ANNUAL REPORT
OF
PROGRAM ACTIVITIES

NATIONAL INSTITUTE OF NEUROLOGICAL DISEASES AND STROKE

FISCAL YEAR 1971
PART II

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

ANNUAL REPORT

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NATIONAL INSTITUTE OF NEUROLOGICAL DISEASES AND STROKE

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PART II

ANNUAL REPORT
July 1, 1970 through June 30, 1971
Associate Director, Collaborative and Field Research
National Institute of Neurological Diseases and Stroke
National Institutes of Health

Through October 31, 1970, leadership of this program area was provided on an acting basis by Dr. Eldon L. Eagles, Deputy Director, NINDS. On November 1, Dr. Warren V. Huber was appointed Associate Director. Dr. Huber was formerly Project Director of the American Neurological Association's Joint Committee for Stroke Facilities, and had served as Chief of Neurology in the Veteran's Administration's Department of Medicine and Surgery. Dr. Huber's appointment marked a re-emphasis of the role of collaborative, directed, and field-type research in the overall effort to accomplish the scientific objectives of this Institute. The intent of this report is not to encapsulate items of information found in the separate branch summaries but to provide an overview of the significant accomplishments and problems of the Institute's Collaborative and Field Research programs during Fiscal Year 1971, and to look briefly, though broadly, toward the future of these programs.

PROGRESS AND ACCOMPLISHMENTS, FY 1971

Increased funds provided by the President's budget and congressional supplements permitted orderly program expansion in epilepsy, funding for a pilot study of cerebral death, and sufficient support to prevent dislocation in other programs. Without these increases significant program opportunities would have been lost.

The Special Projects Branch continued to pursue studies of neurologic disorders, concentrating on controlled clinical studies for the evaluation of two experimental anticonvulsants and one marketed agent. These efforts have demonstrated the value of blood level determinations as an adjunct to anticonvulsant therapy and established methodologies for further anticonvulsant studies. The successful transition of Epilepsy Abstracts from a fully supported Institute publication to a partially subsidized periodical available by subscription has demonstrated that periodical's scientific merit and value. The completion of the evaluation of the 15-year field follow-up of head injured veterans of the Korean campaign, and the evaluation of data from Phase I of the Head Injury Model Construction Program, mark the conclusion of two important aspects of the Institute's directed Head Injury Program. Funds must now be obtained and leadership recruited for continued efforts in this important program area.

The process of planning and developing a collaborative study of the criteria of cerebral death was accomplished by staff of the Office of the Associate Director and the Head Injury Section of the Special Projects Branch. With expert guidance from an ad hoc advisory committee, an acceptable protocol was developed and a coordinator and collaborators were selected. Contracts for the study will be awarded before July 1, 1971.

Results of controlled and limited studies using the assembled data base have continued to come in to the Perinatal Research Branch. Subject area task forces, in collaboration with the Epidemiological and Statistical Advisory Committee, are redirecting staff effort and attention to larger, more significant statistical analyses to identify major trends, developmental patterns, and risk factors. Interesting and significant studies of the influence of infectious diseases in pregnancy and childhood upon the development of the child's central nervous system (CNS) continue.

A multi-institutional study to determine the feasibility of developing an effective sensory prosthesis for the blind has been launched. Currently, the study is focused on the problems of direct stimulation of visual centers of the brain, including the safety thereof, and the reproducibility of stimulation results. The future success of this project is enchanced in large part by the careful, expert, and conscientious project direction being provided by the Chief, Laboratory of Neural Control, Intramural Research, and his assistant.

Epidemiologic studies of a selected group of acute and chronic nervous system disorders continue. Short term studies, particularly of multiple sclerosis, sub-acute sclerosing panencephalitis, and CNS tumors have pointed the way toward a better understanding of the etiology of these diseases.

Collaboration with scientists working in a broad range of the basic biological sciences and in clinical medicine has improved considerably our understanding of the mechanisms and etiology of slow, latent and temperate virus infections. Successful transmission of two sub-acute and chronic degenerative diseases of the nervous system from man to primates has led to the implication of suspect viral agents--a significant step in the march toward understanding the etiology and thereby, hopefully, being able to successfully treat or prevent such types of central nervous system disease.

PROBLEMS - FY 1971

Despite the successes and progress of the past year there are problems affecting program development which remain to be solved.

Tight controls on full-time and permanent employment restricts the recruitment of staff needed to implement and develop important new programs. This has necessitated the most careful allocation of positions as vacancies occur. Only through this mechanism can needed and important shifts in priority be implemented.

