per million of AO, stained adequately for fluorescence microscopy. Living cells stained orthochromatically (green fluorescence). Reversibly injured cells acquired metachromatic (red fluorescent) granules in the cytoplasm. With further injury, the nucleolus and the entire cytoplasm became metachromatic. This degree of injury was irreversible, and the cells degenerated. Spinal ganglion cultures were harder to stain and to observe, possibly because of the dense growth of satellite cells necessary for their health. However, the same sequence of staining events was observed in the cultured neuron. The metachromasy of the irreversibly injured cells was like that seen in fixed cells and was probably due to RNA. The metachromatic granules seen in reversibly injured cells probably were not RNA; in the neuron their shape and distribution were not those of the Nissl substance. In supravitally stained, teased retinal rods, metachromasy was confined to the ellipsoid segment, which contained all the mitochondria but little if any RNA.

b) Spectroscopic studies showed that many polyelectrolytes including natural and synthetic polynucleotides, can bind AO in such a manner as to cause bound AO molecules to associate, or "stack". Stacked AO had metachromatic fluorescence. When the dye-binding sites of the polyelectrolyte were in great excess of the AO molecules, they distributed themselves among the available sites and unstacked. The amount of stacking was a function of the binding site/AO ratios and of the intrinsic tendency of the polyelectrolyte to produce stacking of bound AO. This tendency can be expressed as a stacking coefficient, which varied according to the chemical structure of the polyelectrolyte. Calculations are underway to test an equation which relates the stacking coefficient and the binding site/AO ratio to the molar extinction of bound AO at any given wavelength.

Significance: AO, because of its low toxicity and metachromasy, is a rare tool with which to study the chemical reactivity to living, intact cells. Tissue cultures provide growing, mechanically undisturbed cells whose staining can be observed in detail. Spectroscopic studies are elucidating

Part A. Project Description (cont'd)

the physico-chemical basis of AO staining, and will help to interpret the fluorescence observed in living cells in terms of specific chemical structures.

Proposed course of project: The observations on cultures in Paul chambers were made in collaboration with Dr. Samuel Aronson, Opthalmology Branch, National Institute of Neurological Diseases and Blindness. They are complete and will be prepared for publication.

The observations on cultured neurons will be continued and extended.

The spectroscopic studies on a quantitative theory of AO binding are a collaborative effort with Dr. Dan F. Bradley Laboratory of Physical Chemistry, National Institute of Mental Health. A preliminary report is in press and further reports will be submitted for publication as the calculations are completed.

Part B included: Yes

Serial No. NINDB-NA-DR-1b

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Bradley, D. F. and M. K. Wolf: The Neurochemistry of Polynucleotides, in: Symposium on the Neurochemistry of Nucleotides: Neurology, supplementary volume, 1958 (in press).

Serial No. NINDB-NA-DR-11
1. Neuroanatomical Sciences
2. Section on Development and Regeneration
3. Bethesda, Maryland
4. Same as NINDB-NA-8

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Structure and chemistry of photoreceptor cells

Principal Investigator: Richard L. Sidman

Other Investigators: Ned Feder

Cooperating Units: None

Man Years (calendar year 1958):
Total: 1.0
Professional: 0.7
Other: 0.3

Project Description:

Objectives: To define the structural and chemical properties of photoreceptor cells, in relation to retinal function.

Methods employed: Histochemical and spectrophotometric methods were used. Most studies were carried out with mouse eyes fixed by a new freeze-substitution method of fixation and stained by various histochemical methods. Suspensions of frog, cattle, and monkey retinal rods were prepared by differential centrifugation in sucrose and their absorption spectra were recorded with a double beam recording spectrophotometer.

Major findings: A series of new structural features in rodent retinas were described in a Symposium on Photoreception at the New York Academy of Sciences.

(a) A description was given of the "interstitial zone" which relates the retinal pigment epithelium to the rods and cones. The apical parts of the pigment epithelial cells contain periodic acid-Schiff (PAS) positive granules. The apical border of these cells is microvillous and PAS-positive. The space between this apical border and the outer segments of

Part A Project Description (cont'd)

rods and cones is occupied by an homogenous "interstitial substance" containing acid mucopolysaccharide. These morphological features imply a secretory function for the pigment epithelium, with the secretion directed toward the photoreceptor cells.

(b) The first convincing evidence was obtained for the presence of cone cells in retinas of rats and mice. Their structural and histochemical properties are comparable to those of cones in other vertebrate species. Cones were detected also in the guinea pig retina, in confirmation of a few reports in the older literature.

(c) Internal structure was detected with the light microscope in the rod-bipolar synapse, as described with the electron microscope by several investigators in recent years. After fixation by freeze-substitution, the synapse appeared as a central, dense, round or oval sphere surrounded in turn by an almost clear zone and a dense outer margin. The central sphere contained lipid and a PAS-positive substance, while the surrounding clear zone and marginal zone had different histochemical properties.

(d) A difference between rods and cones was detected during study of the distribution of dehydrogenase enzymes in photoreceptor cells. DPN diaphorase, and presumably the associated dehydrogenases, were found in the ellipsoids of rods and in both ellipsoids and byoids of cones. Succinic dehydrogenase, on the other hand, was found only in ellipsoids in both types of cells. Studies are in progress on a wider range of vertebrate retinas, to determine if this difference between rods and cones is general.

(e) The absorption spectrum of rhodopsin shifted to higher wavelengths when this visual pigment was studied in intact rods than when extracted into solution. Rhodopsin probably exists in a different form or molecular shape in vivo than when extracted.

Significance: It is self-evident that each advance in our understanding of retinal structure and chemistry will aid our understanding of visual function.

Proposed course of project: This project will be

Part A Project Description (cont'd)

terminated early in January, 1959.

Part B included: Yes

Serial No. NINDB-NA-DR-11

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Sidman, R. L., Histochemical studies on photoreceptor cells. Ann. N.Y.Acad.Sci., 1958 (In press).

Honors and Awards relating to this project:

First Richard Stearns Memorial Lecturer, Albert Einstein College of Medicine, New York, May, 1958.

Serial No. NINDB-NA-DR-12

1. Neuroanatomical Sciences
2. Section on Development and Regeneration
3. Bethesda, Maryland
4. Same as NINDB-NA-9

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Development of new histochemical methods.

Principal Investigators: Ned Feder, Richard L. Sidman

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1958):
Total: 0.9
Professional: 0.7
Other: 0.2

Project Description:

Objectives: To devise new methods for the localization and characterization of chemical substances in cells.

Methods employed: A new method of fixation by freeze-substitution was developed. Tissue samples were frozen rapidly at -175°C and then placed in a "substituting fluid" which simultaneously dissolved ice and fixed the tissue at -70°C. Dehydration and fixation were thus achieved with little distortion of tissue structure. The most useful substituting fluids have been osmium tetroxide in acetone and mercuric chloride or picric acid in ethanol, all at -70°C. A variety of nervous, special sensory, and other tissues were studied.

Major findings: A number of new structural features in retina were described in another report. Skeletal muscle was fixed regularly without the contraction and distortion which usually accompany fixation. During study of brain meninges infected experimentally with the fungus Cryptococcus neoformans, a nucleus was demonstrated in the organism for the first time. The nucleus had an eccentric position, a diameter of less than 1μ, and contained DNA like other nuclei. The method served for demonstration of nerve fibers in central and peripheral tissues by both silver stains and supravital methylene blue. The method also allowed preservation of all lipids in tissue,

Part A Project Description (cont'd)

as well as other molecular species difficult to preserve, such as acid mucopolysaccharides.

Significance: The method is probably the best available for the accurate fixation of small tissues and the preservation in them of a wide variety of chemical substances. It is already being used in a number of other laboratories engaged in histochemical studies.

Proposed course of project: A further analysis of the structure and chemical characterization of sense organs and developing brain will be undertaken when the investigators establish their new laboratory at Harvard Medical School.

Part B included: Yes

Serial No. NINDB-NA-DR-12

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Feder, N. and R. L. Sidman: Methods and principles of fixation by freeze-substitution. J. Biophysic. and Biochem. Cytol., 5:593-602, 1958.

Honors and Awards relating to this project: None

Serial No. NINDB-NA-DR-13
1. Neuroanatomical Sciences
2. Section on Development and Regeneration
3. Bethesda, Md. and San Juan P.R.
4. Same as NINDB-NA-13

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Behavior and social organization of rhesus monkeys on Cayo Santiago, Puerto Rico

Principal Investigator: Stuart A. Altmann

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1958):
Total: 0.6
Professional: 0.3
Other: 0.3

Project Description:

Objectives: To observe and record behavior and social organization of Macaca mulatta monkeys on Cayo Santiago with the view of obtaining basic control data for experiments in neurological and psychological deficits of adverse factors in the perinatal period of this species. To collect normal reproductive and gestational data. To observe and study the infant from birth.

Methods employed: Direct observations and recordings in motion pictures and sound tape were made. The great complexity of the social behavior of primates is a result of their extensive repertoire of behavior and of the many ways in which they combine the elements of this repertoire into a wide variety of distinct sequences of behavior, or "courses of action". Thus, in order to maximize the accuracy of predictions of social behavior, it is essential that we obtain estimates of the probabilities of sequences of events. These estimates are based on the relative frequency of each possible course of action. The frequencies are obtained from random samples of social interactions. The sampling that was begun in 1957 was continued until June, 1958.

Part A. Project Description (cont'd)

Major findings: Of primary significance, the organization of a primate society can be expressed as a set of rules that are independent of any particular member of the society and which depend upon the fact that each member of the society passes through essentially the same life history pattern. These rules can be given as a set of probabilities of courses of action.

Analysis of sequential dependencies of social behavior requires a formal model of social organization. A stoichiometrical model, based on the mathematical theory of communication, has been developed.

While the task of analyzing the extensive data that have been collected on the life history pattern and on the organization of the society has not been completed, certain outstanding characteristics are noted.

Individual recognition is highly developed in monkeys and is of considerable importance in determining the status of the individual in the society. For example, a monkey must be able to distinguish between those whom he can dominate in food competition and those who dominate him and are therefore dangerous to challenge. In addition, he must be able to make temporary shifts in these distinctions to take into account the changes in mood of his companions. For example, a playful adult male can be approached safely, whereas the same male, when angry, cannot be. Beyond that, the monkey must continuously revise these predictions as he and his companions mature.

Thus the ontogeny of recognition is of paramount importance in understanding the social behavior of monkeys. The infant monkey's purview expands as a result of his own curiosity and the strong attraction that the infant has for other members of the society. His ability to distinguish dangerous situations and individuals is further facilitated by his mother's interventions in his own behavior and in that of individuals who are associated with him.

One of the most outstanding characteristics of the groups of monkeys now on Cayo Santiago is their remarkable social stability. In this respect, they are in sharp contrast to the highly unstable conditions that were reported shortly after the creation of the colony in 1938. A number of factors seem to be responsible for this change. The consistency of the life history pattern has already been mentioned. The displacement of aggression onto subordinates has considerably replaced the

Part A. Project Description (cont'd)

continuous contests for status that seem to have characterized the colony shortly after its formation; the status of dominant members of the group is now very rarely contested. In addition, aggression now generally involves only threats; overt physical aggression is rare. Beyond that, suppression by dominant males of aggression among subordinates, and the strong attraction of the entire group to these dominant males have tended to reduce disjunctive tendencies. The cohesiveness of the group has been further enhanced by the strong bond between mother and infant and its persistence during the juvenile period, by the tendency to restrict interactions to members of the peer group, and by the marked ability of the monkeys--all except six of whom have spent their entire lives on the island--to recognize individuals and the boundaries of their group.

The annual cycle of reproductive activity that was reported for 1957 was again observed. Once again, extensive sexual activity between adults occurred during the four months beginning in mid-September. Parturition correspondingly occurred from February through May.

Significance: The opportunity to study a colony of rhesus monkeys under free ranging conditions is unique in the new world.

The basic research with the colony of Cayo Santiago has transformed it into one of the best-known groups of free ranging mammals. Despite the fact that rhesus macaques are used more extensively in biomedical research than all other primates combined, many fundamental biological questions about this monkey have never been adequately studied. A continuation of research with the rhesus colony on Cayo Santiago may well provide answers to many of these questions.

Proposed course of project: To complete analysis of data collected.

A short break in observations was occasioned by transfer of the Principal Investigator to Harvard University. A new phase of the work will begin in November 1958, when another investigator comes on duty.

Part B included: No

Serial No. NINDB-NA-DR-14

1. Neuroanatomical Sciences
2. Section on Development and Regeneration
3. San Juan and Cayo Santiago
4. Same as NINDB-NA-14

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Physical measurements of rhesus monkeys from birth to old age

Principal Investigator: S. A. Altmann

Other Investigators: J. Gavan and K. Chandler

Cooperating Units: Medical College of South Carolina

Man Years (calendar year 1958):
Total: 0.8
Professional: 0.5
Other: 0.3

Project Description:

Objectives: To arrive at age estimates of monkeys by establishing standard physical measurements.

Methods employed: The techniques of anthropometry are being used to obtain data on: sitting height, head length, head breadth, foot length, tail length, dentition and weight. The definitions of A. H. Schultz were used. To these, the technique of radiographic recording of skeletal parts is about to be added.

Major findings: It is too early to report results. Data are being collected and will be analyzed.

Significance: Mensuration data on the monkeys of Santiago Island are of primary value as a means of estimating ages. Age determination is essential for study of growth and development, behavioral ontogeny and other studies.

Proposed course of project: To continue the project. To

Part A Project Description (cont'd)

correlate data with that obtained from the caged colonies in Charleston, S.C., by Dr. Gavan who has received extramural support for five years.

Part B included: No

Serial No. NINDB-NA-DR-15
1. Neuroanatomical Sciences
2. Section on Development and Regeneration
3. San Juan, Puerto Rico
4. Same as NINDB-NA-17

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Technique of neurological examination of the monkey (Macaca mulatta).

Principal Investigator: J. Ranck

Other Investigators: Marisa I.R. Ramirez de Arellano, C. M. Combs, D. L. McCrosky, H. N. Jacobson

Cooperating Units: University of Puerto Rico Medical School, San Juan

Man Years (calendar year 1958):
Total: 1.3
Professional: 1.0
Other: .3

Project Description:

Objectives: To design and perfect techniques for carrying out neurological examinations of monkeys from the first day of life to maturity.

Methods employed: Adaptation of standard methods used in the human neurological examination, when possible, recording photographically each step in motion pictures. The monkeys on Cayo Santiago and in the San Juan caged colony are used as subjects.

Major findings: Considerable progress has been made in the infant monkey. A protocol with "check sheets" has gone through several revisions and is now being used in comparing asphyxiated with control animals. It is too early to analyze the data completely. Normal maturational patterns have been established. Deviations have been observed after asphyxia neonatorum, some of which occur with regularity.

Significance: Although the monkey is being used for

Part A Project Description (cont'd)

experimental neurological investigations in many institutions, no satisfactory standards for the neurological examination have been published.

Proposed course of project: To complete the study, and publish results illustrated by motion picture.

Part B included: No

Serial No. NINDB-NA-DR-16

1. Neuroanatomical Sciences
2. Section on Development and Regeneration
3. Bethesda, Md. and San Juan, P.
4. New

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Normal reproductive function in the rhesus monkey

Principal Investigators: Howard N. Jacobson

Other Investigators: None

Cooperating Units: University of Puerto Rico Medical School and USPHS Clinic, San Juan.

Man Years (calendar year 1958):

Total:	0.5
Professional:	0.1
Other:	0.4

Project Description:

Objectives: To obtain data on menstruation in monkeys under standard conditions. To observe whether or not seasonal variations occur. To study spontaneous fluctuation of these cycles and attempt to alter them experimentally.

To obtain data on conceptions in the monkey and determine whether or not seasonal changes in conception rate occur. To study factors influencing the relation between conception (presumably together with ovulation) and the menstrual rhythm of individual monkeys.

Methods employed: The breeding colony in Puerto Rico provides an excellent source of monkeys for study. The animals are fed a standard optimum diet and are inspected daily for evidence of menstrual bleeding. An attempt will be made to alter the usual rhythm by means of implanted induction coils whose current is lead to the nerves supplying the uterus to induce bleeding. Simultaneous examination of the endometrium will be performed.

Part A Project Description (cont'd)

Matings are presently confined to what is considered the most optimal portion of the menstrual cycle. To determine the relationship of conception to the menstrual rhythm chosen couples will be mated outside of the usual period and the results will be obtained by visual inspection of the genital tract.

Major findings: The menstrual cycle of individual monkeys is subject to wide variations. These variations seem to be equally spread throughout the year. There is no systematic relation between regularity of cycles and fertility. A change in the time of mating within the cycle (i.e. on day 11 rather than day 14) tends to alter the time to the so called implantation bleeding. It seems from this data that implantation bleeding is more a function of the animals own menstrual rhythm than it is of length of residence of the embryo in the uterine cavity. If this can be confirmed it will require different explanations for how the bleeding is produced.

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.

Significance: Most of the various hypotheses concerning reproduction in the human have their origin in observations on primates. Our data indicate that some of the conclusions drawn from earlier work, particularly about the autonomy and the fixed character of the hormonal regulation, require additional restrictions and may have to be revamped.

Proposed course of project: To study the relationship between the nervous system and reproduction by means of electrical stimulation of the uterus, by continued study of the nerve distribution in the uterus, and by studying factors limiting the period within a given menstrual cycle during which ovulation and conception will occur.

Part B included: No

Serial No. NIMDB-NA-DR-17

1. Neuroanatomical Sciences
2. Section on Development and Regeneration
3. Bethesda, Maryland, San Juan
4. New

PHS-NIH
Individual Project Report
Calendar Year 1958

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.

Man Years (calendar year 1958):
Total: 0.5
Professional: 0.2
Other: 0.3

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.

Methods employed: A nursery, resembling in many details those in use for the care of human infants, is maintained. Records are kept of daily weights, daily milk intake, temperature, respiration rate. Dentition is recorded. Heart rates are recorded electrically. Grasp reflex is measured routinely. Maturation of the ability to self-feed is assessed.

Major findings: More than 50 monkeys were born in cages and an equal number in the free-range colony. Infant monkeys show a regular pattern of growth and development, but with wide ranges of normals. Any study of infant monkeys must first start with a knowledge of what is normal. Such standards for infant monkeys are not plentiful.