Head and spinal cord injury has been recognized as a serious problem for several years. Despite generous Extramural support and a vigorous Intramural program, important scientific questions remain unanswered; these must be answered if the goal of improved treatment, coupled with improved prevention, is to be attained. Directed collaborative studies aimed at elucidating the pathophysiologic responses of brain and spinal cord tissues to trauma are urgently needed. Until additional funds are available and adequate leadership can be found--and the two needs are interrelated--studies in this important problem area cannot be developed.

The significant FY 1972 budget decrease that has been imposed on the Perinatal Research Branch (PRB) makes it exceedingly difficult to accomplish two important tasks. The first is an orderly completion of the Collaborative Perinatal Project. As a result of the cut, important activities in follow-up, sample maintenance, and quality control at the collaborating institutions and at the coordinating center in the PRB will be curtailed. The second task which must be foregone is the planning for research projects on neurological diseases of childhood; preliminary results of the Collaborative Perinatal Project indicate these would be productive. Although an orderly decrease in budget support and effort by the PRB was planned as the study phased out, the immediate and drastic decrease at this time is quite damaging.

The Collaborative and Field Research Programs of NINDS are, by their nature, heavily dependent upon continuing and active biostatistical participation. The need for biostatisticians in these varied programs goes deeper than simple part-time consultation. To be effective, the statistical staff must be part of the research teams and relate closely to them. Scientific program leaders, charged with the responsibility of promptly producing evidence of research results to fully collaborate with scientists outside of NINDS, must have the explicit and constant control of the processing and analysis of the data upon which they rely. Without such control they have experienced an inability to discharge these responsibilities promptly and effectively. Therefore, it is imperative that such a realistic relationship with the Office of Biometry, NINDS, be established.

LOOKING AHEAD

Fiscal Year 1972 promises to be a difficult year in terms of availability of resources to move forward with existing and planned programs. Budget cuts will effect the Epilepsy, Head Injury, and Cerebral Death Programs as well as the Collaborative Perinatal Project. Considerable resourcefulness will be required to maintain programs which have been so laboriously built and organized. In no way will it be possible to mount new programs in stroke research, in disorders of communication, or to pursue significant head and spinal cord injury research.

As data collection for the Collaborative Perinatal Project moves toward completion, and is supplanted by less expensive, but promising, investigations of neurologic disorders of childhood, funding required by the Perinatal Research Branch will diminish. It is anticipated that this will permit the resumption of active, directed, collaborative research in the field of head and spinal cord injury, and the commencement of similar efforts in the fields of communicative disorders and stroke, with only relatively minor increases in the overall funding level over the next five years.

ANNUAL REPORT
July 1, 1970 through June 30, 1971
Special Chronic Disease Studies for Collaborative and Field Research
National Institute of Neurological Diseases and Stroke
National Institutes of Health

Study of Child Growth and Development and Disease Patterns in Primitive Cultures, and Slow, Latent and Temperate Virus Infections

This section has continued to focus its attention on long term studies of the human biology of the many vanishing primitive societies. Our laboratory research and that of our numerous collaborating investigators is directed to problems which have been phrased under this theme. The neurological development and learning patterns in children in diverse cultural experiments in the human condition has been the major focus of attention. The laboratory studies on human biology, genetics and associated molecular biology, immunology, virology, and biochemistry have all been directed at solving problems which have been carefully chosen from small isolated bands still living in the primitive situation in which these problems may be more appropriately studied than in larger civilized societies.

Our efforts to document the development and neurological patterning in disappearing primitive cultures has resulted in the largest archive of such documentation in the world. The collection and preservation of existing cinema data from Australian aborigines, New Guinean, Oceanic and African aborigine groups, and American Indians, as they live as hunter-gatherers or primitive hoe-and-digging-stick agriculturists provides the only such documentation of the life and behavior of man, as he most probably lived and evolved for over 99% of his evolutionary history, or about one million years.

Kuru, a new disease which we discovered and described in the New Guinea Highlands fourteen years ago, has been the first subacute and chronic degenerative disease of the nervous system of man with a transmission model in animals and an established virus etiology. From analogy with kuru, we selected Creutzfeldt-Jakob disease and other forms of spongiform encephalopathy as likely slow infections. The "hunch" proved right: Creutzfeldt-Jakob disease has now been transmitted to chimpanzees, independently from ten patients, and serial chimpanzee to chimpanzee transmission has also been successful through the third passage. The agent, as that of kuru, is filterable. During this year significant breakthroughs were achieved in the study of these two CNS diseases with the successful transmission of both kuru and Creutzfeldt-Jakob disease from human patients to 3 species of New World monkeys — the spider monkey (Ateles geoffroyi), the squirrel monkey (Saimiri sciureus) and the capuchin monkey (Cebus sp.). Creutzfeldt-Jakob disease was transmitted from affected chimpanzee to yet another New World monkey, the woolly monkey (Lagothrix lagothricha), as well as to squirrel monkeys. Serial passage and viral pathogenesis and characterization studies have been expanded utilizing these more economically purchased and conveniently handled primates. These animals are also being used in isolation studies being conducted on other human slow-infections suspected of being caused by viruses. It is significant to note that during the past year we have received greater than 400 specimens from patients with CNS diseases.

Of this number, approximately 50% (200) are brain biopsy and autopsy specimens and between 50-60 of these are from patients with Creutzfeldt-Jakob disease. We now believe that the two human diseases, kuru and Creutzfeldt-Jakob disease, and the two similar animal diseases, scrapie and mink encephalopathy, which we are also studying, form a group of diseases of similar pathogenesis, which we have called the spongiform viral encephalopathies.

Our work has continued to incriminate the measles virus as the cause of SSPE, but we have not yet found evidence of a second virus in this disease. With the return of Dr. David Asher, from 17 months work in various institutes in the Soviet Union, work has been begun on the problem of chronic progressive forms of encephalitis caused by tick-borne encephalitis virus. Several strains of the virus known to induce chronic infections have been inoculated into rhesus monkeys. The continuation of these studies, in collaboration with our colleagues in the Soviet Union, are providing working models for the in depth studies to demonstrate chronic virus infection as the cause of epilepsy partialis continua or focal epilepsy in children in which viral-like inclusion bodies are found in brain cells. Further, we are pursuing with our Soviet colleagues our studies on epidemic hemorrhagic fever, of the nephro-nephritis type, which occurred in epidemic proportions in civilians and troops during the Korean War and which still occurs at a somewhat lower level of incidence at the present time.

Encouraged by our success with one of the presenile dementias and several cases in the borderland between Alzheimer's and Creutzfeldt-Jakob diseases, we are concentrating on the other presenile dementias and senility with particular emphasis on Alzheimer's and Pick's diseases, parkinsonism-dementia and senile dementia. Our work on the presenile and senile dementias, the isolation of latent viruses from the central nervous system, and comparisons of the many aspects of the pathological processes in kuru and Creutzfeldt-Jakob disease (including the presence of amyloid (senile) plaques and extensive glial hypertrophy with fibrillary tangles in the astrocytes) has led us to be increasingly concerned with possible virus involvement in certain aspects of senile dementias and normal aging.

In our attempts to establish infection as the etiology of other diseases of the CNS of man we have successfully isolated in tissue culture a strain of adenovirus from the brain of a patient with lymphosarcoma. We have shown the virus to be closely related to candidate adenovirus type 32. Furthermore, we have recently demonstrated Cowdry type A intranuclear inclusion bodies, which by EM contain papovavirus-like virions, in in vitro explant cultures of human brain from a patient with progressive multifocal leucoencephalopathy.

We are trying to establish the range of cases with spongiform encephalopathy which may be transmitted; other diseases we are studying by in vitro cultivation of brain and other tissues, and with animal (including monkeys and apes) inoculations, are multiple sclerosis, amyotrophic lateral sclerosis (both sporadic and familial types), parkinsonism-dementia complex, in both the U.S. and Guam, essential parkinsonism, myoclonic epilepsy, Schilder's disease, metachromatic leucodystrophy, and progressive supranuclear palsy. In collaboration with Drs. Michael Alpers and Malcom Simons

of Australia investigations to seek the agent of kuru in cultivated leucocytes of patients and of experimental chimpanzees are under way, and the evaluation of the humoral (thymic) and delayed hypersensitivity (splenic) immune status in kuru in man and in the experimental disease have been made.

Most significant has been our continued isolation of further strains of hidden, masked, or latent viruses from surgically sterile brain and other tissues of both our chimpanzees with transmitted neurological diseases and of human patients. The list of these virus isolates from chimpanzees is now over one hundred virus strains. These fall thus far into ten identified new virus species. Six of these have been thoroughly investigated virologically and have been the subject of both graduate theses and papers in the specialty journals.

All six viruses have been deposited in the American Type Culture Collection. Two of the six viruses, Pan 1 and Pan 2, have now been classified as simian foamy viruses type 6 and type 7, respectively. We have demonstrated that these RNA viruses have an RNA-DNA dependent polymerase suggesting a close relationship to the RNA oncogenic group of viruses.