Significance: The significance of this study lies mostly in its usefulness to others establishing primate colonies. It

Part A Project Description (cont'd)

is also essential in assessing the standards of care of a nursery to be able to compare it to others.

Proposed course of project: To continue collection of data.

Part B included: No

Serial No. NINDB-NA-DR-18

1. Neuroanatomical Sciences
2. Section on Development and Regeneration
3. Bethesda, Md. and San Juan, P.R.
4. New

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: The intrinsic nerve supply to the endometrium in cat, guinea pig, monkey and man.

Principal Investigator: Howard M. Jacobson

Other Investigators: Ivan Pelegrina Sariego

Cooperating Units: University of Puerto Rico Medical School and USPHS Clinic, San Juan

Man Years (calendar year 1958):

Total:	0.6
Professional:	0.3
Other:	0.3

Project Description:

Objectives: To study the course and distribution of nerves in the endometrium of several species of animals. To see whether the nerves are distributed to the vascular elements or supply the glandular elements as well.

Methods employed: The endometrium of adult cats, guinea pigs and rhesus monkeys is stained by the methods of Dastur and of Weddell using methylene blue dye. The dye is injected intra-arterially and locally into the uterus of lightly anesthetized animals. Whole-mounts of tissue are prepared for examination. Counter staining is used to show structural relationships of the nerve distribution. Human uteri are obtained at elective hysterectomy.

Major findings: The endometrium of the cat is densely supplied with fine terminal nerves. These nerves pass through the endometrium and are found approaching the surface epithelium. In certain areas the nerves seem to be arborizing around the

Part A Project Description (cont'd)

bases of the endometrial glands.

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 further demonstration of the nerve supply may help elucidate mechanisms of uterine bleeding.

Proposed course of project: To continue the histological studies and compare results in the several species.

Part B included: No

Serial No. NINDB-NA-DR-19
1. Neuroanatomical Sciences
2. Section on Development and Regeneration
3. Bethesda, Md. and San Juan
4. Same as NINDB-NA-12-1957

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Neurological deficits of asphyxia neonatorum in macaca mulatta.

Principal Investigators: W. F. Windle, J. B. Ranck, C. M. Combs and H. N. Jacobson

Other Investigators: C. J. Bailey, Marisa Ramirez de Arellano, W. Stiehl, J. G. Frontera, D. McCroskey, S. V. Saxon

Cooperating Units: University of Puerto Rico Medical School, San Juan

Man Years (calendar year 1958):
Total: 1.5
Professional: 1.1
Other: 0.4

Project Description:

Objectives: To determine acute and chronic neurological changes caused by asphyxia neonatorum in the macaca mulatta.

Methods employed: Fetuses of known gestational age were delivered at Caesarian section shortly before full term from mothers mated under controlled conditions. Fetuses were asphyxiated by removal of the uterine contents without opening the chorionic sac. Animals were asphyxiated for varying lengths of time. Others served as controls. Those asphyxiated infants not able to breathe spontaneously were resuscitated by oxygen insufflation of the lungs by intermittent positive pressure until they ventilated themselves. Experimental and control animals were raised with identical maintenance and testing procedures, except when illness or severe damage interfered. Extensive use was made of motion picture photography and other recording devices providing

Part A. Project Description (cont'd)

opportunity for review and comparison of experiments. Neurological examinations were performed regularly.

Animals whose survival seemed unlikely and a few others were killed for pathological study. One infant was asphyxiated by unintentional retention of the head within the vaginal canal during a spontaneous breech delivery.

Major findings: Of eight infants asphyxiated for 11.5 minutes or less and which then breathed spontaneously, 5 showed no abnormalities after recovery from the acute period. Three animals had deficits in sucking, one of which also had deficits for 10 days in righting, motor dexterity and tonicity.

Of 13 infants, asphyxiated for 7 to 17 minutes and requiring resuscitation, all had deficits in sucking and the quality and extent of voluntary motion was affected. However, no differences from the controls were noted in voluntary motion after 3 weeks in the 3 infants surviving that long.

Nine resuscitated infants had deficits in righting. Eight resuscitated infants had deficits in reaction to dropping. Flame-shaped petechial hemorrhages were seen in the retina in 8 infants. Six infants had deficits in localizing sound. Two infants had hypotonic musculature for a week. In one of these the hypotonia alternated with generalized rigidity.

Other abnormalities in resuscitated infants observed only once included a III nerve palsy, a 3 per second tremor lasting 7 days, status epilepticus, papilledema, and loss of control of body temperature.

Significance: Experimental evidence that asphyxia neonatorum in a primate can produce symptoms, comparable to certain ones described in human neurological disorders of infancy, has been obtained for the first time.

Proposed course of project: To continue and extend this project. Attempts are being made to produce more surviving animals and to test them psychologically. Physiological and chemical studies of cardiovascular and respiratory functions will be related to the neurological studies.

Part B included: Yes

Serial No. NINDB-NA-DR-19

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Windle, W. F.: Editor, "Neurological and psychological deficits from asphyxia neonatorum". Springfield, Illinois, Charles C Thomas, 1958.

Honors and Awards relating to this project:

Serial No. NIMDB-NA-DR-20
1. Neuroanatomical Sciences
2. Section on Development and Regeneration
3. Bethesda, Maryland
4. New

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Psychological effects of asphyxia neonatorum in rhesus monkeys.

Principal Investigators: C. J. Bailey, Sue V. Saxon

Other Investigators: None

Cooperating Units: University of Puerto Rico Medical School, San Juan; U.S. Public Health Service Clinic, San Juan

Man Years (calendar year 1958):
Total: 0.7
Professional: 0.4
Other: 0.3

Project Description:

Objectives: To construct a large battery of tests that can be administered to young monkeys. To determine the effects of neonatal asphyxia on the performance of monkeys in these tests. To correlate the results of the psychological studies with histopathological observations on the brains.

Methods employed: Pregnant monkeys were delivered at term by means of cesarean section. In some cases, infants were retained in the intact membranes in order to asphyxiate them; in other cases they were allowed to breathe immediately and served as controls. Asphyxiated infants were resuscitated, if necessary, by intratracheal insufflation with oxygen. Activity, curiosity, emotionality, and learning ability were studied by means of the psychological test battery.

Major findings: Most of the time was spent on designing, constructing, and testing the battery of tests. A series of tests was decided upon that involves training the animals every day for the first 2 years of life. So far only 8 pairs

Part A Project Description (cont'd)

of monkeys have started this test battery, the oldest having finished about one-third of the testing program, and the youngest about one-tenth. Although it is still too early to determine what the final outcome of this testing program will be, so far there appears to be no consistent difference between the asphyxiated and normal animals.

Significance: Not enough animals have progressed far enough in the testing program to decide whether the monkey differs from other animals, such as the rat, guinea pig, and cat, in its reaction to asphyxia neonatorum.

Proposed course of project: The psychological test battery is still being perfected. Some tests will be dropped and new ones invented. Present animals will proceed with the battery, and others, particularly those with a more severe asphyxia, will be started.

Part B included: No

Serial No. NINDB-NA-DR-21
1. Neuroanatomical Sciences
2. Section on Development and Regeneration
3. Bethesda, Md. and San Juan, P.R.
4. Same as NINDB-NA-15-1957

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Psychological and histopathological deficits of asphyxia neonatorum in guinea pigs.

Principal Investigator: C. J. Bailey

Other Investigators: W. F. Windle
Marisa I. R. Ramirez de Arellano

Cooperating Units: University of Puerto Rico School of Medicine, San Juan
U.S. Public Health Service Clinic, San Juan

Man Years (calendar year 1958):
Total: 0.8
Professional: 0.6
Other: 0.2

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 mazes and (e) histopathological correlations. Littermate controls were used.

Part A. Project Description (cont'd)

Major findings: Using the Becker alternation maze, differences between controls and experimental animals at 8 to 13 weeks of age were not statistically significant in respect to time of running and number of errors; there was a difference in respect to repetitive errors significant beyond the .01 level. In another group of animals started in the Becker maze in the first two weeks of life, the controls were significantly faster in running the maze than the asphyxiated littermates ($p = .05$). Using a closed field water maze, the asphyxiated guinea pigs (now 12 to 19 months old) made more errors than their littermate controls ($p = .01$). In order to test retention the animals relearned each maze. There was no consistent difference in the asphyxiated and nonasphyxiated pigs. In order to test the effect of stress on asphyxiated guinea pigs, one group relearned the Becker maze with an electric shock on its entire floor, except in the goal box. No difference could be detected between the experimental and control animals. Although there were significant correlations among various measures of degree of asphyxia and degree of neonatal neurological deficits, there were no significant correlations among the measures of degree of asphyxia and performance in the mazes.

Significance: Asphyxia neonatorum in the guinea pig--previously shown by Windle and Becker to produce neurological and psychological deficits correlated with structural brain damage in the first 8 to 10 weeks of life--now appears to have produced effects on adult ability to learn simple problems.

Proposed course of project: The acute and chronic physiological and experimental psychological aspects have been completed. Though many interesting leads could be pursued profitably, the time of the investigators must be devoted to primates next year. The histopathological studies will be continued at Bethesda and correlations drawn where possible.

Part B included: No

Serial No NINDB-NA-DE-32
1. Neuroanatomical Sc[illegible]
2. Section on Develop[illegible]
and Regeneration
3. Bethesda, Md. and San [illegible]
P.R.
4. New

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Centers and pathways involved in induced cerebellar seizures.

Principal Investigator: C. M. Combs

Other Investigators: D. L. McCroskey

Cooperating Units: University of Puerto Rico School of Medicine
U.S. Public Health Service Clinic San Juan

Man Years (calendar year 1958):
Total: 0.8
Professional: 0.4
Other: 0.4

Project Description:

Objectives: To obtain a clearer understanding of the cerebellar role in movement by determining the structures involved in the phenomenon of the long-lasting induced cerebellar seizures. To then study the effects upon the cerebellar seizures of electrical stimulation and lesions in the involved structures.

Methods employed: Initial experiments are being performed on cats. The seizures are produced with permanent implanted electrodes in the chronic animal. During the seizures recordings are made from other deep electrodes. Subsequently stimuli are delivered or lesions are placed in the latter structures to study the resultant effects upon the cerebellar seizure.

Stereotactic mapping with deep electrodes is being followed by the cathode ray oscillograph and the electroencephalograph. Seizure-producing electrical stimuli are delivered to the exposed cerebellar cortex.

Part A. Project Description (cont'd)

Major findings: This project has been started but too recently for a report of results.

Significance: Abnormalities of posture, coordination and quality of voluntary movements have been observed after asphyxia neonatorum. Physiological studies on role of the cerebellum in these are needed for adequate understanding of the phenomena.

Proposed course of project: To carry out the stated plan. To extend the study to normal monkeys and those suffering neonatal asphyxia.

Part B included: No

Serial No. NINDB-NA-NC-1
1. Neuroanatomical Sciences
2. Section on Neurocytology
3. Bethesda, Maryland
4. Same as NINDB-NA-18-1957

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Ultrastructure of the nervous system.

Principal Investigator: Sanford L. Palay

Other Investigators: Angelo Bairati
E. E. Manuelidis
Spencer Gordon

Cooperating Units: Dept. of Anatomy, University of Milan
Dept. of Pathology, Yale University

Man Years (calendar year 1958):
Total: 1.9
Professional: 1.4
Other: 0.5

Project Description:

Objectives: To study the fine structure and organization of nervous tissue, particularly the synapse, the protoplasm of nerve cells and their processes, and the neuroglia, with special attention to the correlation between structure and function.

Methods employed: The principal instrument employed is the electron microscope. Tissues are prepared for examination by fixation in osmium tetroxide, embedding in methacrylate, and sectioning.

Major findings: A study of the terminals on the giant Mauthner cell in the medulla of the gold fish showed that the boutonlike endings on this cell resemble those in mammals in that they contain myriad vesicles and a cluster of mitochondria. The intrasynaptic cleft is approximately the same diameter. The large club-endings on the lateral dendrite differ from the usual terminals in the following ways: The mitochondria are very small and are aligned opposite the synaptic cleft. The vesicular component is rather limited and is clustered at the sides of the ending instead of at the synaptic cleft. The meaning of these differences is not immediately clear.

Part A. Project Description (cont'd)

A study of axoplasm in the large myelinated fibers of the goldfish medulla, and in peripheral nerves of the rat and cat demonstrates that there are three longitudinally oriented structures in axoplasm: 1. long, extremely slender mitochondria, 2. their membrane-bound canaliculi, and 3. slender threads, termed neurofilaments. In the goldfish, the neurofilaments are the most prominent feature of the large axons. They are neatly aligned parallel with one another and fill almost the entire axon. They are approximately 120Å in diameter. In the smallest axons, the neurofilaments are less numerous and the canalicular elements are the most prominent structure. However, in the size range between the biggest and the smallest, there is no regular relation between diameter of fiber and ratio of filaments to canaliculi. Also, in the fish, there is no regular relation between the thickness of the myelin sheath and the diameter of the axon. That the filaments are not artifacts of fixation is demonstrated by a study with polarized light optics of the fresh nerves and medullary tissue. The axoplasm shows a positive extrinsic birefringence and a slight positive intrinsic birefringence which are not significantly changed by fixation, embedding, and sectioning. The neurofilaments in mammalian peripheral nerves are like those in the large nerve fibers of goldfish medulla, but certain differences are immediately evident. The filaments are not so concentrated or so regularly disposed as in the goldfish and their diameter is approximately half, i.e. 60Å.

Significance: These studies are part of an intensive program to explore the nervous system at the electron microscopic level in order to establish criteria for an adequate fine structural histology of these tissues. Until this is done a clear concept of the interrelations between neurons, neuroglia, and their respective processes cannot emerge. The geography must precede exploitation of the resources.

Proposed course of the project: Preliminary reports of the results on Mauthner cell synapses and on axoplasm are being prepared for publication. The study of the Mauthner cell will be continued, because there are morphological types of synapses on the Mauthner cell other than the ones we have studied so far. Furthermore a study of dendrites will be carried out to learn their characteristics in contrast to those of the axon. A study of the relationship between the dorsal root ganglion cell and its surrounding capsular cells is to be initiated in collaboration with Dr. Rosenbluth. An investigation of the extracellular space in the mammalian central nervous system will be carried on in collaboration with Dr. Spencer Gordon.

Part B included: Yes

Serial No. NINDB-NA-NC-1

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Palay, S. L.: An electron microscopical study of neuroglia in "Biology of Neuroglia," W. F. Windle, Ed., Springfield, Charles C Thomas, pp. 24-38, 1958.

Palay, S. L.: The morphology of synapses in the central nervous system. Exp. Cell Research, Supplement 5, 275-293, 1958.

Palay, S. L.: The morphology of secretion, in "Frontiers in Cytology," S. L. Palay, Ed., New Haven, Yale University Press, pp. 305-342, 1958.

Honors and Awards relating to this project:

Serial No. NIMDB-NA-NC-2
1. Neuroanatomical Sciences
2. Section on Neurocytology
3. Bethesda, Maryland
4. Same as NIMDB-NA-19-1957

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Enzymatic reactions of gamma-aminobutyrate (γ-AB) catalyzed by brain tissue.

Principal Investigator: R. W. Albers

Other Investigators: J. M. McKhann
R. A. Salvador

Cooperating Units: Laboratory of Clinical Chemistry, NIMDB
Laboratory of Neurochemistry, NIMDB

Man Years (calendar year 1958):
Total: 0.8
Professional: 0.5
Other: 0.3

Project Description:

Objectives: To establish and characterize the enzymatic transformations of γ-AB and related compounds which occur when these compounds are incubated with brain tissue under appropriate conditions.

Methods employed: Spectrophotometry, fluorimetry, isotopes chromatography and related techniques.

Major findings: A soluble enzyme which oxidizes succinic semialdehyde to succinic acid has been purified from brain mitochondria. The reaction catalyzed by this enzyme requires diphosphopyridine nucleotide as hydrogen acceptor.

Some evidence was obtained for the possible importance of γ-AB in the energy metabolism of the brain. Comparison of oxygen consumption and oxidation of C^{14}-γ-AB in homogenates of brain tissue indicate that a significant fraction of the respiration of brain tissue *in vitro* may be associated with the reactions involving γ-aminobutyrate.

Significance: The area of metabolism described above is f undoubted importance in cerebral metabolism. The elucida- ion of these reactions is a fundamental necessity for an nderstanding of the operation of the neural mechanism and the erangements of metabolism which may cause certain forms of eurological disease.

Proposed course of project: Further work is planned for btaining information about the rate of metabolism of γ-amino- utyrate *in vivo*. This work will require the development of a echnique for introducing C^{14}-γ-aminobutyrate into the endogenous ool of brain γ-aminobutyrate. This will be approached in three ays:

(1) Continuous intravenous infusion;

(2) injection of a radioactive precursor of γ-aminobutyrate hich will penetrate the blood-brain barrier rapidly;

(3) the use of very young animals in which the blood-brain arrier is not yet established.

art B included: Yes

Serial No. NINDB-NA-MC-2

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Albers, R. W. and R. A. Salvador: Oxidation of succinic semialdehyde by soluble dehydrogenase from brain. Science 128:359 (1958).

Honors and Awards relating to this project:

Serial No NINDB-NA-M-3
1. Neuroanatomical Sciences
2. Section on Neurocytology
3. Bethesda, Maryland
4. Same as NINDB-NA-21-(75)

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Quantitative histochemical distribution of glutamic decarboxylase in the nervous system

Principal Investigator: R. W. Albers

Other Investigators: R. O. Brady

Cooperating Units: Laboratory of Neurochemistry, NINDB

Man Years (calendar year 1958):
Total: 0.9
Professional: 0.5
Other: 0.4

Project Description:

Objectives: To determine the neuroanatomical localization of glutamic decarboxylase in the nervous system.

Methods employed: Liquid scintillation counting; Lowry ultramicroanalytical techniques.

Major findings: The distribution of glutamic decarboxylase has been determined in gross areas of the central nervous system and at the histological level in several areas of the brain and spinal cord of the rhesus monkey. The enzyme is virtually absent in all areas of white matter, and in the neurohypophysis and pineal gland. It is relatively high in certain histological areas of the grey matter and quite low in others.

Significance: Neuropharmacological studies have indicated that gamma-amino butyrate is a powerful inhibitor of synaptic transmission. Since large amounts of gamma-amino butyrate and γ-AB decarboxylase are found uniquely in the central nervous system, the more precise localization of this distribution is of fundamental importance in establishing the physiological role of this system.