In 1969 we isolated two strains of virus from the lungs and brain, respectively, from sheep with progressive pneumonia in Montana. We have now demonstrated that both viruses are serologically indistinguishable from maedi, a lymphoreticular progressive pneumonia, and visna, a primary demyelinating disease of sheep in Iceland. Moreover, visna, maedi and the virus of Montana sheep progressive pneumonia are all RNA viruses associated with an RNA-DNA dependent polymerase. Further characterization of these viruses and their relationship to the RNA oncogenic group of viruses having these properties are under way.

In many cases, two or more viruses have been isolated from the same brain. We are forced to speculate that activation of these latent agents may be a process responsible for some subacute or chronic neurological diseases. We are attempting to study the activation of measles virus to produce SSPE, of chickenpox virus to produce herpes zoster in later life, of herpes simplex virus and the simian herpes viruses to produce central nervous disease, and of a papova-like virus to produce progressive multifocal leucoencephalopathy. Cytomegalo virus and EB (Epstein-Barr) virus activation to produce the post-transplantation of "pump" syndrome in most subjects receiving organ transplants has been studied by Dr. Lang at Duke University. We are now interested in using this work as well as a model for the chronic and slow infections with which we work. Our current hypothesis is that similar processes may underlie the pathogenesis of kuru, Creutzfeldt-Jakob disease, and other spongiform encephalopathies and, perhaps, even be involved in some presenile and senile dementias.

We therefore continue attempts at isolating virus strains from long-maintained sterile tissue explants and trypsinized cell suspensions, using the techniques of cell fusion and co-cultivation. These are being applied to the degenerative central nervous system disorders listed above, as well as to other chronic diseases. The electron microscopy, in collaboration with Dr. Peter Lampert, and the immunological techniques, including fluorescent antibody, are being used to localize virus-like particles, gamma glob-

ulin, components of complement, and antigen-antibody complexes in tissue and in sera of patients with these diseases; and extensive serological investigations of serum and spinal fluid from patients with these diseases for antibodies against a wide range of microbial antigens has been continued.

Nutritional studies, and studies on reproduction and fertility, on the selective advantage and establishment of genetic polymorphisms, of unusual and odd alternative ways of employing the central nervous system in its higher cerebral function of language learning and use, computation (number sense and calculation with a numbers system), psychosexual culturally-modified behavior, and cognitive style, are providing data on alternative forms of possible neurological functioning for man, of which we would remain unaware and for moral, ethical and political reasons be unable to produce or investigate in the clinic or laboratory, once the natural cultural experiments in primitive human population isolates had all finally been amalgamated into the modern civilized cultural veneer which is now imposed upon almost all members of the community of man.

Growth and development studies in primitive cultures have yielded new evidence for incredibly delayed puberty and slow growth rates in certain peoples, particularly the short-of-stature populations in the Highlands of New Guinea. Here we find people with the mean age of menarche over eighteen years, and with male puberty of eighteen at twenty years. These are thus the slowest growing populations on earth, with the most delayed puberty; two thirds of their life span is spent in reaching maturity. Preliminary evidence suggests high levels of pituitary growth hormone in such populations, in both pituitary glands obtained at autopsy and in the serum. Growth rate seems to be proportional and age of puberty inversely proportional to mean adult stature.

Congenital mental defect and a wide range of neurological problems associated with the most severe foci of endemic cretinism in the world, in the Highlands of West New Guinea (West Irian, Indonesia), are under further investigation. Our investigation of an accumulation of cases of familial periodic paralysis in certain of the Pacific Islands continues.

The discovery of new genetic factors, including haptoglobins and hemoglobins, with elucidation of their biochemical structure and their later use as markers in human population genetic studies, has been a by-product of these investigations. Similarly, the discovery of immunologically virgin populations without exposure to respiratory and enteroviruses which are ubiquitous in the civilized world, has permitted: 1) fundamental investigation on the immune response in man; 2) investigations possible no where else on the persistence of immune response after natural measles infection and live attenuated measles virus immunization in the absence of circulating virus, yielding data important to the diagnosis of understanding of delayed slow measles encephalitis (subacute sclerosing panencephalitis, SSPE), and 3) establishing the serological identity of the agent of the 1918 influenza pandemic to aid in the genetic-historical elucidation of the serial mutation of influenza virus. By virtue of limited travel during their entire lifetime and intensive exposure to their natural ecology, members of primitive groups serve as unequaled sentinel populations for revealing the focal micro-

bial agents that infect man in their environment. Thus, for infections ranging from Chagas disease and toxoplasmosis to arbovirus infections, primitive groups offer unusually fruitful subjects for investigation. In toxoplasmosis, filariasis, yaws and malaria, as well as in the arbovirus encephalitides, we have studies in progress.

Our studies on the antigenic composition of the virus causing the 1918-22 influenza pandemic and on the immunologic response to various closely related influenza viruses, including the new 1968 Hongkong Asian influenza (the latest A2 variant which might better have been designated A3) have now been published. However, the opportunity of supervising and participating in investigations of the influenza epidemic which swept through the Territory of Papua and New Guinea, and through West New Guinea in late 1969 has brought additional material for further study on virus evolution of influenza into our laboratory. Incidental to this work our field investigations also provided the opportunity to study new virgin soil measles epidemics and to find new isolated populations free of many nearly ubiquitous respiratory viruses.

We planned, organized and selected participants for Theme III on Pathogenesis of Slow Virus Diseases of the Central Nervous System at the VIth International Congress of Neuropathology in Paris in September, 1970. This theme provided reviews and new concepts on virus-host relationships and significant new data on the pathogenesis of cellular injury associated with persistent viral infections, immunological aspects of slow infections and characterization and properties of subacute spongiform virus encephalopathies. New and significant findings of the neuropathology of these diseases were presented and reviewed. The papers presented were published in the Proceedings of the VIth International Congress of Neuropathology, Masson and Cie, Paris.

Our various laboratory investigations have thus evolved around the theme of elucidating medical problems in the cultures of primitive man, or man living in small, isolated traditional communities, by cautiously selecting such problems of immunological, virological, biochemical, genetic and nutritional interest which are pertinent to the long-term studies of growth, behavior and human biology in these groups.

Serial No. NDS (CF)-65 OAD 1282
1. Collaborative & Field Research
2. Office of Associate Director
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Study of Child Growth and Development and Behavior, and Disease Patterns in Primitive Cultures.

Sub-Project I: Study of the developmental patterning of the human nervous system (cybernetics of human development).

a. Analysis of culturally determined methods of approach to symbolic representation from drawings and art forms of children and adults in primitive societies.

b. Analysis of the development in writing of adolescents in preliterate cultures.

c. Analysis of child care and behavior patterns in primitive cultures from photographic recording (development of techniques and methods).

d. Investigation of nonrecurrent phenomena (objectives and selectivity used in documentation of aperiodic phenomena to preserve maximum information).

e. A research archive for ethnopediatric film investigation of styles in the patterning of the nervous system.

Sub-Project II: Human evolutionary studies in isolated primitive groups.

a. Kuru.

b. Motor neuron disease and other degenerative diseases in East and West New Guinea, and in other inbred Pacific Islands populations.

c. Blood group genetic studies of Australasian (Melanesia and Micronesia), and South American indigenous groups.

d. Red cell enzyme, serum factor and leucocyte type pleomorphisms among these groups.

e. Analysis of dermatoglyphic variance.

f. Epidemiologic and ecologic investigations of kuru: with expanding, manipulatable computer records system.

Sub-Project III: Studies of isolated Micronesian populations.

a. Child development and behavior on Ulithi, Ifalik and Lamotrek atolls, and on Fais Island.

b. Response to live measles virus vaccine in immunological virgin populations without circulating measles virus (special attention to response in susceptible adults and pregnant women and their offspring). Follow-up studies to determine persistence of antibody response and to observe these populations for possible long-term neurological sequellae following use of attenuated live vaccine virus.

c. Influenza A2 virgin soil epidemics (epidemiological, clinical and immunological response and discovery of populations without previous experience with Type A or Type B influenza. Follow-up studies of these populations as sentinel populations for detection of epidemics due to new strains of influenza virus.

d. Studies of infectious disease patterns in remote individual populations.

e. Genetic characterization of the population of the Western Caroline Islands.

f. The antigenic identification of the 1918-29 influenza virus from residual antibody in aged persons in isolated populations free of influenza since the pandemic.

Sub-Project IV: Studies of isolated New Guinea populations.

a. Slow growth, delayed puberty and early aging in Melanesians of short stature.

b. CNS defects in areas of intense endemic goitrous cretinism in Highland populations.

c. Child development and behavior patterns in the Asmat, Tjitak, Auyu, Kayagar, Western Dani, Kaure, Toure groups of West New Guinea; the Kukukuku (Anga), Eastern Highlands peoples, the Biami, Etoro and Waragu people of the Great Papuan Plateau of Papua and New Guinea; and, the West Nakanai, Mangsing and Mamusi of New Britain.

d. Infectious disease--including encephalitis--studies in diverse, ecologically isolated New Guinea populations.

e. Study of the pattern of the A2 Hongkong influenza epidemic in East and West New Guinea in 1969-70.

f. Genetic and demographic characterization of New Guinea populations.

g. Hereditary and genetic disease patterns in New Guinea.

h. The Anga (Kukukuku): an intensive longitudinal study of growth and development, behavior, disease patterns, human genetics, communication, in an archaic mountain population of New Guinea.

i. Research cinema films of behavior patterns of children in New Guinea.

j. Study of the hazards of lowland resettlement of Highland groups in New Guinea.