Part I Project Description (cont'd

Proposed course of project Work terminated and manuscript has been submitted for publication

Part B included: Yes

Serial No NIMDB-NA-[illegible]

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Albers, R. W. and R. O. Brady: The distribution of glutamic decarboxylase in the nervous system of the rhesus monkey. (In press)

Serial No. NINDB-NA-NC-4
1. Neuroanatomical Sciences
2. Section on Neurocytology
3. Bethesda, Maryland
4. Same as NINDB-NA-22-1957

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Micro-radiometric measurement of decarboxylase reactions.

Principal Investigator: R. W. Albers

Other Investigators: R. O. Brady

Cooperating Units: Laboratory of Neurochemistry, NINDB

Man Years (calendar year 1958):
Total: 0.9
Professional: 0.6
Other: 0.3

Project Description:

Objectives: To devise a method which will permit the measurement of carbon dioxide produced by enzymes in samples of tissue weighing ten micrograms or less.

Methods employed: Ultra-micro analytical techniques of the Lowry type; liquid scintillation counting.

Major findings: A radiometric method for the measurement of glutamic decarboxylase activity in brain tissue has been developed and applied to samples of brain tissue weighing as little as 3 micrograms.

Significance: The method permits quantitative histochemical studies of decarboxylases. An enumeration of the decarboxylating reactions and their products serves to illustrate the importance of a study of decarboxylating enzymes with respect to neurological diseases:

Glutamic decarboxylase produces γ-amino butyric acid;
histidine decarboxylase produces histamine;
5-hydroxy tryptophane decarboxylase produces serotonin;
3,5-dihydroxyphenylalanine decarboxylase produces a precursor of non-epinephrine.

Part A Project Description (cont'd)

The underlying specificity of the method may allow its application to macro-analysis of samples of blood, etc., where other methods are difficult to apply.

Proposed course of project: Work terminated and manuscript has been submitted for publication.

Part B included: Yes

Serial No. NINDB-NA-NC-4

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Albers, R. W. and R. O. Brady: The distribution of glutamic decarboxylase in the nervous system of the rhesus monkey. (In press)

Serial No. NINDB-NA-NC-5
1. Neuroanatomical Sciences
2. Section on Neurocytology
3. Bethesda, Maryland
4. Same as NINDB-NA-23-1957

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: A fluorimetric micromethod for the determination of succinic semialdehyde.

Principal Investigator: R. W. Albers

Other Investigators: R. Salvador

Cooperating Units: Laboratory of Neurochemistry, NINDB

Man Years (calendar year 1958):
Total: 1.0
Professional: 0.7
Other: 0.3

Project Description:

Objectives: To develop a sensitive method to permit the measurement of succinic semialdehyde (SSA) in tissue samples of 10 micrograms or less.

Methods employed: Fluorimetric, ultramicro-analytical and related techniques.

Major findings: SSA may be measured by means of the fluorescence of a derivative formed by reaction with diaminobenzoic acid. The sensitivity of the method is adequate and the method can be applied to the measurement of the gamma-aminobutyrate transaminase activity of brain tissue.

Significance: The development of this method was a requirement for an adequate study of the metabolism of gamma-aminobutyrate. Further development of the ultramicro method has permitted an investigation of the possible association of gamma-aminobutyrate transaminase with certain neuroanatomical structures, and thus to a hypothesis for the physiological role of gamma-aminobutyrate.

Page 2 Serial No. NINDB-MA-MC-5

Part A. Project Description (cont'd)

Proposed course of project: Work terminated and manuscript has been submitted for publication.

Part B included: Yes

Serial No. NINDB-NA-NC-5

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Salvador, R. A. and R. W. Albers: The distribution of glutamic-γ-aminobutyric transaminase in the nervous system of the rhesus monkey. (In press)

Serial No. NINDB-NA-NC-6
1. Neuroanatomical Sciences
2. Section on Neurocytology
3. Bethesda, Maryland
4. Same as NINDB-NA-26-1957
and NINDB-NA-28-1957

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Neurosecretion in the rodent.

Principal Investigator: Milton W. Brightman

Other Investigators: None

Cooperating Units: National Cancer Institute

Man Years (calendar year 1958):
Total: 0.9
Professional: 0.6
Other: 0.3

Project Description:

Objectives: To determine the morphological effects on the neurosecretory system of: (a) physiological stimuli, i.e. suckling, and (b) x-irradiation.

Methods employed: (a) Lactating female mice were allowed to nurse litters for various intervals and changes in neurosecretory content of their hypothalamo-hypophysial systems were noted.

(b) Large doses of 220 KV x-rays which were directed to the hypothalamus, were given to adult rats. Alterations in the brain, spleen and peripheral blood were followed.

Major findings: (a) There is a release of neurosecretory material, which may be closely associated with oxytocin, from the neurohypophysis of a significant number of females.

(b) The neurosecretory nuclei are no more susceptible to x-irradiation than are adjacent nuclei. The earliest changes in the brain are an increase in vascular permeability to trypan blue and the formation, in the irradiated zone, of petechiae and of perivascular globules consisting of a glycoprotein. Neuroglial

Part A. Project Description (cont'd)

and neuronal elements are relatively refractory. A terminal leucocytosis demonstrates that the hemopoietic tissue is still able to respond (e.g. to systemic infection). The structure of the spleens was unaltered.

Significance: Insight into the factors which influence the function of the hypothalamo-neurohypophysial system.

Proposed course of the project: Part (b) has been submitted for publication. Part (a) is to be submitted for publication.

Part B included: No

Serial No. NINDB-NA-NC-7
1. Neuroanatomical Sciences
2. Section on Neurocytology
3. Bethesda, Maryland
4. Same as NINDB-NA-29-1957

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Extraneuronal cholinesterase of the vertebrate central nervous system.

Principal Investigator: M. W. Brightman

Other Investigators: R. W. Albers

Cooperating Units: None

Man Years (calendar year 1958):
Total: 1.1
Professional: 0.8
Other: 0.3

Project Description:

Objectives: To make a systematic study of the sites and probable function of extraneuronal cholinesterase.

Methods employed: The application of the Koelle histochemical method has been extended to 9 species. The development of pseudocholinesterase (PChE) activity in the chick embryo was quantified by a spectrophotometric method.

Major findings: (A) The extraneuronal cholinesterases occur 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.

(B) As early as the fifth day of incubation, there is appreciable PChE activity which is thus attributable to the spongioblast. The embryo becomes susceptible to a number of selective PChE inhibitors at the time of greatest increase in number of neuroglia cells.

Significance: Findings of species differences in the localization of extraneuronal cholinesterases afford an approach to the elucidation of the function of these enzymes. There appears to be a relation between the advent of PChE activity and myelogenesis in the embryo.

Part A. Project Description (cont'd)

Proposed Course of project: The work on species differences in localization of these enzymes is being presented for publication.

Part B included: Yes

Serial No. NINDB-NA-NC-7

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Brightman, M. W. and R. W. Albers: A major component of neurohypophysial tissue associated with antidiuretic activity. J. Neurochem. (in press).

Honors and Awards relating to this project:

Serial No. NINDB-NA-EP-1

1. Neuroanatomical Sciences
2. Section on Experimental Neuropathology
3. Bethesda, Maryland
4. Same as NINDB-NA-30-1957

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Pathogenetical factors in the development of myelopathies.

Principal Investigator: Jan Cammermeyer

Other Investigators: Helen Ramsey, Mignon Malm and Stanislaw Ziemnowicz

Man Years (calendar year 1958):

Total:	1.8
Professional:	1.4
Other:	0.4

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: Volumetric and karyometric measurements were performed on normal spinal cords of several species. Techniques were standardized which made it possible to prepare microscopical sections of uniform appearance.

Major findings: Three series of investigations have been conducted and their results will be adapted to experiments on the spinal cord:

(a) On the basis of new methods developed here it was possible to measure rapidly the volume of the entire cord, its regions and segments; repeated examinations were made on the intact specimens. The percentage size of each region was found

Part A Project Description (cont'd)

to vary with species, e.g., monkey, dog, cat, rabbit and opossum. Considerable individual variations were found within each species.

A further use of this volumetric method led to the observation that the volume of the spinal cord can be estimated according to an equation based on a correlation between the volume of the spinal cord and either body weight or length of leg bones. This method was tried with equal accuracy in monkeys, cats and rabbits.

(b) The combined volumetric and morphological control of the various steps in the preparation of histological sections led to a revision of the entire procedure used for neuropathological investigations. The histological material is now obtained free of several of the unwanted changes which usually occur in the spinal cord. With the precautions adopted, particularly those concerned with fixation and removal of the spinal cord, a number of nonspecific cell changes were avoided. As part of this project, Dr. Mignon Malm, is proceeding with standardization of autoradiographic techniques. Proper handling of tissue through fixation and embedding has given a basis for determining the normality of spinal cord architecture, cell appearance and nuclear size in monkeys, cats, rabbits, chinchillas, rats and mice. The shape and size of neuroglial nuclei in the cat, were found to be influenced by the histological techniques used; this influence was greater on cells of the thoracic region than on those of the cervical region. When a poor fixative was employed (e.g., the widely recommended fixation by submersion of the specimen with 10% formalin) the ill effect was different through various regions of the spinal cord as judged by the number of dark neurons and pyknotic neuroglia cells through the segments of the spinal cord.

(c) Two karyometric surveys were performed on two types of material, the one aimed at the functional significance of neuronal nuclei and the other concerned with individual variations in the size of neuroglial nuclei.

Neurons in the brain of chronically reserpinized monkeys were found to contain enlarged nuclei and nucleoli. This cytological reaction was regarded as evidence of a functional abnormality of temporary nature. No permanent pathological

Part A Project Description (cont'd)

changes were noted in the central nervous system including the spinal cord.

The other karyometric study of neuroglial nuclei in the cat demonstrated that they were of the same size through the cervical and thoracic regions of the spinal cord. However, the size of astrocyte nuclei changed as a linear function to the volume of the spinal cord.

Significance: The evaluation of the significance of the research results already on hand will be divided into three divisions which indeed form an integral part of the overall project.

(a) Volumetric studies of the spinal cord combined with measurements of long bones of the extremities enable us now to determine whether the spinal cord size in a given case might have changed as the consequence of an experiment. This has not been possible to establish until the present technique was developed here. Another asset to this technique is that we are in a position to select animals of equally sized spinal cord after having measured the length of the animal's extremities, this may prove helpful for the planning of experiments with pharmacological agents in order to study their effect on central nervous systems of similar size.

(b) Before any experiments could be performed a much needed control of our histological methods had to be completed. The careful formulation of a standard preparation of histological tissues has almost completely eliminated the presence of artefactual cell changes from our microscopical sections. Thus, we are now more confident about the results of microscopical investigations in particular about those of the spinal cord. Previously, the study of this organ more than any other was hampered by the occurrence of numerous artefactual cell changes. Since a large number of investigators have interpreted these cell changes as representing an intra vitam process, many erroneous conclusions have been reached about results of experiments. The standardization of histological methods formed an important part of autoradiographic investigations in order to identify properly the site of tagged substances and the amount of deposition. Paramount to all morphological study is to preserve as much as possible the size of tissue structures, their relative distance and distribution of particles.

Part A Project Description (cont'd)

(c) The observation that the size of glial nuclei varied with the techniques used and the size of animals pointed out two important facts, namely, (1) morphological studies aimed to identify changes in nuclear size must use material treated according to the best techniques available, and (2) comparison between the size of cells in an experimental and a normal animal is permissible only when the animals are of similar size.

The significance of analysing the size of nuclei, nucleoli was brought out in a study with chronic administration of reserpine to monkeys. Any changes in nuclear size is usually taken as an evidence of changes in intracellular metabolism of nucleic acid. Thus, the karyometric results might indicate that this nuclear function is deranged throughout the nervous system as a consequence of chronic reserpine treatment. Different parts of the brain had responded with different intensity to the treatment. Concomitantly with the cellular changes the animals exhibited the syndrome characteristic of human parkinsonism. From these studies we have also learned that the only material useful for studies on cell function is the one which has been handled with the greatest care. Many papers dealing with similar subjects have used techniques which are not well suited for such studies and their statements have to be reevaluated on the basis of new experiments.

Proposed course of project: Future experiments will be conducted along two separate lines:

(a) Extraspinal factors concerned with spinal cord functions will be studied in joint experiments with Dr. H. Ramsey on the epidural space. These experiments will form a continuation of two nearly completed studies by Dr. H. Ramsey, one on the topography of the epidural fat of the cat and another study on epidural fat of the monkey and rabbit in comparison with that of the cat.

(b) Intraspinal factors involved in spinal cord functions will be studied in several experiments, these experiments will be directed towards three main aspects of experimental neuropathology.

Experimental neuropathology frequently requires anesthesia, trepanation and use of dehydrating agents, therefore a preliminary study with Dr. Ziemmowicz was planned for the purpose of examining

Part A Project Description (cont'd)

to what degree the spinal cord reacts to some of these experimental conditions. Some cats underwent craniotomy in anesthesia and others were treated with urea for varying time and dosage. The brain and spinal cord material was measured volumetrically and then prepared histologically. The material is being readied for microscopical examination.

Another set of experiments will be centered on a correlation between degree of morphological changes and functional deficits of neurons. Animals will perform a certain amount of motor activity shortly before they are killed. Others will receive different drugs which elicit functional abnormalities as after injection with anesthesia, reserpine, acetyl pyridine, curare, and insulin. A series of animals will be subjected to liver damage of different types, these experiments will be controlled by repeated biochemical examinations of the blood. Through these studies we hope to be in a position to account for some of the peculiarities of spinal cord pathology and differences in clinical manifestations between the rostral and caudal regions. Although these studies are mainly concerned with the spinal cord, the brain will be examined too, and the conclusions to be reached will be applicable to the entire central nervous system.

Experiments will be directed towards the factors regulating transportation of substances to the neurons. For this purpose Dr. Mignon Malm has been currently engaged in studying the distribution of isotopes through the brain and spinal cord. Furthermore, a morphological study on the distribution of neuroglia and vessels has been contemplated that we may lay the foundation for a discussion about the inherent factors involved in control of transportation of substances through the nervous tissue. Histological material has been prepared for microscopical examination of the spinal cord, in monkeys, cats, rabbits, chinchillas, rats and mice.

Part B included: No

Serial No. NINDB-NA-EP-2
1. Neuroanatomical Sciences
2. Section on Experimental Neuropathology
3. Bethesda, Maryland
4. Same as NINDB-NA-32-1957

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Distribution of fat in the epidural space in mammals.

Principal Investigator: Helen Ramsey

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1958):
Total: 1.0
Professional: 0.7
Other: 0.3

Project Description:

Objectives: To study the anatomical distribution of fat in the spinal epidural space in several species, to show the variation with age and other factors, to study the histology of epidural fat and to compare this with the histology of other adipose tissue.

Methods employed: Epidural fat was colored in vivo by oral administration of Sudan III dissolved in oil. Animals were killed by perfusion-fixation; the vertebral column was removed and the bone decalcified. Dissections to demonstrate the epidural fat in its proper relations were photographed for comparative study.

Major findings: Complete series of specimens have been accumulated for adult cats, kittens 1 day to 24 weeks of age, rabbits and monkeys. A few specimens of several other species have been studied. Epidural fat in each of the species examined showed a different, characteristic pattern of distribution.

Part A Project Description (cont'd)

Distribution of fat in the epidural space of the cat was shown to follow a distinct metameric pattern characterized by longitudinal lateral masses connected by segmental dorsal masses. The pattern was laid down during the first 12 weeks after birth. Histologically this tissue differed from adipose tissue elsewhere in the body, little fibrous connective tissue being present. Its composition and its relative freedom from attachment make it well adapted to its function.

Significance. This is the first complete description of the disposition of fat throughout the epidural space. Epidural fat serves as a lubricant and protection for the spinal cord, dura, nerve roots and blood vessels against their bony surroundings, particularly during movement of the vertebral column. Since these vulnerable structures are contained within a closed space and influenced by pressure changes in it, all structures of the space assume some physiological significance as well.

Proposed course of project: A completed manuscript on the anatomy and histology of the epidural fat in the cat will be submitted to an anatomical journal. Data on the epidural fat of the rabbit and the monkey are being analyzed and will be readied for publication in the near future. Experiments involving damage to structures of the epidural space will become part of another project.

Part B included: No

Serial No NINDB-NA-EP-3
1. Neuroanatomical Sciences
2. Section on Experimental Neuropathology
3. Bethesda, Maryland
4. New

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Structure of brains of monkeys in which the pituitary gland had been irradiated with high-energy deuterons.

Principal Investigator: J. Cammermeyer

Other Investigators: Cornelius A. Tobias
Gertrude Van Wagenen

Cooperating Units: University of California
Yale University Medical School

Man Years (calendar year 1958):
Total: 1.0
Professional: 0.6
Other: 0.3

Project Description:

Objectives: To survey extent of structural change in brains of animals previously subjected to beams of high-energy deuterons focussed on the pituitary gland.

Methods employed: Four female rhesus monkeys were irradiated on the California Betatron prior to sexual maturity. Several years later they were killed by the technique of perfusion-fixation. The brains were removed and examined for gross pathology. Appropriate portions were sectioned serially and studied for microscopical defects.

Major findings: Two brains were perfectly normal. One brain showed moderate changes in hypothalamic nuclei directly connected with the pituitary. One brain showed severe degenerative changes in the parts of the brain adjacent to the pituitary; these changes were restricted to the pathway of the beam.

Part A. Project Description (cont'd)

Significance: The study of carefully prepared brains following deuteron irradiation helps towards a better understanding of the biological effects that the organism may suffer after exposure to a variety of radioactive sources in particular at high altitudes. The encouraging aspect of the morphological results is a confirmation of the idea formulated by the California scientists that this type of radiation, when properly applied, may be used as a tool to destroy tissues which are not easily accessible.

Proposed course of project: A report was given to the investigators at the University of California. They will incorporate the findings with their data and prepare a final report.

Part B included: No

Serial No NINDB-[illegible]
1. Neuroanatomical Sc[illegible]
2. Section on Functional
Neuroanatomy
3. Bethesda, Maryland
4. Same as NINDB-NA-34-[illegible]

PHS-NIH
Individual Project Report
Calendar Year 1958

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 1958):
Total: 0.9
Professional: 0.5
Other: 0.4

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. (3) To determine whether or not the cochlear nucleus possesses efferent neurons that innervate the hair cells of the cochlea.