Sub-Project V: Studies of isolated New Hebrides and Solomon Islands populations.

a. Child development and behavior in Tongariki, the Banks and Torres Islands and Espiritu Santo.

b. Tongariki: an intensive study of human evolution in the Shepherd Islands.

c. Human genetics and disease patterns survey in the Banks and Torres Islands.

d. Seroepidemiology of infectious disease in New Hebrides.

Sub-Project VI: Studies of Australian Aborigines.

a. Arbovirus seroepidemiological studies of Aboriginal groups in Cape York.

b. Survey patterns of infectious disease in Aboriginal groups in the Haast Bluff, Cape York, the Kimberley and Bentnik-Mornington Islands groups.

Sub-Project VII: Studies of Central and South American Indians.

a. Human genetics and disease patterns of Guayaki and Chaco Indian tribes, and the Mennonite colonists in the Chaco of Paraguay.

b. Child growth and development of Guayaki and Ai'yore Indians of Paraguay.

c. Child growth and development of Aroyo (Moro) Indians of Bolivia and Paraguay.

d. Population genetics, disease patterns and child growth and development of the Waunan Indians of Columbia and Ecuador.

Sub-Project VIII: Developmental, genetic and disease patterns in primitive populations of Asia, Africa and in Polynesia.

Sub-Project IX: Experimental developmental neuropediatrics in infantile programming: an empirical approach to the language of information input into the nervous system.

Sub-Project X: Ciphers and notation for the coding of sensory data for neurological information processing:

a. Notational systems for human movement.

b. Ciphers and notation for human form (physiognomy, physique, palm printing, ear form, hair).

c. Theoretical studies in notational problems in mathematics, linguistics, music, dance.

d. Alphabets: a theoretical investigation into their relation to linguistics, and the application to non-linguistic information.

e. Form recognition: neuroanatomic and genetic determination of preferential recognition, and interrelationships of the problems in computer programming and in the arts.

f. The ciphering and coding of visual data as solved in the visual arts (drawing, painting and sculpture).

Sub-Project XI: Racial distribution and neuroanatomic variations in the structure of the human brain.

Principal Investigator: D. Carleton Gajdusek, M.D.

Other Investigators: Clarence J. Gibbs, Jr., Ph.D., Paul W. Brown, M.D., Raymond Roos, M.D., David M. Asher, M.D., Michael Alpers, M.D., Vincent Zigas, M.D., Francoise Cathala, M.D., Richard Marsh and Robert Cornelius, D.V.M., John Hooks, Ph.D., E. Richard Sorenson, Nancy Rogers, Mint Basnight, Helene Gilbert, Judith Meyer and Richard Benfante.

Project Description:

The Section for the Study of Child Growth and Development, and Behavior, and Disease Patterns in Primitive Cultures has continued all projects listed in the previous Annual Reports, with expansion of collaborating investigators as reflected in the authorship and studies of the publications listed. The titles of sub-projects and their subdivisions are sufficiently explicit to constitute the project description.

Sub-Project I. Study of the developmental patterning of the human nervous system (cybernetics of human development).

Principal Investigator: D. Carleton Gajdusek, M.D.

Other Investigators: Michael Alpers, M.D., Paul Brown, M.D., Vincent Zigas, M.D., E. Richard Sorenson, Judith Meyer, Peter Fetchko and Donald Rubinstein.

Cooperating Investigators: Dr. Margaret Mead, American Museum of Natural History, New York; Dr. Ted Schwartz, Univ. California, Los Angeles; Alan Lomax, Columbia Univ., New York; Mr. and Mrs. Mark Jablonko, Columbia Univ.; Dr. Paul Ekman and W.V. Friesen, Langley Porter Neuropsychiatric Inst., San Francisco; Dr. Peter Kundstadter, Univ. Washington, Seattle; Kal Muller, Univ. Arizona, Tucson; Thomas Kiefer, Dr. Edwin Cook, Univ. California, Davis; Dr. Gordon Gibson, Smithsonian Inst.; Dr. Robert MacLennan, Inter. Agency for Cancer Res., France; Dr. Maurice Godelier, Sorbonne; William H. Bloxam, Wayne Dye, John MacGregor, Father David Gallus, Father F. Trenkenshuh, O. Kooyers, Dr. C.K. Dresser, West New Guinea; Timothy Asch, Brandeis Univ.; N. Chagnon, Univ. Michigan; James Bruce, Pasadena, Calif.; Elizabeth and Perry Kennedy, Univ. Buffalo.

Sub-Project II. Human evolutionary studies in isolated primitive groups.

Principal Investigator: D. Carleton Gajdusek, M.D.

Other Investigators: Paul Brown, M.D., C.J. Gibbs, Jr., Ph.D., M. Alpers, M.D., F. Cathala, M.D., D. Asher, M.D., N. Rogers, M. Basnight, J. Hooks, Ph.D.

Cooperating Investigators: Dr. Stephen Fazekas, CSIRO, Sydney; Eric French, and Cyril Curtain, M.D., CSIRO, Melbourne; Dr. Malcolm Simons, Royal Children's Hospital, Melbourne; Dr. R.T. Simmons, John J. Graydon, Alan Duxbury and Frank Warbuton, Commonwealth Serum Laboratories, Melbourne; and Dr. Robert Kirk, John Curtain School of Medicine, Canberra, Australia; Dr. Vincent Zigas, Dr. Richard Hornabrook and Dr. Adolph Suweri, Public Health Department, Territory of New Guinea.

Sub-Project III: Studies of isolated Micronesian populations.

Principal Investigator: D. Carleton Gajdusek, M.D.

Other Investigators: Paul Brown, M.D., C.J. Gibbs, Jr. and J. Anthony Morris, Ph.D., David Asher, M.D., Francoise Cathala, M.D., Michael Alpers, M.D., Vincent Zigas, M.D., John Hooks, Ph.D., Nancy Rogers, Mint Basnight, Richard Benfante.

Cooperating Investigators: Dr. Leon Rosen, Gordon W. Wallace, NIAID, Honolulu, Hawaii; Dr. Jacob Brody, NINDS; Chris Plato, NICHD; Dr. Kwang Ming Chen, National Taiwan Univ., Tapei; Newton Morton, Population Genet. Laboratory, Honolulu; Dr. Stephen Fazekas, Sydney; Dr. Antonio Golbuu, Jose Torres, Eddie Iderug, NINDS Research Center, Guam.

Sub-Project IV: Studies of isolated New Guinea populations.

Principal Investigator: D. Carleton Gajdusek, M.D.

Other Investigators: Drs. Paul Brown, Raymond Roos, Michael Alpers, Vincent Zigas, C.J. Gibbs, Jr., David Asher, Francoise Cathala and John Hooks; Judith Meyer, Richard Benfante, Mint Basnight and Helene Gilbert.

Cooperating Investigators: Dr. C.K. Dresser, West New Guinea; William Bloxam, John MacGregor, Richard Hornabrook, John Mathews, Territory of New Guinea; Edwin Cook, Univ. California, Davis; Ted Schwartz, Los Angeles; Dr. R. MacLennon, France; Dr. John Hotchin, Department of Health, New York; Paul Ekman, San Francisco; R.T. Simmons, J.J. Craydon, C.C. Curtain, Australia; David Kitchin, M.D., Columbia Univ.; Alexander Bearn, M.D., Rockefeller Inst., New York; Maurice Godelier, Ph.D., Paris; E. Beck, P. Daniel, Inst. Psychiatry, London; Chris Plato, NICHD; Roger Rodrigue, M.D., Temple Univ.; and J. van Delden, New Guinea.

Sub-Project V: Studies in isolated New Hebrides and Solomon Islands populations.

Principal Investigator: D. Carleton Gajdusek, M.D.

Other Investigators: Paul Brown, M.D., David Asher, M.D., C.J. Gibbs, Jr., Ph.D., Nancy Rogers, Mint Basnight and Helene Gilbert.

Cooperating Investigators: Dr. H. Lehmann, Univ. Cambridge; Dr. Robert Kirk, Australian National Univ., Canberra; Dr. Jean Guiart, Sorbonne, France; Dr. James MacGregor, Honiara; Dr. Roger Greenough, Dr. William Rees, New Hebrides; Chris Plato, NICHD.

Sub-Project VI: Studies of Australian Aborigines.

Principal Investigator: D. Carleton Gajdusek, M.D.

Other Investigators: Paul Brown, M.D., Michael Alpers, M.D., C.J. Gibbs, Jr., Ph.D., Nancy Rogers, Mint Basnight and Helene Gilbert.

Cooperating Investigators: Drs. R.T. Simmons, J.J. Graydon, A. Duxbury and F. Warbuton, Commonwealth Serum Laboratories, Melbourne; C. Curtain, Sydney; E. Beck, London; and W.C. Leyshon, NIDR.