Part A. Project Description (cont'd)

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). The employment of these techniques on experimental subjects in which small isolated lesions are properly placed makes it possible to reveal important complexly arranged neuronal connections.

Major findings: Histological preparations of 35 experimental animals (cats and chinchillas) have been completed but the series is not yet sufficient to complete the picture of the afferent projection arrangement in the cochlear nucleus.

Significance: More exact knowledge of the auditory system is essential for a foundation upon which to design intelligent physiological experimentation and for interpretation of the results that lead to understanding of the neuromechanism of hearing.

Proposed course of project: To extend the study of the afferent system in a similar fashion to higher levels of the auditory system including the auditory cortex.

Part B included: No

Serial No. NINDB-NA-FN-2
1. Neuroanatomical Sciences
2. Section on Functional Neuroanatomy
3. Bethesda, Maryland
4. Same as NINDB-NA-35-1957

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: A correlative histopathologic and genetic study of the hearing mechanism in a strain of congenital deaf guinea pigs.

Principal Investigator: G. L. Rasmussen

Other Investigators: None

Cooperating Units: Dr. George Jay, geneticist of the Laboratory Aids Branch, is responsible for the breeding and study of the inheritance of deafness. He has supplied one hundred guinea pigs to this section during the year 1957.

Man Years (calendar year 1958):
Total: 0.8
Professional: 0.4
Other: 0.4

Project Description:

Objectives: (1) To determine the factors of inheritance responsible for the hearing defect. Dr. Jay is the principal investigator of this phase of the project. (2) The chief aim of the investigator of this section is to find, if possible, the underlying morphological defects which lead to regression of the hearing mechanism and ultimate deafness.

Methods employed: Serial sections of the petrous bones and brain stems of animals ranging in age from birth to two and one half year, are prepared by various appropriate techniques for the study of the different tissues and cytological elements. Myelin sheaths and axonal degeneration

Part A Project Description (cont'd)

techniques are also employed to determine the stages of neuronal degeneration.

Major findings: A histological study of the petrous bones of 65 animals of the NIH 'waltzing strain' is nearly complete. The pathological changes noted thus far are essentially similar to those described by Lurie (1941) from a smaller series of Waltzers secured from another colony. The hair cells are the most susceptible cellular elements of the organ of Corti being the first to disappear entirely by the end of the first week. Early atrophic changes are noted in the stria vascularis as a thinning of the capillar bed and covering epithelium but the atrophy is not complete even in the older animals of more than two years of age. Other cellular elements of the organ of Corti fade away more slowly and in a certain sequential fashion. All remnants of the organ of Corti have disappeared in animals of five months of age. The cochlear neurons slowly atrophy later on but a few resistant afferent neurons persist in the oldest animal studied.

Of particular interest is the fate of the efferent cochlear fibers located in the juxtaganglionic spiral of the cochlea. These fibers whose cells of origin are more remotely located in the CNS are not entirely resistant to this hereditary disease. These fibers also drop out after the first year and no trace of them is found in the two year old animal.

Thus far in the study no morphological defect has been found which might account for the regressive changes in the cochlea.

Significance. Observations resulting from this project should help to understand (a) similar type inherited deafness occurring in human and (b) the resultant progressive pathological changes that subsequently occur in all structures of the cochlea.

Proposed course of project: To complete the study during 1959 and to report results.

Part B included: No

Serial No. NINDB-NA-FY-3
1. Neuroanatomical Sciences
2. Section on Functional Neuroanatomy
3. Bethesda, Maryland
4. Same as NINDB-NA-38-1957

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: An experimental study of the medial longitudinal fasciculus of the brain stem and spinal cord.

Principal Investigator: Leo C. Massopust Jr.

Other Investigators: G. L. Rasmussen

Cooperating Units: None

Man Years (calendar year 1958):
Total: 0.9
Professional: 0.6
Other: 0.3

Project Description:

Objectives: To determine the origin and termination of fibers in the medial longitudinal fasciculus in central nervous system with particular attention to its termination on cells of the anterior horn of the spinal cord.

Methods employed: The making of lesions at different levels of the medial longitudinal fasciculus and in the various nuclei of origin of its fibers. Analysis of neuronal connections by the use of axonal and terminal degeneration methods described by Nauta-Gygax 1954.

Major findings: A noteworthy finding since the last annual report is summarized in the Anatomical Record of 1958. This concerns a component of the medial longitudinal fasciculus which arises from the midbrain in an area occupied by the interstitial nucleus of Cajal. The fibers course in the dorso-medial part of the medial longitudinal fasciculus; turn sharply ventrad at the level of the facial colliculus and terminate in the main inferior olive.

Part A Project Description (cont'd)

Significance: This study will furnish information on the termination of descending pathways from the brain stem to anterior horn cells. It should be of value to those neurophysiologists studying the electronic behavior of anterior horn cells which are affected by the termination of descending pathways from the brain stem.

Proposed course of project: This project was terminated in July of this year. The manuscript is in the final stages of preparation.

Part B included: No

Serial No. NIMDB-NA-FN-4
1. Neuroanatomical Sciences
2. Section on Functional Neuroanatomy
3. Bethesda, Maryland
4. Same as NIMDB-NA-37-1957

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Neuronal connections and the functional significance of the interpeduncular nucleus.

Principal Investigator: Leo C. Massopust, Jr.

Other Investigators: G. L. Rasmussen

Cooperating Units: None

Man Years (calendar year 1958):
Total: 0.8
Professional: 0.5
Other: 0.3

Project Description:

Objectives: (a) To determine the neuronal connections of the interpeduncular nucleus, particularly the efferent connections about which little is known. (b) To determine, if possible, the functional significance of this nucleus which may involve both visceral processes and/or somatic motor processes.

Methods employed: (a) Small electrolytic lesions are to be confined to the interpeduncular nucleus itself in order to avoid injury to surrounding structures which would complicate interpretation of the results. This ventrally placed nucleus has heretofore been destroyed from a dorsal approach which necessarily destroyed many structures along the electrode path. (b) The employment of physiological tests in both acute and chronic preparations to determine the functional integrity of certain visceral mechanisms by means of recording electronically alterations in temperature regulatory ability, vasomotor changes, heart rate changes and others. Further, any abnormal behavior due to destruction of the nucleus will be studied. (c) The experimental material

Part A. Project Description (cont'd)

will be prepared according to certain axonal and terminal degeneration technics in order to bring out the resulting degeneration.

Major findings: Due to the technical difficulties involved in the surgical approach to the interpeduncular nucleus, only one of 12 animals operated upon possessed a well-placed lesion in the nucleus. More successful experiments need to be done.

Significance: This project will provide basic anatomical information on the efferent fiber connections of this nucleus, a subject about which little experimental data exists. Further, it will provide some knowledge of the possible functional significance of this nucleus.

Proposed course of project: To inactivate project during 1959.

Part B included: No

Serial No. NINDB-NA-EN-5
1. Neuroanatomical Sciences
2. Section on Functional Neuroanatomy
3. Bethesda, Maryland
4. New

PHS-NIH
Individual Project Report
Calendar Year 1958

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 1 July, 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 1958):
Total: 1.0
Professional: 0.8
Other: 0.2

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 technic for degenerating axons is applied to intramedullary material and the Sudan black and Marchi methods are employed on the peripheral portion of the acoustic nerve.

Part A. Project Description (cont'd)

Major findings: No major results to report. Serial sections of the brain stem and inner ears of three pigeons and one Caiman have been prepared by appropriate histological techniques but observations at this time are too incomplete to present definite anatomical details concerning the presence, origin or course of the efferent cochlear bundle in these forms.

Significance: The disclosure of an efferent component to the cochlea has resulted in much speculation concerning its role in the hearing process as well as its ultimate central and peripheral terminations. While the efferent bundle has been shown to be present in essentially constant form throughout the mammalia, its presence has not been determined in lower forms. A study of the efferent cochlear bundle in lower forms with a simpler auditory apparatus will lead to a better understanding of the functional significance of this bundle and provide phylogenetic evidence upon which to base future physiological and anatomical considerations.

Proposed course of project: This project will continue under the present research fellowship grant until 1 July, 1959. However, it may be necessary to continue study beyond this time in order to complete histological preparations and observations. The results of this project will be used in the preparation of a dissertation for the degree of Doctor of Philosophy and subsequently published.

Part B included: No

Serial No. NINDB-NA-FN-6
1. Neuroanatomical Sciences
2. Section on Functional Neuroanatomy
3. Bethesda, Maryland
4. New

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: A study of an efferent component of the vestibular nerve arising from the medulla oblongata.

Principal Investigator: Grant L. Rasmussen

Other Investigators: Richard Gacek

Cooperating Units: None

Man Years (calendar year 1958):
Total: 0.7
Professional: 0.4
Other: 0.3

Project Description:

Objectives: To learn more about the origin and particularly the mode of termination of the efferent vestibular fibers, i.e., to determine whether or not the hair receptor cells receive efferent as well as the well-known afferent fiber terminals.

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 are studied in a series of 31 experimental animals (24 cats and 7 chinchillas). Silver and methylene blue vital stains will be employed for demonstrating the ultimate termination of the efferents.

Major findings: During the past year a myelinated nervous component of the vestibular nerve has been revealed which is comparable in many morphological respects to the

Part A. Project Description (cont'd)

efferent cochlear bundle. For the first time vestibular efferents have been traced from a central origin and throughout the vestibular nerve and all its branches as far as the receptor organs of the vestibular labyrinth. The essential findings have been reported by abstract in the Anatomical Record, 130:361-362, 1958.

Significance: As yet no physiological study has been attempted to elucidate the function of the vestibular efferents. However, one may speculate on their functional significance on the basis of what is known about the cochlear efferent bundle which has been demonstrated to exert a regulatory or inhibitory influence on the auditory nervous input (Galambos).

Proposed course of project: To continue the study in order to settle the important question concerning the ultimate termination of the efferents in the vestibular receptor organs

Part B included: No

Summary Report of Laboratory of Biophysics
Calendar Year 1958

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, John E. Gebhart, and Ernest E. Whitcomb, have progressed towards this objective, in part with the collaboration of Jose del Castillo, Clinical Neurophysiology, NINDB, Seymour L. Friess, 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 tentative conclusions from the measurements, analyses, and computations now available for the squid axon are that the internal current electrodes of 20 ohm cm. resistance as driven from the 0.2 ohm amplifier outputs in routine use have allowed a reasonably adequate control of the membrane potential over a 5 mm. length. The effects of the external electrode geometry on the control do not seem to be serious but these and the effects of an unidentified membrane resistance have not been satisfactorily resolved.

An indication of the effect of such an internal electrode is that it can reduce the impulse transit time from the normal $\frac{1}{2}$ msec. for a cm. to less than $\frac{1}{2}$ μsec.

In the course of this work the need to measure membrane current, without introducing an appreciable potential difference, has been met by the invention and use of a new feedback circuit and the stabilized wide band electrometer preamplifier for faithful measurement of microelectrode potentials has been improved with about a 10 fold reduction in noise and ripple.

Frankenhaeuser's method for measurement of the potential inside a node of Ranvier from an adjacent node has been successfully adapted to use the improved electrometer preamplifiers and operational amplifier techniques available here. A preliminary examination of the characteristics of the node showed that

when the difficulties of controlling the membrane potential through the high internodal resistance were overcome, ionic current characteristics similar to those of the squid axon were found and were in agreement with independent results.

Although some progress has been made, there is still the need for a careful study of several methods, including those originally used by Hodgkin and Huxley, for the separation and empirical representation of the sodium and potassium components of the squid membrane ion current. It has, however, been possible to show, by variation of the initial conditions, that the original representation of the potassium current transient is only satisfactory under limited conditions while the assumption of the independence of the two components has received further support.

The finding that in 0.5 M external KCl the squid membrane has the predicted negative resistance characteristics to permit and all-or-none action potential suggests that the normal distinction between sodium and potassium is only relative. Yet the survival of a high peak sodium conductance to the end of a long experiment showed that this membrane mechanism can be very rugged. Preliminary investigations with ethanol, ether, chloroform, acetone, dioxane, eserine, and cevadine indicate that the potassium conductances were unaffected while the sodium conductances were markedly decreased by some and increased by none.

In contrast to eserine, Holothurin, from the sea cucumber, blocked the frog sciatic nerve without change of velocity and on a single node also, irreversibly and independently of pH. New derivatives of the ethylene diamine type showed that, although a cis form blocked when the trans form did not, more cis diamine units were no more effective.

Further analog computations of Hodgkin-Huxley equations with modified time constants have given action potentials with recovery plateaus that are in better agreement than before with those produced by some membranes.

The error in the earlier SEAC digital computer code has been found and the recomputations on the IBM 704 completed. Computations of the entire uniformily propagating action potentials at 6.3°C. and 18.5°C. have been completed, and the calculated net ionic fluxes per impulse found to have approximate agreement with experimental results. A digital code has been constructed to compute the more difficult problems of the initiation of an impulse in a medullated axon with modified Hodgkin-Huxley membranes at the nodes. Preliminary computations show both the origin of an impulse at an electrode and its propagation with a constant velocity along the axon.

NATIONAL INSTITUTE OF NEUROLOGICAL DISEASES AND BLINDNESS

Basic Research Program

Laboratory of Biophysics

Estimated Obligations for FY 1959

Total: $156,500
Direct: 130,000
Reimbursement: 26,500

Individual Projects 1 through 5

Serial No. NINDB-B-1
1. Laboratory of Biophysics
2. ------
3. Bethesda, Maryland
4. Continuation of 1957 project

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Ionic Permeabilities of the Squid Giant Axon Membrane.

Principal Investigators: Moore, J. W., Cole, K. S.

Other Investigators: Taylor, R. E., del Castillo, J. (Clin. Neurophys.)

Cooperating Units: Section on Clinical Neurophysiology, NINDB, Marine Biological Laboratory, Woods Hole, Massachusetts

Man Years:

Total:	4.6
Professional:	3.8
Other:	0.8

Project Description:

Objectives: The interpretation of nerve function in terms of fast ionic transports and the elucidation of the structures and mechanisms by which these transports are controlled.

Methods Employed: The instrumentation remains, fundamentally, the same as that first used at Woods Hole in 1947 to measure directly the ion current crossing the squid axon membrane between a long axial electrode within the axon and an external concentric electrode after a controlled change of potential difference across the membrane. Micropipette-calomel and external-calomel electrodes have been used for measurement of the membrane potential and control of the membrane current to minimize the effect of internal and external electrolytic resistances, and to simplify the axial electrode design, construction and operation. The Laboratory chopper-stabilized broad-band electrometer has made the drift of membrane potential measuring equipment during an experiment negligible. A new current measuring circuit, which introduces a negligible potential drop across its input, has been developed in the Laboratory, effectively used at Woods Hole during the 1958 squid season, and submitted for publication.

Major Findings: The preliminary investigations with ethanol, ether, chloroform, acetone, dioxane, eserine, and cevadine have shown that the steady state potassium conductances were relatively unaffected whereas the peak sodium conductances were markedly decreased by some but increased by none. In apparent contrast is the finding of an unchanged peak sodium conductance at the end of a long experiment. The procaine results previously reported have been submitted for publication.

Personnel have not been available to make satisfactory progress on the general problem of the routine separation and empirical representations of the sodium and potassium components of the membrane ion currents. However, considerable evidence has been obtained in support of the original assumption that these components are independent of each other. The potassium current transient can be expressed in the Hodgkin-Huxley form only if an exponent of at least twenty-five is used in place of the original value of four. This in turn implies an even smaller reverse potassium conductance than previously suspected and leads to a membrane mobility of 10^{-4} that in water casts doubt upon the utility of the formulation. The finding of a negative resistance characteristic with the axon in 0.5 M KCl has been submitted for publication and is not only predictable by the original equations and a rational basis for the observed all-or-none action potentials but also suggests that the usual distinction between sodium and potassium as well as lithium may be more quantitative than qualitative.

Some years ago, when it became possible to obtain and maintain axons having up to 6 ma/cm^2 of peak sodium, the first of several complications appeared in the ionic current pattern that had not been seen in the original experiments with peak sodium currents of about 1 ma/cm^2. During the 1958 squid season, the effects of resistance in the output circuit of the control amplifier and of the surface impedance of the axial electrode upon the potential difference across the membrane at the control point and at other points within the measuring chamber were investigated with various arrangements of additional external and internal axon electrodes.

Amplifier resistances well below one ohm cm. and axial electrodes of 20 ohm cm., effective, gave a control of the membrane potential to within a few millivolts. Amplifier and/or electrode resistances of about one ohm cm. and 75 ohm cm. respectively allowed some anomalies

while for somewhat higher resistances the membrane potential error could be tens of millivolts within millimeters of the control microelectrode. These experimental and analytical approaches are not yet complete but it is expected that the phenomena are to be rather well explained in physical terms. The effects of the external electrode geometry do not seem to be serious but they and the effect of an unidentified membrane resistance have not been satisfactorily resolved. In spite of these reservations, it seems probable that the membranes of strong axons have been routinely measured under conditions not dangerously far from those assumed and sought and that they have characteristics similar to those of the axons that were easier to control.

Significance to NINDB Research: The recently developed concept and the measurements of the sodium and potassium ion movements across the squid axon membrane offer a new, highly specific, and general approach to the factors underlying normal and pathological nervous processes which cannot as yet be investigated directly in higher animals and man.

Proposed Course of Project: The detailed and extensive analysis of the present records will be continued with the objectives of

1. a reasonably complete and critical examination of the original Hodgkin-Huxley formulations,
2. more satisfactory and objective techniques for the empirical formulations of the membrane characteristics,
3. the resolution of the conventional physiology, pathology and pharmacology of nerve into the ion permeability variables,
4. establishing of practical experimental routines and general planning of further experimental work,
5. providing bases for theoretical investigations of the mechanisms of ion permeabilities.

Part L Honors, Awards, and Publications

Publications other than abstracts from this project:

Schwan, H. P and Cole, K S., "Electrical physiology- Alternating current admittance of cells and tissues Accepted for publication in Medical Physics Vol. III Chicago, Otto Glasser, Ed., Year Book Publishers.

Individual Project Report
Calendar Year 1965

Part A.

Project Title: Ionic Permeabilities of Nerve Membranes
Theoretical Investigations

Principal Investigator: FitzHugh, R.

Other Investigators: Moore, J. W., Taylor, R. E., and Antosiewicz, H. A.

Cooperating Unit: Computation Laboratory, National Bureau of Standards

Man Years:
Total: 1.5
Professional: 1.2
Other: .3

Project Description:

Objectives: To investigate the bases, consequences and extensions of ionic permeability concepts and [illegible] by mathematical analysis and computation.

Methods Employed: Mathematical analysis, using the theory of nonlinear differential equations and digital and analog computers. The electronic analog computer in this laboratory has become an indispensable tool for this project, although many problems remain which require the use of the digital computer of the National Bureau of Standards.

Major Findings: The error in the earlier SHAC computation of the Hodgkin-Huxley equations for the squid axon membrane has been found definitely to result from a coding error permitting overflow in the computation of the transcendental functions near their singularities. This resulted in the appearance of two spurious singularities in the phase space for the current clamp case. Near these points, a threshold saddle point, profound [illegible] diverging phase plane trajectories. Recomputations on the National Bureau of Standards IBM 704 of the nerve action potential have shown that intermediate sized impulses can be obtained by a sufficiently accurate

adjustment of the stimulus intensity, thereby changing a result which had originally been expected from the form of the equations. The accuracy required (better than $1/10^8$) is, however, experimentally unattainable, and the Hodgkin-Huxley equations adequately represent an all-or-none phenomenon.

Computations of the complete Hodgkin-Huxley propagated action potential for 18.5° C. and for 6.3° C. were finished with velocities of 18.7 m/sec. and 12.3 m/sec respectively and the net transfers per impulse of potassium, sodium, and leakage ions computed to show the effect of temperature on them. The figures for potassium showed only a rough agreement (30% difference) with the experimental results of Shanes and gave a Q_{10} of 0.86, compared with Shanes' value of 0.

Coding has been completed and a few exploratory computations have been made with the 704 for the case of a non-space-clamped noded nerve fiber, with Hodgkin-Huxley membranes at the nodes, so modified as to have the capacitance and resting conductance found in frog nerve. These show the initiation of an impulse at a stimulating electrode, and its consequent propagation with constant velocity. The difference between the two velocities already obtained, 13.6 m/sec. for a 0.01 msec. stimulating current pulse, and 11.9 m/sec. for an infinite duration pulse, suggest, however, that a truly constant velocity has not yet been reached, and further computations are needed.

A large number of computations of the complete Hodgkin-Huxley equations under various conditions and modifications have been made with the Berkeley analog computer. The analysis in terms of the interaction of two sub-systems of the model, previously reported, has been verified and extended to explain the effects of multiplying the time constants by various factors. One such modified system, with the time constant for the potassium activation 100 times normal and that for the sodium inactivation 0.35 times normal, nearly duplicates experimental plateau action potentials found with the drug TEA (Tasaki and Hagiwara). The membrane conductance changes are less accurately reproduced, and these are being studied further.

The oscillations obtained with the equations under a space current clamp are the result of the instability of a singular point in the phase space. Thus the range of constant current values which produce an infinite train of impulses can be predicted from calculations of stability. In addition, a small range of

Part A. Project Description (cont'd.)

current intensities result in finite trains of impulses, but the mathematical basis for this phenomenon will require further study. Under a resistive clamp, an intermediate condition between current and voltage clamp, oscillations are obtained when the clamping resistance varies from infinity down to a value between 300 and 500 ohm cm., while a threshold phenomenon is still obtainable as low as 50 ohm cm. This latter value sets an approximate theoretical upper limit to the permissible series resistance for voltage-clamp measurements. Preliminary calculations, assuming an ideal electronic control system, show that anomalous membrane currents, including "notches" and "oscillations", can occur in a Hodgkin-Huxley axon and an ideal electronic control system. With a necessarily simplified geometry, these anomalous currents are found for a high resistance axial electrode alone or in combination with non-uniform properties along the axon such as fiber diameter, condition of the membrane or variations of the current electrode impedance. These computations are similar to observations of our own and others on real axons and are being continued for a more detailed comparison.

Significance to NINDB Research: The concept and the measurements of the sodium and potassium ion movements across the squid axon membrane offer a highly specific, and general approach to the factors underlying normal and pathological nervous processes which cannot as yet be investigated directly in higher animals and man. An analysis of the mathematical properties of the Hodgkin-Huxley equations makes possible a better understanding of their possible modifications and their limitations, than is obtainable by a purely physical interpretation.

Proposed Course of Project: The analog computer will be used to solve the Hodgkin-Huxley equations under a number of physiologically important conditions, especially the changes of excitability near threshold. The analysis of their mathematical properties, which has already been useful in understanding experimental results, will be continued, especially to investigate further the conditions under which oscillations can arise for both perfect and imperfect space clamps. An attempt will be made to modify the equations on the basis of recent voltage-clamp experiments on the frog node, so as to provide a mathematical model for this important case. The study of propagation of an impulse in a non-space-clamped noded nerve will be continued.

Part B included -- yes

Part C. Honors, Awards and Publications

Publications other than abstracts from this project:

Cole, K. S., Antosiewicz, H. A., and Rabinowitz, P.
Automatic computation of nerve excitation, correction.
J. Soc. Indust. Appl. Math 6 106, 1958.

Cole, K. S. Nervous system: Excitation and propagation
of nerve. Accepted for publication by Medical Physics
Vol. III, Chicago, Otto Glasser, Ed., Year Book Publishers

1. Laboratory of Biophysics
2. [illegible]
3. Bethesda, Maryland
4. Continuation of 1957 proj.

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Correlation of Acetylcholinesterase Inhibition With Nerve Action

Principal Investigator: Whitcomb, E. R.

Other Investigators: Moore, J. W. and Friess, S. L. (NNRI)

Cooperating Units: Naval Medical Research Institute
Chemistry Department, Mount Sinai Hospital

Man Years:
Total: 1.0
Professional: .8
Other: .2

Project Description:

Objectives: To determine the inhibition spectrum of acetycholinesterase drugs and to determine their blocking action on the nerve impulse.

Methods Employed:
1. Frog Sciatic Nerve: A length of desheathed frog sciatic nerve is exposed to a compound of selected geometric and electron distribution. The time course and extent of the depression of the A fiber action potential and the change of its conduction time are compared with the in vitro anticholinesterase activity of the agent.

2. Single Fiber: A single fiber from the toad sciatic nerve is isolated and mounted across an air gap for recording from a single node. The node is stimulated directly at the rate of 1/sec. The time course and amplitude of the threshold and the action current of the node are studied as functions of concentration and pH levels of the drug solution and unsaturated electron distribution of the drug.

Major Findings:

1. The effects of holothurin, a neurotoxin obtained from the sea cucumber, were studied using the desheathed bullfrog sciatic nerve and the node of a single toad

[illegible] nerve preparation, compounds like eserine reduce the velocity at the action potential is decreased. When applied to the single node, holothurin abolished the action current irreversibly in 3-5 minutes at a concentration as low as 0.5 mM. This irreversible effect was accompanied by a first rise in threshold. The effectiveness of this neurotoxin is not altered by pH level in the pH 6.7 - 7.3 range. Again this is in contrast to eserine which is approximately 1/30 as potent, blocks in a reversible manner and with an effectiveness that is dependent on the pH level.

The properties of holothurin are being studied in collaboration with NHRI and the Mount Sinai Hospital, Chemistry Department. A report, "Some Pharmacologic Properties of Holothurin, an Active Neurotoxin from the Sea Cucumber", of its properties is now being prepared for publication in the "Journal of Pharmacology and Experimental Therapeutics".

3. In connection with studies in stereochemical influences on the hydrolysis of cyclic acetates, the effectiveness of the following compounds to block the conducted impulse in the bullfrog desheathed sciatic nerve was determined

a) tetramethylstreptamine-2HCl

$C_{10}H_{24}N_2O_4Cl_2$ m.w. 307.15

b) tetramethyltetracetylstreptamine

$C_{18}H_{30}N_2O_8$ m.w. 402.26

c) mycinosamine

$C_8H_{17}NO_5$ m.w 217.14

d) scyllo inosamine

$C_8H_{17}NO_5$ m.w. 217.14

e) desoxystreptamine

$C_{10}H_{22}N_2O_3$ m.w. 218.19

displayed some alkyl ester grouping, this eliminated two important anticholinesterase centers.

a) ...was more effective with the ...
...
b) ...increasing the ...

...
...did not increase effectiveness in ...
...was conducted in collaboration with NMRI. The report 'Further Response of Acetylcholinesterase and of Conduction in Bullfrog Sciatic Nerve to the Pharmacodynamics of Amine Inhibitors. II.' is being prepared for publication in Archives Internat. Biophysics.'

2. A series of experiments was conducted on the bit...
...node of a squid fiber.

a) ...to correlate the concentration of tertiary ...
...effects with the degree of effect on the threshold and magnitude of the action current and
b) ...to determine if the pH level could alter the response of the node to the tertiary form of ...

The experiments so far observed have obscured any quantitative relationships. However, it was found that

a) ...with increasing concentrations of the tertiary ...form of ..., the action current was progressively reduced and the threshold increased.
b) ...time for ... to exert its maximum effect ... as long as 60 minutes in some trials.

(c) Complete recovery of the action current and threshold can be obtained after treatment in appropriate solutions but is not yet routinely possible.

Significance to NINDB Research: These studies aid in evaluating the role of acetylcholinesterase in nerve function and in characterizing the condition for one type of nerve impulse blockade.

Proposed Course of Project: To continue using the desheathed frog sciatic nerve as a means for studying the effectiveness of the propagated nerve impulse. To modify the single node recording procedure in an attempt to obtain a quantitative relationship between the in vivo properties of anticholinesterase compounds and their effect on the action current and/or threshold. To combine the results of these two procedures to define the role of acetylcholinesterase.

[illegible] Continued -- no

Serial No. NHNB-B-4
1. Laboratory of Biophysics
2. ---------
3. Bethesda, Maryland
4. Continuation of 1957 proj

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Membrane Potentials of a Lobster Giant Axon.

Principal Investigator: Dalton, J. C.

Other Investigator: Taylor, R. E.

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

Project Description:

Objective: To test the possibilities of the lobster giant axon as a preparation to be used in general studies of ion permeabilities, to compare this axon with other nerve preparations which have already been investigated, and to use the preparation in studies of the actions of certain pharmacological agents with special reference to membrane oscillations and the production of repetitive activity.

Methods Employed: Resting potentials and spike potentials from the lobster giant axon are obtained by means of an intracellular microelectrode and recorded by conventional electrophysiological instruments. A circulation and cooling system permits changes in ionic composition and concentration, and the admission of drugs. Electrical stimulation of the nerve may be external or internal.

Major Findings: Any one of the three giant axons in the lobster circumesophogeal commissures makes a rather simple, hardy preparation for the present investigations It appears to have good possibilities for further experimentation on the types of ionic problems which have been under investigation in this Laboratory.

The investigation of the effect of various agents has been continued and concluded. Ethyl alcohol, acetone and probably dioxane cause repetitive activity, a slowed return of the spike potential, without oscillations, towards a reversibly decreasing resting

Part A. Project Description (cont'd.)

potential. The effects of ether and chloroform were similar except that an apparent inhibitory action usually prevented repetitive activity. The action of veratridine was also similar but not completely reversible. The effects of procaine were a linear function of the amount of the tertiary form over the pH range 6.5 to 8.75 investigated. The resting potential decreased and the action potential decreased and lengthened but without change of form in the late phases.

Significance to NINDB Research: These investigations are one aspect of the general problems of ionic permeabilities which are under investigation in this Laboratory, and, as such, are a mode of attack on the underlying questions of general nerve function. They are useful in providing additional evidence both of the common features and of the singularities of nerve phenomena. They have a deeper significance in that they present a possible transition between the ionic permeability analysis now only available for the exotic squid axon and the neurological problems of higher animals and man.

Proposed Course of Project: The exploratory work on the lobster axon is largely completed and has been discontinued with the return of the principal investigator to inactive duty. Some more detailed work on procaine may be undertaken and as new techniques can be developed or present procedures miniaturized, the ionic currents in the lobster axon membrane will be determined -- to be tested as the common underlying basis of the conventional phenomena now being investigated.

NS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Dalton, John C. Effects of External Ions on Membrane Potentials of A Lobster Giant Axon. Accepted for publication in J. Gen. Physiol.

Section No. NINDB-A-5
1. Laboratory of Biophysics
2. [illegible]
3. Bethesda, Maryland
4. Continuation of 1957 proj

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Ionic Permeabilities of Nodal Membrane

Principal Investigators: Moore, J. W., del Castillo, J. (Clin. Neurophys.)

Cooperating Unit: Section on Clinical Neurophysiology, NINDB

Man Years:

Total:	0.2
Professional:	0.2
Other:	0

Project Description:

Objectives: The measurement and the analysis of the ionic components of the membrane current in the single frog node to attempt an interpretation of the many phenomena characteristic of this most useful present representative of medullated nerve fibers.

Methods Employed: A technique developed by Frankenhaeuser for the frog node has been successfully adapted to use the improved electrometer preamplifiers and operational amplifier techniques developed here during the squid axon investigations. With it, the external internodal leakage resistance is effectively increased to allow an accurate measure of the nodal membrane potential. An analogous technique was also developed to minimize the external leakage of the controlling current, injected into the nodal membrane through one other internode.

Major Findings: A preliminary study of the accuracy of the voltage clamp on the node showed the difficulty of feedback control through the high internodal axoplasm resistance. With easily obtainable values of this resistance, the node potential and current showed violent oscillations. With enough further reduction of this resistance, adequate potential control and continuous current characteristics were found and were similar to those of the squid axon membranes. A manuscript "An Electronic Electrode" by Moore, J. W. and del Castillo, J. has been submitted for publication in the Institute of Radio Engineers Convention Record.

Part A. Project Description (cont'd.)

Significance to NINDB Research: Medullated nerve fibers are such important components of neurological systems that it is not only interesting but practically important to know if -- and if so, to what extent -- the concepts and information now available only for the squid axon also apply to the nodal membrane.

Proposed Course of Project: Frankenhaeuser and Dodge have established the basic similarities of the nodal and squid axon membranes. The project may be held in abeyance until accumulated results on squid axons are analysed and codified.

Part B included - No

LABORATORY OF NEUROPHYSIOLOGY

Wade H. Marshall, Chief

The productivity of the Laboratory of Neurophysiology has continued at a high level and either directly includes, or touches upon many areas of knowledge.

The Section on Limbic Integration and Behavior is rolling in the grand manner under the inspiring leadership of Dr. Paul MacLean. A study of midline nuclei of the limbic system is underway. These systems are very important for integrated behavior. This research is particularly pertinent for the general field of alcoholism (Korsakoff's syndorme aspect of alcoholism), and for certain aspects of the schizophrenic syndrome. Dr. MacLean's laboratory studies on this and other projects are being largely done on the squirrel monkey, the use of which he has developed into a very comprehensive tool for correlated observations on physiology and behavior. Another important project is the investigation of relation of visual apparatus to limbic system.

This Section is carrying out an extensive series of experiments involving biochemical differences in various regions of the brain using animals ranging from mouse to monkey. Specific chemical poisons affect different regions according to dominance of differing metabolic processes. These researches contribute to basic knowledge of the brain and have specific implications for problems of alcoholism and drug addiction. A study on brain mechanisms involved in sexual activity is underway in the squirrel monkey. The precise mechanisms in the brain involved in sexual activity have remained elusive until very recent years, and these studies promise to contribute real information on brain mechanisms of this important function. It has long been realized that the persistance and relative aperiodicity of the human sexual appetite, as compared with lower primates, was one of the strong socially integrating forces in the evolution of human society. The importance of sex in psychiatry requires no elaboration.

In the Section on General Neurophysiology certain studies are being carried out which complement the above discussed researches on the limbic system. The large pyramidal cells in the hippocampus are attractive elements on which to employ unitary electrical analysis. These neurons are otherwise uniquely arranged for experimental analysis in that the basilar dendrites are arranged dorsally and laterally and long apical dendrites are directed centrally. Work is going forward to analyze the direct electrical excitation, and antidromic excitation through basilar dendrites and orthodromic excitation through the apical dendrites. This section has also concluded work begun in the previous year on the action of curare on the neocortex. No

specific action was found and this has been so reported in the Transactions of the American Neurological Association. Investigation of the direct cortical response is being continued. The direct cortical response, first investigated by Adrian, has had a peculiar history. It has been considered to represent, primarily, electrical activation of dendritic appendages of cortical neurons. It has been used extensively as a criterion of cortical reactiveness by investigators doing experiments involving local perfusion of cortical vessels or perfusion of the whole brain. If this reaction constitutes an adequate test it should be sensitive to anoxia. Our tests have shown that it, in fact, is very resistance to anoxia compared to the anoxic sensitivity of known synaptic transfer reactions. Work is also proceeding to test the widely held notion that activation of neuron soma or axon process is not necessarily involved in the direct cortical response. The direct cortical response of the hippocampus is under similar investigation. Work is also going forward on direct estimation of K ion transfers in the neocortex during action of drugs (Gamma Amino Butyric Acid).

During the summer the head of this section made a scientific mission trip to Brazil to clear up some disagreements about spreading depression with Dr. Aristides Leao, the discoverer of the phenomenon. Dr. Leao clearly showed that tetanic stimulation of the callosal system of the rabbit can initiate SD. This is an important point which we have failed to confirm and about which there were no reports of confirmation from the several other laboratories which have worked on spreading depression. Some of our special technics, the triangular pore electrode assembly for direct cortical response work and the Bak high input impedance amplifier were introduced to Dr. Leao's laboratory. Six lectures and seminars were given by me in Brazil. At the end of my tour of duty I was awarded by the Brazilians a trip to the Indians in the Upper Xingu Basin as a member of a special medical mission. I made some curious observations which may be worked up later in a formal publication. Among these observations is the identification of what will probably be accepted as the most ancient transquilizing agent. I was also impressed with the many opportunities for various kinds of research among the primitives of Brazil where several decades of courageous and kind treatment of the Indians by Brazilians has made the Indians friendly and cooperative.

Dr. Freygang is currently working on ion exchange patterns in membranes of single muscle fiber preparations. There are some distinct advantages in using such preparations and he will continue this work during the coming calendar year in Cambridge University, England in collaboration with Professor Hodgkin.

Further work has been done on Freygang's hypothesis advanced last year and based on excellent laboratory work that the dendrites and a large part of the soma dendritic membrane in certain classes of neurons are not electrically excitable (see last years report M-NP-GN-3). This work has been confirmed by further

investigations by Freygang and Frank using Frank's technic of placing one electrode inside a neuron and another electrode just outside of the membrane. These experiments done on the anterior horn cells of the cats' spinal cord confirmed Freygang's conclusions made on the principle cells of the cats' lateral geniculate. The major portion of the soma membrane reacts only passively during activation and if the axon is discharged by the activation the soma membrane is not electrically excited but remains passive. This is a very important result in the current progress of knowledge. It is now generally accepted that some nerve cells operate in this manner and that others do not. In the former type, it turns out that the extracellular potential is an IR drop across a passive membrane and that this current is the first derivitive of the potential recorded intracellularly.

Drs. Frank and Fuortes are proceeding with use of clamping technics which show that the membrane resistance does not fall to a negligible value during the peak of the spike which is in line with all above work of Freygang and Frank.

Drs. Frank and Sprague (University of Pennsylvania) have found support for the idea that what is called direct inhibition probably occurs through short process interneurons.

Dr. Frank and Dr. Paton (Royal College of Physicians and Surgeons) devised methods for administering small quantities of drugs near neurons while recording from electrodes just outside the cell and within it. This technic is useful for tests of specific chemical transmitter agents. They found no evidence that gamma amino butyric acid had any such specific action on anterior horn cells of the spinal cord. Dr. Frank has been particularly honored by an appointment as a member of a scientific mission to visit Russian laboratories.

The work of Dr. Tasaki and his collaborators involving the execution of critical experiments on the transmission of the nerve impulse is now widely recognized as a major contribution to science. The principle that active electrochemical forces maintain activity in the nerve membrane, which is most clearly demonstrated in the two stable state experiment now seems to have been proven. It appears that our confidence in, and support of Dr. Tasaki has been justified in every particular and that his ideas are either accepted or seriously examined and considered with great respect throughout the world.

This group is also launching a general attack following up their evidence that production of the nerve action potential is not a simple physical phenomena but involves active biochemical processes. To this end they have another large scale program in action, using intracellular glass pH electrodes, intracellular and extracellular chemical manipulation and electron paramagnetic resonance spectroscopy for detection of free radicals.

An investigator working as a guest in Dr. Tasaki's laboratory at Woods Hole turned up a very important decisive argument involving the two stable state concept of the nerve membrane. Briefly this experiment showed that a hyperpolarizing response could be propagated if the external K ion was made very high.

Dr. Tasaki and his collaborators are engaged in a general attack on the processes underlying initiation of sensory nerve impulses in the retina, the cochlea and the skin, on all of which Dr. Tasaki has made important contributions for many years.

This section has in the past year seriously attempted to do electrical analysis of neuron and glial cells grown in tissue culture. The results were sufficiently promising to make continuation of this work an urgent matter. In tissue culture preparations the cells can be chemically manipulated and the electrodes can be placed under visual observation. This latter point is very important. Dr. Chang has continued the observations on glial cell excitability in the cat brain.

Drs. Tasaki and Spyropoulos, who have been invited to write the section on nerve excitability for the 1959 edition of Annual Reviews of Physiology, were also invited to participate in the International Biochemical Congress in Vienna during August of this year. Dr. Spyropoulos accepted the invitation and also took the opportunity to visit several important laboratories in Europe to gather better information for the Annual Review. His reception was most impressive and, according to Dr. Spyropoulos, indicated the very great respect with which his chief, Dr. Tasaki, is regarded throughout Europe.

The Section on Cortical Integration is proceeding with methods to store on tape patterns of electrical activity from a 25 electrode array, recodeing them into brief diphasic pulses and feeding this pattern back into the array as a stimulus pattern. The Section Chief has proceeded with further work on psychology and physiology of isolation, some of which was done as a consultant of the military on space travel problems.

Dr. Strumwasser is proceeding with studies on CNS mechanisms in hibernation. Hibernation is an important scientific subject providing many opportunities to make crucial observations on temperature and time factors in the CNS as well as fundamental metabolic studies. It also provides important information pertinent to clinical research on hypothermia techniques for surgery. Dr. Strumwasser is also proceeding with unitary electrical analysis of visual and auditory integrating mechanisms. This work is being done on the frog brain, and is already yielding important information on fundamental problems of attention, habituation, discrimination and extinction.

NATIONAL INSTITUTE OF NEUROLOGICAL DISEASES AND BLINDNESS

Basic Research Program

Laboratory of Neurophysiology

Section on Brain Stem

Estimated Obligations for FY 1959

Total: $19,000

Direct: 18,500

Reimbursement: 500

Individual Projects - None

NATIONAL INSTITUTE OF NEUROLOGICAL DISEASES AND BLINDNESS

Basic Research Program

Laboratory of Neurophysiology

Section on the Special Senses

Estimated Obligations for FY 1959

Total: $85,000

Direct: 70,500

Reimbursement: 14,500

Individual Projects 3, 4, and 5

NATIONAL INSTITUTE OF NEUROLOGICAL DISEASES AND BLINDNESS

Basic Research Program

Laboratory of Neurophysiology

Section on the Spinal Cord

Estimated Obligations for FY 1959

Total: $76,500

Direct: 63,500

Reimbursement: 13,000

Individual Projects 1a, 3 and 4

Serial No. NINDB-NP-SS-1a
1. Lab. of Neurophysiology
2. Special Senses Section
3. Marine Biological Lab.,
Woods Hole, Mass.;
Univ. of Mich., Ann Arbor,
Mich., and Bethesda, Md.
4. Combination of 1 and 2, 1957

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: The chemistry of neural activity

Principal Investigators: I. Tasaki and C. S. Spyropoulos

Other Investigators: J. Chang, A. Bak, M. Ezzy and R. Sands.

Cooperating Unit: Laboratory of Physics,
University of Michigan, Ann Arbor

Man Years (calendar year 1958):
Total: 2.5
Professional: 1.5
Other: 1.0

Project Description:

Objectives: Experimental studies carried out in this laboratory cast some doubt to the generally accepted view that production of action potential in the nerve fiber is essentially a physical phenomenon. The main objective of this project is to find out the relationship between biochemistry of the nerve and the process of action potential production.

Methods Employed: The method of intracellular injection has been used to study the effects of various chemicals upon the squid giant axon. The method of measuring the intra-cellular pH with a micro-glass electrode has been developed. The single fiber technique was employed to investigate the mechanism of action of various chemicals upon the vertebrate nerve fiber. The method of "electron paramagnetic resonance spectroscopy" was used to investigate whether or not there is

production of free radicals associated with nervous activity.

Major Findings:

(1) Using an electrochemical model of the nerve (iron-nitric acid or cobalt-hydrochloric acid system), the mechanism of action potential was investigated. The results obtained indicated that many known properties of the nerve fiber membrane can be satisfactorily reproduced in the model. The similarity between the electrical behavior of the model and that of the nerve membrane is considered to support the two-stable state hypothesis of action potential production. This hypothesis postulates that there are two chemically stable configurations in the excitable membrane and that initiation and abolition of the action potential represents transitions between the two states. The membrane potential, steady and transient, is assumed to be determined by the configuration of the membrane and the ionic environment.

(2) Using an extremely small glass electrode, it was possible to determine the pH of the axoplasm directly. Both a calomel cell and a glass electrode (made from glass capillaries supplied by Beckman Co. of California) were introduced into the axoplasm of the squid giant axon and the intracellular pH was determined by measuring the potential difference between the two intracellular electrodes. The intracellular pH was found to be 7.4 with a probable error of 0.1 in pH unit. The effect of various agents upon the intracellular pH was investigated by this direct method.

(3) With a view to comparing the process of action potential production in lower unicellular animals with that in the well-differentiated nerve fiber, the properties of the "hyperpolarizing response" in a protozoa, Noctiluca, were investigated. With two microelectrodes inserted into the cell sap of the protozoa, it was found that the passage of an inward current through the cell membrane could induce an all-or-none response which represented a transient lowering of the intracellular potential. This

PHS-NIH
Individual Project Report
Calendar Year 1958

hyperpolarizing response was associated with a simultaneous reduction in the membrane resistance. This response could not be eliminated by replacing 95% of the sodium ions in the surrounding sea water with choline or potassium ions.

(4) The nature of the hyperpolarizing response in the squid giant axon (discovered by J. Segal) was investigated by the method of measuring the membrane impedance during activity. It was shown that the process of production of a hyperpolarizing response represented simultaneous variations in the membrane-emf. and in the membrane resistance. The results of this investigation were interpreted as supporting the two-stable state hypothesis.

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:

So far, the attempt to demonstrate free radicals during neural activity was unsuccessful. It is planned, however, to repeat EPR (electron-paramagnetic resonance) spectroscopic observations under different experimental conditions.

Using squid giant axons, the relationship between the oxidative metabolism and the hyperpolarizing response in the nerve membrane will be investigated. The investigation of the effects of various chemicals upon the process of action potential production will be continued both on the squid giant axon and on the vertebrate medullated and non-medullated nerve fibers.

Part B included Yes

Part B: Honors, Awards and Publications — Serial No. NINDB-NP-SS-1a, page 4

Publications other than abstracts from this project:

Tasaki, I. and Bak, A. Discrete threshold and repetitive responses in the squid axon under voltage clamp. Am. J. Physiol., 193: 301-308, 1958.

Tasaki, I. and Spyropoulos, C. S. Non-Uniform response in the squid axon membrane under 'voltage-clamp'. Am. J. Physiol., 193: 309-317, 1958.

Tasaki, I. and Spyropoulos, C. S. Membrane conductance and current-voltage relations in the squid axon under 'voltage-clamp'. Am. J. Physiol., 193: 318-327, 1958.

Tasaki, I. and Bak, A. Current-voltage relations of single node of Ranvier as examined by the voltage-clamp technique. J. Neurophysiol., 21: 124-137, 1958.

Hagiwara, S. and Tasaki, I. A study of the mechanism of impulse transmission across the giant synapse of the squid. J. Physiol., Lond., 143: 114-137, 1958.

Brady, R., Spyropoulos, C. S. and Tasaki, I. Intra-axonal injection of biologically active materials. Am. J. Physiol., 194: 207-213, 1958.

Tasaki, I. Conduction of the nerve impulse. Chapter in: Handbook of Physiology, (in press).

Tasaki, I. Physiological properties of the myelin sheath and of the node of Ranvier. Chapter in: Progress in Neurobiology. Korey, S. R. and Nurnberger, J. I., eds. (in press).

Honors and Awards relating to this project:

Dr. Tasaki was invited to participate in the symposium on "Membrane States: Excitation and Inhibition" held at Washington University in St. Louis, Missouri.

Drs. Tasaki and Spyropoulos were invited to participate in the Second Int. Symposium on the Mechanism of Excitation held at Humboldt University in Berlin (neither doctor was able to attend this symposium.).

Part B: Honors, Awards, etc. (cont.)

Serial No. NINDB-NP-SS-1a, page 5

Dr. Spyropoulos was made a member of the Marine Biological Laboratory, Woods Hole, Mass.

Dr. Spyropoulos was a participant in the International Biochemical Congress, Vienna, Austria

Drs. Tasaki and Spyropoulos were invited to write the chapter on Nerve Excitation and Conduction for the Annual Review of Physiology, 1959.

Serial No. NINDB-NP-SS-3
1. Lab. of Neurophysiology
2. Special Senses Section
3. Bethesda, Maryland
4. Continuation of 1957 project

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Investigation of the sensory mechanism.

Principal Investigator: I Tasaki

Other Investigators: C. S. Spyropoulos, M. E. Ezzy

Cooperating Units: none

Man Years (calendar year 1958):
Total: 1.16
Professional: .66
Other: .50

Project Description:

Objectives: The main objective of this project is to clarify the mechanism whereby nerve impulses are initiated in the sensory endings, particularly in the cochlea, in the retina and in the skin.

Methods Employed: (1) In the investigation of the cochlear mechanism, guinea pigs are used. The surgical procedure used to expose the cochlea has been developed previously in Dr. H. Davis' laboratory (cf. e.g. Tasaki et al. J. Acoust. Soc. Am., 1954, 26: 766). Comparing the cochlea of so-called waltzing guinea pigs with that of normal animals, the source of the endolymphatic D.C. potential has been explored. (2) Using excised eyes of gold fish and carp, so-called graded retinal potential were recorded by the technique described by Svaetichin (Acta Physiol. Scand., 1956, 36, suppl. 134). It is planned to investigate the photochemical processes in the fish retina by the use of the electron-paramagnetic resonance spectroscopy (in collaboration with Dr. R. Sands of the University of Michigan). (3) Using the technique for recording electric responses from individual non-medullated fibers in the cutaneous nerve (see p. 126 in Tasaki, Nervous Transmission, 1952), the properties of the non-medullated nerve fibers

arising in the toad skin have been investigated.

Major Findings:

(1) The histological examination of the guinea pig cochlea used in the previous physiological investigation was completed in Dr. G. Rasmussen's laboratory. It was shown that there were no hair cells in the cochlea of the waltzing guinea pigs in which nearly normal endolymphatic D.C. potential had been recorded. This finding proves that the endolymphatic potential in the cochlea is not generated by the sensory cells. This is in agreement with the previous finding attributing the source of the potential to the stria vascularis.

(2) In preliminary experiments on the fish retina, results were obtained indicating that the graded retinal potential may represent an extra-cellular potential. No definite relationship was observed between the resting potential (recorded with a microelectrode) and the possibility of obtaining graded retinal potentials in response to light stimulation. The microelectrode could be moved a considerable distance without losing the recorded potentials.

(3) The threshold, chronaxie, the duration of the action potential and the conduction velocity were measured on a large number of individual non-medullated cutaneous nerve fibers. The analysis and a statistical treatment of the results obtained are in progress.

Significance to Neurology Research: This investigation is expected to contribute to the understanding of the process of sensory perception.

Proposed Course of Project: Emphasis will be placed on the investigation on the retinal and cutaneous sensory mechanism. An attempt will be made, in collaboration with the physicists in the laboratory of physics in the University of Michigan, Ann Arbor, to test whether or not there is production of free radicals in photic stimulation of the retina. It is planned to study the nature of adequate sensory stimuli in the skin for evoking action potentials in various cutaneous non-medullated axons.

PHS-NIH
Individual Project Report
Calendar Year 1953

Part A. Proj. Desc. (cont.) Serial No. NINDB-NP-SS-3,
page 3

An attempt will be made to correlate the number and the sizes of the non-medullated axons in a Remak bundle with the electric responses recorded from the same bundle.

Part B included Yesxxx

Part B: Honors, Awards, and Publications

Serial No. NINDB-NP-SS-3, page 4

Publications other than abstracts from this project:

Tasaki, I. and Spyropoulos, C. S. Stria vascularis as source of endochlear potential. J. Neurophysiol., March (in press), 1959.

Honors and Awards relating to this project:

Dr. Tasaki was invited to attend a Symposium on Electrophysiology of the Visual System.

Serial No. NINDB-NP-SS-4
1. Lab. of Neurophysiology
2. Special Senses Section
3. Bethesda, Maryland
and Galveston, Texas
4. New

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Physiological studies on the nervous elements in tissue culture.

Principal Investigator: J. J. Chang and I. Tasaki

Other Investigators: W. Hild and M. Wolfe

Cooperating Units: Laboratory of Neuroanatomical Sciences, Tissue culture section; Tissue Culture Laboratory, University of Texas-Medical Branch, Galveston, Texas.

Man Years (calendar year 1958):
Total: .83
Professional: .83

Project Description:

Objectives: The technique of tissue culture provides us with a unique opportunity of investigating the function of the nerve cells and the glial elements in the vertebrate central nervous system under direct visual observation. Furthermore, the investigation of the gradual change in the activity of these elements during the course of their development from the immature stage is expected to give us a better understanding of the behavior of mature cells. The main objective of this project is to study physiological properties of the nerve and glia cells in tissue culture and to compare these properties with those of the cells in vivo.

Methods Employed: The method of recording intracellular potentials with hyperfine glass pipette electrodes is used in the study of the nervous elements in tissue culture as well as in the in vivo investigations. Phase-contrast microscopy combined with the use of micromanipulators specially designed for the present purpose enables us to observe the position of the microelectrode in the nerve and glial cell impaled.

Part A. Project Des. (cont.) Serial No. NINDB-NP-SS-4,
page 2

Major Findings:

It was found that the astrocytic glias in tissue culture develop a slow "electric response" to direct stimulation with extracellular electrodes. The nerve cells in the same culture media (stimulated in a similar manner) produce short action potentials of the order of 1 msec in duration. The duration of the glial response is roughly 3000 times as long as that of the action potential of the nerve cell. The amplitude of the glial response varies with the stimulus intensity; using strong stimuli responses of 30 - 40 mv can be evoked in a reproducible manner.

Electric stimulation of the astrocytic glia was found to evoke a slow contraction of the cell. It was necessary to use a time lapse camera to demonstrate this slow contraction. The duration of the contraction phase was 1.4 to 3.4 min., and that of the relaxation phase was 6 - 16 min.

Slow electric responses which resemble the glial response observed in the tissue culture material were demonstrated with a hyperfine microelectrode pushed into the cat cerebral cortex. It was inferred that the glia cells in vivo are capable of developing slow electric responses as the astrocytes in vitro are.

Significance to Neurology Research: This investigation is expected to lead us to better understanding of the function of the brain.

Proposed Course of Project: In the previous experiments on the tissue culture material, a considerable difficulty was encountered because of the presence of a strong layer of plasma clot in which the cells to be studied are imbedded. It is planned to repeat the previous experiments using tissue culture material in which collagen gel is used to fix the cells on cover slips.

The interaction between the nerve cells and the glia in the cerebral cortex will be investigated. Measurements of the electric impedance of the cerebral cortex following a strong electric shock is expected to reveal some aspects of the behavior of the glias in the cortex.

Part B included Yesxxx

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications — Serial No. NINDB-NP-SS-4, page 3

Publications other than abstracts from this project:

Hild, W., Chang, J. J. and Tasaki, I. Electrical responses of astrocytic glia from the mammalian central nervous system cultivated in vitro. Experientia, Basle, 14: 220-221, 1958.

Tasaki, I. and Chang, J. J. Electric response of glia cells in cat brain. Science, 128: 1209-1210, 1958.

Chang, J. J. and Hild, W. Contractile responses to electrical stimulation of glial cells from the mammalian central nervous system cultivated in vitro. J. Cell. Comp. Physiol. (in press).

Honors and Awards relating to this project:

Serial No. NINDB-NP-SC-3
1. Lab of Neurophysiology
2. Spinal Cord Section
3. Bethesda, Maryland
4. New

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Generation of impulses in spinal motoneurons.

Principal Investigators: K. Frank and M. G. F Fuortes

Other Investigators: M. Becker and P. Nelson

Cooperating Unit: This project has been carried out with the cooperation of the Ophthalmology Branch of NINDB. Dr. M. G. F. Fuortes has devoted considerable time to the work of this section.

Man Years
Total: 3.0
Professional: 1.3
Other: 1.7

Patient Days: None

Project Description:

Objectives: To determine which parts of the motoneuron contribute the various components of the recorded action potential and the quantitative aspects of the changes each part undergoes.

Methods Employed: Concentric micropipettes have been developed which can be advanced independently and which are small enough to permit both to be introduced inside a single motoneuron. By measuring the currents through the outer pipette required to maintain the potential of the inner pipette at any desired level, inferences can be drawn regarding the extent of firing over various cell parts. Some of the advantages of voltage clamping techniques can thus be realized in studying the behavior of motor horn cells.

Major Findings: Only preliminary findings are available so far. These indicate that the earlier assumption that the membrane resistance falls to a negligible value during the peak of the spike is false. A transient in clamping current occurs during cell excitation which has two components like the "A" and "B" parts of the unclamped

Part A. Major Findings (cont.) Serial No. NINDB-NP-SC-3

potential. The "A" current transient follows antidromic excitation over a wide range of clamping voltages indicating that it arises from an area only partially clamped with this technique. Repetitive firing of the "A" area follows clamping at depolarizing potentials. A relatively long time delay (1 msec) occurs between application of a depolarizing clamping potential and the development of the first "A-B" current transient.

Significance to Neurology Research: This is fundamental neurophysiological research designed to further development of hypotheses capable of more successful prediction of the observed behavior of the nervous system.

Proposed Course of Project: This technique of voltage clamping in the motoneuron will be continued and extended to the analysis of synaptic activity. It is too early to say whether further information can be obtained from this method on the repetitive activity of motoneurons, but if it appears feasible it will be exploited. On the basis of the results of this study it is planned to construct electrical models of the motoneuron for further comparative measurements.

Part B included Yes___ No X

Serial No. NINDB-NP-SC-4
1. Lab of Neurophysiology
2. Spinal Cord Section
3. Bethesda, Maryland
4. New

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A

Project Title: Effects of locally applied drugs on single spinal motoneurons.

Principal Investigators: K. Frank and W. Paton

Other Investigator: M. Becker

Cooperating Units: None

Man Years:
Total: 1.5
Professional: 0.6
Other: 0.9

Patient Days: None

Project Description:

Objectives: To test certain drugs for possible transmitter and inhibitor actions on spinal motoneurons.

Methods Employed: Concentric micropipettes are used to record simultaneously from inside and just outside a spinal motoneuron. Test drugs placed between inner and outer pipettes are applied to the surface of the cell by iontophoresis. Consequent changes in membrane potential, excitability, membrane resistance and spike generation are monitored by the inner pipette. Comparisons of drug-induced changes with those following natural synaptic excitation may permit identification or elimination of a test drug as a possible chemical transmitter substance.

Major Findings: No drug has been found which mimics either synaptic excitation or inhibition when applied with this technique. It was found that a number of factors affect the movement of drugs in the outer pipette: Iontophoretic field applied, hydrostatic head, capillary forces and electro-osmosis. Because the control of these factors requires a separate minor study for each drug tested to determine the form and manner of applying it, the method

Part A. Major Findings (cont.) Serial No. NINDB-NP-SC-4

is considered too cumbersome for use as a general survey technique. Preliminary findings on gamma amino butyric acid (GABA) indicate that this drug applied outside the cell reduces first excitatory postsynaptic potential (EPSP), resting membrane potential, then inhibitory post-synaptic potential (IPSP) and finally changes the cell membrane so that the outer pipette falls inside and the cell is destroyed. There is no indication that this drug is the physiological inhibitor substance acting in the spinal cord.

Significance to Neurology Research: Identification of transmitter substances would be a major step in the elucidation of basic mechanisms in the nervous system.

Proposed Course of Project: Since this technique has not proven practical as a general survey method it has been temporarily discontinued. Should other techniques indicate that a particular drug is a likely candidate as a chemical transmitter or inhibitor substance, this project can be reactivated to test the drug.

Part B included Yes___ No X

Serial No. NINDB-NP-SC-5
1. Lab of Neurophysiology
2. Spinal Cord Section
3. Bethesda, Maryland
4. New

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: "Direct" contralateral inhibition.

Principal Investigators: K. Frank and J. Sprague

Other Investigators: M. Becker and C. Terzuolo

Cooperating Unit: Department of Anatomy, University of Pennsylvania Medical School

Man Years:
Total: 1.5
Professional: 0.7
Other: 0.8

Patient Days: None

Project Description:

Objectives: To determine if there is reasonable time for an inhibitory interneuron to act in the so-called "direct" inhibitory pathway.

Methods Employed: Measurements were made of the latencies of postsynaptic potentials elicited in motor horn cells by stimulation of ipsilateral and contralateral dorsal roots in the S_2-S_3 region of the cat's spinal cord. The possibility of an interneuron acting in this "direct" inhibitory pathway can be weighed against the time available for such action.

Major Findings: After all other times have been accounted for, there remains an extra latency of 0.3 to 0.65 msec in the inhibitory pathway over that of the excitatory pathway. It is considered a very distinct possibility that this extra time, while short, is occupied by conduction of the inhibitory volley through an inhibitory interneuron.

Significance to Neurology Research: The claim of Eccles' group in Australia that inhibitory and excitatory synaptic knobs cannot occur on branches of the same afferent cells

Part A. Significance to Neurology Research (cont.) Serial No. NINDB-NP-SC-5

and that this requires an interneuron in the inhibitory pathway is supported by the present study.

Proposed Course of Project: No further work on this project is contemplated.

Part B Included Yes_____ No X

ANNUAL REPORT

LABORATORY OF NEUROCHEMISTRY

1958

Robert B. Livingston, M.D.

Acting Chief

Until more space becomes available to the laboratory, it will not be feasible to establish the one or two desirable additional sections and give a more adequate working space for the two present Sections. Until such a time, we have had to abandon our search for a Laboratory Chief. During the year, we submitted plans to the NIH which reflect our space and budgetary considerations pertinent to the expansion of this Laboratory. We are hopeful that a new building will be constructed here to accommodate some of the growing needs of the Basic Research Program and that such construction will be available for occupancy sometime during 1964. The Laboratory of Neurochemistry will at that time be due for major expansion. It is our plan in the meantime, after construction appropriations have been committed, to recruit and assemble together the necessary personnel to make this the most effective and resourceful establishment for the investigation of neurochemical problems.

Not a moment is to be lost in this enterprise because advances in biochemistry and related achievements in other complementary mental and neurological disciplines are ripe for imaginative exploitation by a strong team of neurochemists. Although the Laboratory then will be much expanded and as yet only two elements of the total breadth of interest in this field are represented, it is clear from what we already have within the Laboratory and within the Program that this expansion of neurochemistry will represent growth from strength within this essential discipline.

1. The Section on Physical Chemistry. Dr. Alexander Rich, former Chief of the Section on Physical Chemistry, was attracted away, much to our regret, to the post of Associate Professor at the Massachusetts Institute of Technology.

Both before and after his leaving, Dr. Rich was considerably torn, as were we, by his departure. Perhaps the outstanding differential between the two positions, from Dr. Rich's point of view, was that he had never before participated in university life except as a student. Now it would be possible for him to join an excellent faculty, extend his teaching at which he is very adept, as a bona fide tenure Professor, and surround himself with a humming drove of graduate students. Although we regret Dr. Rich's leaving, we are proud of the example of our quality he will represent at MIT. Moreover, the lesson is well taken that we should do all in our power to justify the feeling among our senior staff that they already belong to a first rate faculty; and to continue to build up the NIH as a high quality teaching instrumentality. For many of our staff, these attitudes and functions relating the individual to his colleagues and to the Institutes are of central importance.

We were very fortunate in being able to recruit, as a new Chief in the Section on Physical Chemistry, Dr. Sidney A. Bernhard. Dr. Bernhard received his Ph.D. Degree from Columbia University in 1951; under a NRC Postdoctoral Fellowship he received further Laboratory training with Prof. Linus Pauling at the California Institute of Technology concerning the theory of specific molecular interactions applied to biological systems (1951-1953) and with Dr. M. Gutfreund and Prof. F.J.W. Roughton at the University of Cambridge, England, on applications of this theory to enzyme systems (1953-1954). Dr. Bernhard has had a variety of teaching experiences. He transfers to the NIH from the Naval Medical Research Institute where he was doing research in the Division of Physical Biochemistry. Dr. Bernhard was already before this transfer quite closely associated with the Section on Physical Chemistry and with the Laboratory of Cellular Pharmacology. He is especially interested in the physical-chemical basis of action of enzymes, the characterization of active sites on the enzymes, and in the physical-chemical manipulations which may account for enzymatic action.

Dr. Bernhard took a leave of absence during the first six months of his appointment in order to

investigate the possibilities of multienzymatic participation in specific catalysis, at the Department of Biophysics, Weizmann Institute of Science, Rehovoth, Israel. Dr. Bernhard will return to begin laboratory work at the NIH early in February, 1959. In the meantime, working closely with other members of this staff, Dr. Bernhard has planned a certain amount of reorganization of the Section for more appropriate space utilization. As a closely collaborating group, Drs. David Davies, Dan Bradley and Stanley Glauser are continuing without abatement their individual research programs and especially those which will assist Dr. Rich in the completion of studies in which he was a collaborator. At the same time they have begun phasing in activities conjoining the interests of Dr. Bernhard. Actually the shift represents less a matter of program discontinuation and substitution but rather a shift of emphasis in a continuing program.

Dr. Gary Felsenfeld, much to our regret, also left the Section on Physical Chemistry in May, 1958, to become an Assistant Professor of Biophysics at the University of Pittsburgh. Dr. Felsenfeld during his stay here made many important contributions the last of which involved theoretical considerations regarding the likelihood of the double stranded DNA molecule and its analogues to suffer errors in molecular replication by omission or commission or misalignment of strands.

The Section on Physical Chemistry has continued its investigation of the important nucleic acids DNA and RNA for which it has already received an international recognition. For this purpose, they have manufactured a number of synthetic polynucleotides from an enzyme of high yield and quality. This has permitted a thorough examination of the structure of polyadenylic acid. Several different complexes of polynucleotides have been re-examined and verified by the Section, giving increased confidence that RNA-like molecules can assume a helical configuration similar to DNA. Further evidence has been elicited for the mechanisms by which the DNA may be separated into two strands which then rapidly recombine with matching substrate materials to form new strands as in genetic replication. New concepts of the hydrodynamic behavior of large and complex molecules have been proposed on the basis of studies on DNA and RNA. These same molecules have also been examined carefully from the point of view of their linking with acridine orange and other dyes. In collaboration with the Laboratory of Neuroanatomical Sciences, this study has been extended to the vital, and hopefully differential, staining of the natural nucleotides within nerve cells in tissue culture.

One of the most important aspects of biologically active protein systems relates to the way in which the protein partly or wholly surrounds metallic ions and the relationship which obtains between the metal and its protein coordination group. This has been a major pursuit of the Section. Thus, the relationship of cobalt to vitamin B_{12} and iron to cytochrome C is being carefully examined.

2. The Section on Lipid Chemistry. The Section on Lipid Chemistry, under the leadership of Dr. Roscoe O. Brady, has made notable advances in understanding the synthesis and metabolism and the requirements for such activities in lipid constituents of the brain. Of special note is Dr. Brady's recent discovery that aldol condensations of the Knoevenagel type, formerly recognized only in organic chemical systems, take place in the synthesis of sphingosine and fatty acids. Undoubtedly, such reactions will be found to play a key role in the lengthening of carbon chains in a large number of important biochemical substances. It is already suggestive that this reaction occurs in the formation of certain steroids. The discovery of such an important general reaction offers an advantageous example of the fruits of basic research.

The enzymatic synthesis of sphingosine from palmitic aldehyde and serine is now established. Similarly, the stepwise condensation of 2-carbon fragments in the biosynthesis of fatty acids has been shown to take place with activated molecules of malonyl coenzyme A. Dr. Brady has demonstrated the steps and requirements for formation and activation of the malonyl compound. One of the lipid constituents of the nervous system having a high turnover rate, the inositol phosphotides, has been shown to be formed through intermediary cytidine diphosphate diglycerides. A further basic study relates to the investigation of the mechanisms of formation of compounds which contain aromatic rings. Again, a gereral key to many biochemical problems is being sought.

Dr. Bernard W. Agranoff, in the Section on Lipid Chemistry, is currently being supported by the Institute for a year's training with Professor Feodor Lynen at the Institute for Cellular Chemistry in Münich, Germany. Since his training with Professor Lynen will include work on the determination

of oxidation and reduction of lipids by means of certain dye techniques (available only in Professor Lynen's Institute), we are looking forward to Dr. Agranoff's return and the fruitful application of these techniques to research within the Section on Lipid Chemistry.

Section on Lipid Chemistry

R. O. Brady, Chief

Research completed in this section during the past year has resulted in the discovery of a new biological mechanism for the lengthening of carbon chains. The newly-described reaction is an aldol condensation of the Knoevenagel type which had not been previously detected in biological systems. Its fundamental role in physiological processes was first elucidated in the course of investigations on the enzymatic synthesis of sphingosine, the basic component of materials called sphingolipids which are important constituents of the myelin sheath of nerves. The biosynthesis of sphingosine is catalyzed by enzymes present in brain tissue and the essential reaction is the condensation of palmitic aldehyde with an appropriately activated molecule of the amino acid serine. The serine is activated by the formation of a Schiff base-metal complex between serine, pyridoxal phosphate (vitamin B_6) and manganese ions. Under these conditions, the methylene carbon atom (carbon 2) of serine becomes negatively charged and is in a favorable condition for condensation with the carbonyl carbon atom of palmitic aldehyde which carries a positive dipole moment. The product of this reaction is dihydrosphingosine which subsequently is oxidized to sphingosine.

It was apparent from the contributions of several workers in the field of lipogenesis that the mechanism of biological formation of fatty acids remained unexplained. Experiments recently completed in this section indicate that the biosynthesis of fatty acids probably also occurs via an aldol condensation of the Knoevenagel type. The necessary reactants for this process are aliphatic aldehydes containing an even number of carbon atoms which condense with the activated methylene carbon of malonyl coenzyme A. The product of the reaction is a β-hydroxy fatty acid derivative of coenzyme A whose chain length has been increased by 2 carbon atoms, the carboxyl carbon of malonyl coenzyme A being displaced in the course of the condensing reaction. The resulting compound is dehydrated, and the unsaturated derivative is reduced with a molecule of triphosphopyridine nucleotide. The thiol ester of the newly-produced saturated fatty acid is reduced with another molecule of triphosphopyridine nucleotide to the respective aliphatic aldehyde and is thus capable of undergoing further condensation with another molecule of malonyl coenzyme A. The validity of this reaction scheme depends upon the ability of tissues to catalyze the formation of the required malonyl coenzyme A from acetyl coenzyme A and carbon dioxide. We have recently demonstrated the enzymatic carboxylation of acetyl coenzyme A to form malonyl coenzyme A in the presence of magnesium ions and adenosine triphosphate.

Another important contribution from this section during the past year is the elucidation of the mechanism of the biosynthesis of inositol phosphatides. These materials are present in

relatively high concentration in brain and nerve tissue. They are especially important because of their high metabolic turnover rate. Accordingly, these materials have been implicated as participants in the physiological processes carried on by nerve tissue. It was discovered that inositol phosphatides are formed through the participation of a new class of reactive intermediary metabolic compounds called cytidine diphosphate diglycerides (CDP-diglycerides). These compounds are composed of glycerol, 2 molecules of fatty acids, and the metabolically important nucleotide, cytidine diphosphate. Inositol reacts with CDP-diglyceride in the presence of the appropriate enzyme system to form inositol phosphatide. Cytidine monophosphate is split off in the course of the reaction.

Another project under investigation at the present time is the study of the formation of the gluco-cerebroside class of sphingolipids in tissues obtained from patients with Gaucher's disease. Our initial investigations indicate that splenic tissue obtained from these patients does catalyze the synthesis of cerebrosides. This finding therefore probably represents a definitive contribution towards the elucidation of the etiology of this disease.

There is evidence which indicates that sphingosine exhibits the properties of an anti-coagulant. We are beginning investigations to determine the level of free sphingosine in the plasma of normal individuals and patients with certain hemorrhagic diatheses in order to try to determine if such conditions are related to abnormalities of sphingolipid metabolism.

Other problems currently under investigation in this section deal with the mechanism of the formation of cholesterol and compounds which contain aromatic rings. Studies have recently been initiated on the possible antigenicity of certain sphingolipids. Serum obtained from patients with multiple sclerosis will be examined for the presence of specific antibodies against these lipid antigens.

Basic Research Program

Laboratory of Neurochemistry

Section on Lipid Chemistry

Estimated Obligations for FY 1959

Total: $86,500

Direct: $72,000

Reimbursement: $14,500

Individual Projects - 1, 6, 7, 13-15

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Biosynthesis of Sphingolipids

Principal Investigator: Roscoe Brady

Other Investigators: Eberhard Trams

Cooperating Units: None

Man Years
Total: 2.0
Professional: 1.0
Other: 1.0

Project Description

Project: Investigation of the formation of complex cerebral lipids containing sphingosine.

Objectives: To elucidate the pathway of formation of sphingolipids and to determine the rate of turnover and metabolic fate of these substances.

Methods employed: With the use of new enzymatic techniques developed in this laboratory for the investigation of the formation of sphingolipids, experiments have been conducted on the mechanism of formation of sphingosine, the basic constituent of complex cerebral lipids of the sphingolipid class. Discoveries in this laboratory have demonstrated the pathway of formation of sphingolipids called cerebrosides. Investigations have been initiated on the formation of abnormal glucocerebrosides which accumulate in the reticuloendothelial cells of patients with Gaucher's disease. Preliminary studies along these lines indicate that splenic tissue obtained from these patients does catalyze the synthesis of glucocerebrosides.

Part A, continued:

Major findings: The initial phase of research on the biological formation of sphingosine has been completed. The results have led to the discovery of a new reaction for the biological lengthening of carbon chains. This reaction is an aldol condensation of the Knoevenagal type, a reaction familiar to organic chemists which had not been previously detected in biological systems. The biosynthesis of sphingosine is catalyzed by enzymes in brain tissue and the principal reaction is a condensation between a molecule of palmitic aldehyde with an appropriately activated molecule of the amino acid serine. Serine is activated by the formation of a Schiff base-metal complex between serine, pyridoxal phosphate, and manganese ions. Under these conditions, the methylene carbon atom, carbon 2, of serine becomes negatively charged and is thus in a favorable condition for condensation with the carbonyl carbon atom, carbon 1, of palmitic aldehyde which carries a positive dipole moment. The immediate product of this condensation reaction is dihydrosphingosine which is subsequently oxidized to sphingosine.

Significance to Neurological Diseases research: Since sphingolipids comprise a large part of the components of the myelin sheath of nerves, research dealing with the mechanism of the formation of these compounds is of utmost importance for the undertaking of a rational approach towards the treatment of demyelinating disease states. Furthermore, the finding that tissues obtained from patients who are afflicted with lipodystrophic conditions such as Gaucher's disease do apparently form these materials in situ must lead to a re-evaluation of the concepts concerning the etiology of these diseases and should ultimately provide a basis for appropriate corrective measures.

Proposed course of project: It is proposed to continue investigations on the formation of complex lipids. Tissues obtained from patients with Gaucher's disease, Niemann-Pick's disease and, if possible, Tay-Sachs disease will be employed. Furthermore, investigation of the metabolism of complex lipids in normal and diseased states will be undertaken. In this regard, carbon-labeled radioactive sphingosine is at present being prepared and the fate of this compound will be investigated.

Part B included Yes X No ___

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards and Publications

Publications other than abstracts from this project:

1. Brady, R. O. and Koval, G. J. The enzymatic synthesis of sphingosine. J. Biol. Chem., 233: 26-31, 1958.

2. Brady, R. O., Formica, J. V., and Koval, G. J. The enzymatic synthesis of sphingosine II. Further studies on the mechanism of the reaction. J. Biol. Chem., 233: 1072-1076, 1958.

3. Burton, R. M., Sodd, M. A., and Brady, R. O. Studies on the biosynthesis of galactolipids. Neurology, 8, Supplement 1, 84-85, 1958.

4. Burton, R. M., Sodd, M. A., and Brady, R. O. The incorporation of galactose into galactolipids. J. Biol. Chem., 233: 1053-1060, 1958.

Honors and Awards relating to this project:

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Biosynthesis of Aromatic Compounds

Principal Investigator: Roscoe Brady

Other Investigators: H. W. Siegelman

Cooperating Units: Plant Physiology Laboratory, Pioneering Research Group, U. S. Department of Agriculture, Beltsville, Md.

Man Years:
Total: 0.4
Professional: 0.2
Other: 0.2

Project Description:

Project: Investigation of the mechanism of formation of aromatic compounds.

Objectives: To discover the enzymatic pathway of formation of aromatic compounds which do not arise via the sedoheptulose diphosphate pathway.

Methods employed: The enzymatic synthesis of complex aromatic molecules will be investigated using standard enzymological methods as well as radioactive tracer techniques.

Major findings: It has been ascertained that labeled acetate is a preferential precursor for the biological formation of compounds containing benzene rings. There exists, particularly in plant tissue, a biological route for benzene ring formation other than via sedoheptulose, quinic, shikimic acids, etc.

Part A, continued

Significance to Neurological Diseases research: Recent evidence indicates that biologically-active quinones are required for various oxidation-reduction processes. The indispensability of these materials for electron transport suggests that they might exert a considerable role in the conduction of the nerve impulse.

Proposed course of project: After the preparation of a satisfactory enzyme system for the formation of the benzene ring compounds, it is proposed to investigate this reaction process particularly with regard to the liklihood that the reaction also occurs by way of an aldol condensation of the Knoevenagal type.

Part B included Yes____ No X

Serial No. NINDB-NC-7
1. Neurochemistry
2. Lipid Chemistry
3. Bethesda, Md.
4. Continuation

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Metabolism of Inositol

Principal Investigator: Bernard Agranoff

Other Investigators: None

Cooperating Units: None

Man Years:
Total: 2.0
Professional: 1.0
Other: 1.0

Project Description

Project: Investigation of the metabolism of inositol.

Objectives: To learn the nature of the biosynthetic pathway for the formation of inositol-containing compounds in nerve and brain tissue.

Methods employed: A spectrophotometric method for the determination of inositol has been developed in this laboratory. In addition, the metabolism of radioactive inositol has been investigated in whole animals, tissue slices, and appropriate enzyme preparations prepared from tissue extracts.

Major findings: The pathway of the enzymatic incorporation of inositol into inositol lipids has been established. This incorporation has been found to be dependent upon the presence of cytidine nucleotides and magnesium ions. It has been discovered in the course of these investigations that inositol phosphatides are formed through the participation of a new type of reactive intermediary metabolic compound consisting of cytidine diphosphate diglycerides. These compounds are composed of glycerol, two molecules of

Part A, continued

fatty acids and the metabolically important nucleotide, cytidine diphosphate. Free inositol reacts with cytidine diphosphate diglyceride in the presence of the appropriate enzyme system to form inositol phosphatide. Cytidine monophosphate is split off in the course of the reaction. There has been no previous description of an exactly analogous biochemical reaction of this nature. Investigations in other laboratories have recently confirmed our finding of this mechanism for the formation of inositol phosphatides.

Significance to Neurological Diseases research: Since inositol-containing lipids have the highest turnover rate of all brain phospholipids, a study of the metabolic pathways for the formation and fate of these materials is particularly important. It has been postulated that the turnover of this material is related to metabolic processes peculiar to nerve cells.

Proposed course of project: In addition to demonstrating the pathways of the formation of inositol phosphatide, it is apparent from the studies completed that inositol is incorporated into compounds other than inositol lipids. In other words, inositol may be linked with peptides or perhaps nucleotides. The identification of some of these materials must be undertaken, and it has been demonstrated that the processes whereby inositol is incorporated into these materials is distinctly dissimilar from the sequence of reactions required for the formation of the lipid inositol phosphatides. The pathway of the formation of compounds other than lipid inositides will be investigated. Furthermore, an attempt will be made to isolate naturally-occurring cytidine diphosphate diglycerides. Their identification so far has rested upon studies with labeled compounds. Further investigation into the metabolic reactivity of these materials will then be undertaken.

Part B included Yes X No ___

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Agranoff, B. W. and Brady, R. O. Studies with tritium labeled inositol. Neurology, 8: Supplement 1, 79-80, 1958.

2. Agranoff, B. W., Bradley, R. M., and Brady, R. O. The enzymatic synthesis of inositol phosphatides. J. Biol. Chem., 233: 1077-1083, 1958.

3. Agranoff, B. W. Low Level tritium counting techniques, pp. 220-222. Chapter in: Liquid Scintillation Counting, New York, Pergamon Press, 1958.

Honors and Awards relating to this project:

Serial No. NIMDB-MC-13
1. Neurochemistry
2. Lipid Chemistry
3. Bethesda, Md.
4. New Project

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: The Effect of Sphingosine on Blood Coagulation

Principal Investigator: Professor Eugen Hecht

Other Investigators: Roscoe Brady

Cooperating Units: None

Man Years
Total: 1.3
Professional: 1.0
Other: 0.3

Project Description

Project: Investigation of the anti-coagulant activity of sphingosine.

Objectives: To learn how sphingosine reacts with the components required for the process of blood clotting to delay this physiological phenomenon.

Methods employed: Purified sphingosine will be investigated in specific reactions required for the clotting of blood to attempt to establish the locus of the anti-coagulant properties of sphingosine.

Major findings: Sphingosine has been identified as a normally-occurring anti-coagulant. Also, it has been found that sphingosine forms inclusion compounds with a number of long chain lipid derivatives. It is conceivable that the mechanism of action of sphingosine is that of a complexing agent which removes from the sequence of reactions involved in blood clotting, a normally-occurring component required for coagulation.

Significance to Neurological Diseases research: The finding that breakdown products of the myelin sheath exhibit potential anti-coagulant properties merits investigation.

Part A, continued

Proposed course of project: A micro-analytical determination for the estimation of the amount of sphingosine in plasma of normal and certain cases of hemolytic diseases and manifestations of deficiencies in the clotting process will be examined. The use of the isotope dilution technique with tritiated sphingosine will be employed for this determination.

Part B included Yes____ No X

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Enzymatic Synthesis of Fatty Acids

Principal Investigator: Roscoe Brady

Other Investigators: None

Cooperating Units: None

Man Years
Total: 1.5
Professional: 0.5
Other: 1.0

Project Description

Project: Investigation of the mechanism of the formation of long chain fatty acids.

Objectives: Recent experiments in this laboratory have indicated that the enzymatic synthesis of long chain fatty acids occurs by an aldol condensation of the Knoevenagel type between aliphatic aldehydes and malonyl coenzyme A. The present objectives are to purify the enzyme systems responsible for this series of reactions and to identify the intermediary reactants in this process.

Methods employed: A synthetic organic procedure has been developed for the preparation of radioactive malonyl coenzyme A. This material will be used as substrate for the investigation of the condensation reaction. Aliphatic aldehydes from two to fourteen carbon atoms in length will be examined for their ability to participate as substrates in the condensing reaction. Conventional enzyme fractionation procedures will be used to prepare the requisite enzyme systems to be examined.

Major findings: It was demonstrated in this laboratory that fatty acid synthesis occurs via an aldol condensation of the Knoevenagel type. The necessary reactants for the process

Part A (continued)

are aliphatic aldehydes containing an even number of carbon atoms which condense with the activated methylene group of malonyl coenzyme A. The product of the reaction is the β-hydroxy fatty acid derivative of coenzyme A. The chain length has been increased by two carbon atoms. The terminal carbon of malonyl coenzyme A is displaced in the course of the condensing reaction. The resulting β-hydroxy compound is dehydrated and the unsaturated derivative is reduced with a molecule of triphosphopyridine nucleotide. The thiol ester of the newly formed saturated fatty acid is condensed with a second molecule of triphosphopyridine nucleotide to form the respective aliphatic aldehyde. The aldehyde is thus in position to undergo a further condensation with another molecule of malonyl coenzyme A.

Significance to Neurological Diseases research: The [illegible] of lipids in myelin sheath of nerves is unconcealed. The formation of these lipids requires to a major degree the participation of long chain fatty acids containing between sixteen and twenty four carbon atoms. For a satisfactory investigation of myelin sheath formation, it is obvious that a knowledge of the mechanism of the formation of fatty acids should be available. Accordingly, the fundamental nature of this contribution is evident.

Proposed course of project: In addition to the preparation of radioactive malonyl coenzyme A, we have recently demonstrated that tissue extracts contain enzymes which catalyze the formation of malonyl coenzyme A from acetyl coenzyme A and carbon dioxide in the presence of adenosine triphosphate and magnesium ions. It is thought that this reaction represents the fundamental process by which malonyl CoA is made available for fatty acid synthesis. The demonstration of this process in tissue extracts is thus of considerable significance. It is proposed to attempt to purify the enzyme system which is responsible for this reaction to study the stoichiometry of the process. In the course of these studies, we will attempt to obtain insight into the mechanism of action of the vitamin biotin. Biotin has been implicated in carboxylation reactions although the exact nature of its participation is at the present time unexplained.

Part B included Yes X No ____

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Brady, R. O. The enzymatic synthesis of fatty acids by aldol condensation, Proc. Nat. Acad. Sci., 44, 993-998, 1958.

Honors and Awards relating to this project:

Serial No. NINDB-NC-15
1. Neurochemistry
2. Lipid Chemistry
3. Bethesda, Md.
4. New Project

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Biosynthesis of Cholesterol

Principal Investigator: Roscoe Brady

Other Investigators: Eberhard Trams

Cooperating Units: None

Man Years
Total: 0.8
Professional: 0.3
Other: 0.5

Project Description

Project: To investigate the mechanism of conversion of mevalonic acid to cholesterol.

Objectives: To elucidate the nature of the condensation reaction between molecules of mevalonic acid for the formation of farnesene and squalene which are precursors of cholesterol.

Methods employed: Phosphorylated intermediates in the proposed sequence of reactions between mevalonic acid and squalene will be prepared. The mechanism of the condensation reaction will be investigated by suitable tracer techniques.

Major findings: The project has just been initiated.

Significance to Neurological Diseases research: The importance of cholesterol as a component of the myelin sheath of nerves and its relation to arteriosclerosis are well known. The knowledge of the mechanism of its formation may be of considerable benefit for the study of appropriate therapeutic agents to facilitate its formation in certain conditions or retard its accumulation in others.

Part A, continued

Proposed course of project: Model reactions will be investigated in vitro using appropriately activated intermediary compounds derived from mevalonic acid. Studies will be undertaken on the activation of carbon atoms which participate in the actual carbon-carbon condensation reaction for the formation of squalene and ultimately cholesterol.

Part B included Yes____ No X

NATIONAL INSTITUTE OF NEUROLOGICAL DISEASES AND BLINDNESS

Basic Research Program

Laboratory of Neurochemistry

Section on General Neurochemistry

Estimated Obligations for FY 1959

Total: $253,000

Direct: $210,000

Reimbursement: $43,000

Individual Projects - 12 through 13

Individual Project Report
Calendar Year 1958

Part A.

Project Title: Visuo-Motor Coordination in a Lower Vertebrate.

Principal Investigator: Robert B. Livingston, M. D. and Magdolna A. Iranyi, M D.

Other Investigators: None

Cooperating Units: The Director of the U. S. National Zoological Park (Dr. Theodore H. Reed).

Man Years (calendar year 1958):

Total:
Professional: 1.0
Other: 0

Project Description:

Objectives: To determine the anatomical pathways lying between the retina and the eye motor nuclei, and to examine behavioral changes resulting from interference with different parts of those pathways. Lower vertebrates provide some advantages in having more limited circuits relating to visuo-motor coordination; yet the Chameleon must depend upon such circuits to catch its food, living flies which it captures by throwing its tongue long distances. The anatomical organization of the eye motor nuclei and the extraocular muscles in the Chameleon appear to follow the usual vertebrate scheme. Nevertheless the Chameleon possesses an extreme degree of independence of eye movements and yet is able to direct both globes toward the same target. It has generally been supposed that the animal was able to utilize binocular as well as uniocular double vision. This study represents an example of our continuing interest in the mechanisms of sensorimotor coordination.

Major Findings: [illegible] significant differences between bipolar and unipolar food-catching ability in the chameleon. This appears to be true whether one eye is surgically patched or whether one eye is removed.

Our anatomical findings have confirmed and extended those of three previous investigators and our own previous findings relating to structures intermediate between the eyes and the eye motor apparatus. The major and most direct connections are now recognizable.

Significance to the Program of the Institute: Studies of this kind are basic to an understanding of sensorimotor coordination.

Proposed Course of Project: Until it will be possible to initiate further electrophysiological studies on the chameleon visuo-motor circuits this project will be given a low priority.

Part B included: No

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Vestibular Influences on Spinal Mechanisms

Principal Investigator: Bo E. Germandt, M. D.

Other Investigators: Magdolna Iranyi, M. D., Sid Gilbert, M. D., and Robert B. Livingston

Cooperating Units: None

Man Years (calendar year 1958):
Total: 1.2
Professional: 1.2
Other:

Project Description:

Objectives: To gain a better understanding of the nervous integration which takes place between vestibular and spinal mechanisms in the final composition of muscle tone, posture, and locomotion. By this study it will be possible to learn certain general principles relating to the function of the nervous system. The study is an example of our continuing interest in the mechanisms of sensorimotor coordination.

Methods Employed: Stimulation of the vestibular is by the technique of Germandt and Anderson in cats. Natural stimulation as by acceleration of the columns in the semicircular canal does not produce synchrony of impulses to permit an easy tracing of signals along the functionally important central pathways. have been recorded from a variety of spinal nerves, roots and small filaments of the ventral roots. input stimulation has been applied to the various peripheral nerves and dorsal roots.

Major Findings: Determination has been made of the nature and extent of vestibular evoked responses recorded throughout the surface and interior of the lower mesencephalon and upper spinal cord. An analysis has been made of the gross functional pathways followed by vestibular evoked responses from their point of entry on one side of the brainstem to their outflow via spinal nerves along both sides of the neuraxis and of the patterned responses of both gamma and alpha ventral root fibers. There are differences in the character of the spinal output of vestibular origin as distributed to the upper and lower limbs. Vestibular impulses give rise to facilitatory and inhibitory patterns which seem to be linked to mechanisms relating to reciprocal spinal activities and which involve complex interactions with local spinal reflexes. Vestibular impulses crossing to the contralateral side at the bulbar level are sufficient but are not necessary for the contralateral spinal effects. Elimination of both bulbar and local segmental crossings leaves evidence for intermediate functional cervico-thoraco-lumbar crossings. Surprisingly, the contralateral vestibular nuclei do not themselves respond. Moreover, they can be removed without interfering with either ipsilateral or contralateral spinal responses to vestibular stimulation. Other evidence reinforces the importance of reticulo-spinal pathways and vestibulo-reticular integration.

Spinal vestibular responses are markedly influenced by neck muscle proprioceptors. Lengthening of the dorsal neck muscles markedly inhibits or abolishes such responses. Impulses elicited by vestibular and peripheral nerve stimulation interact within certain limited regions of the cerebellum. Removal of the cerebellum or application of silver nitrate crystals to the pertinent cerebellar regions produces an immediate augmentation of the spinal vestibular response, demonstrating a tonic inhibitory cerebellar influence.

Significance to the Program of the Institute: 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 integration between the classical and the diffusely projecting systems in animals without central anesthesia is of especial current value.

DATE DUE

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