Sub-Project VII: Studies of Central and South American Indians.

Principal Investigator: D. Carleton Gajdusek, M.D.

Other Investigators: C.J. Gibbs, Jr., Michael Alpers, M.D., Mint Basnigh t, Nancy Rogers and Helene Gilbert.

Cooperating Investigators: W.C. Leyshon, NIDS; J.L. Sever, NINDS; K. Walls, CDC, Atlanta; R.L. Anderson, WRAIR; J.A. Morris, DBS, Arthur Steinberg, Western Reserve Univ., Cleveland; C.C. Curtain, CSIRO; Elizabeth and Perry Kennedy, Buffalo.

Sub-Project VIII: Developmental, genetic, and disease patterns in primitive populations of Asia, Africa and Polynesia.

Principal Investigator: D. Carleton Gajdusek, M D.

Other Investigators: Frank D. Schofield, M.D., Nairobi; Peter Kundstadter, Seattle; Thomas Kiefer, Univ. California; L. Vogel, M.D., Nairobi; Kok Ann Lim, M.D., Singapore; Chris Plato, NICHD.

Sub-Project IX: Experimental developmental neuropediatrics in infantile programming: an empirical approach to the language of information input into the nervous system.

Principal Investigator: D. Carleton Gajdusek, M.D.

Other Investigators: Michael Alpers, M.D., E. Richard Sorenson, Judith Meyer, Don Rubinstein.

Cooperating Investigators: Paul MacLean, M.D., NINDS; Paul Ekman, San Francisco

Sub-Project X: Ciphers and notation for the coding of sensory data for neurological information processing.

Principal Investigator: D. Carleton Gajdusek, M.D.

Other Investigators: E. Richard Sorenson, Michael Alpers, M.D., Judith Meyer

Cooperating Investigators: Dr. R.L. Kirk, Canberra; Patricia Hunt, Univ. California, Berkeley; Paul MacLean, M.D., NINDS; Chris Plato, NICHD; Alan Lomax, New York.

Sub-Project XI: Racial distribution and neuroanatomic variations in the structure of the human brain.

Principal Investigator: D. Carleton Gajdusek, M.D.

Cooperating Investigators: Elisabeth Beck, Peter Daniel, M.D., Institute of Psychiatry, London; Paul Yakovlev, M.D., Richard Sidman, M.D., Dr. Kemper, Dr. H. Hamlin, Harvard Univ., Boston; Dr. Peter Lampert, Univ. California, La Jolla; Dr. K. Earle, AFIP; Dr. P. MacLean, NINDS; Drs. R. Hassler and H. Stephan, Max-Plank Inst., Frankfurt; Dr. G. Inke, Stony Brook, New York; Dr. A. Hopf, Neustadt, Germany.

Publications:

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Serial No. NDS (CF)-62 OAD 969
1. Collaborative-Field Research
2. Office of Associate Director
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Slow, Latent, and Temperate Virus Infections of the Central Nervous System of Man and Animals

Sub-Project I: Attempts to isolate transmissible agents from subacute and chronic diseases of the nervous system

Sub-Project II: Characterization and pathogenesis of kuru virus

Sub-Project III: Characterization and pathogenesis of Creutzfeldt-Jakob disease virus

Sub-Project IV: Studies on the characterization and nature of scrapie, mink encephalopathy and visna virus and the relationship of visna virus to the virus of progressive pneumonia of sheep in Montana

Sub-Project V: Studies on Australian antigen and antibody in chimpanzees, monkeys and primate handlers

Sub-Project VI: Fluorescent antibody studies on the intracellular localization and identification of viral antigens in vivo and in vitro in tissues from patients with subacute diseases of the CNS

Sub-Project VII: Tissue and cell culture in vitro studies of viral induced slow infections of man and animals

Sub-Project VIII: Characterization and distribution of two new foamy viruses isolated from chimpanzees tissues grown in vitro and their relationship to the RNA oncogenic group of viruses

Sub-Project IX: Characterization of newly identified adenoviruses isolated from chimpanzee tissues grown in vitro

Sub-Project X: Attempts to demonstrate a viral etiology for chronic encephalitis with focal epilepsy

Sub-Project XI: Isolation, epidemiology and pathogenesis of mourning dove pox

Sub-Project XII: Infectious and contagious disease management in a primate colony

Sub-Project XIII: Slow Virus Symposia, VIth International Congress of Neuropathology, Paris, France

Sub-Project XIV: Studies on the ecology, epidemiology and pathogenesis of arbovirus infections of man and animals

Principal Investigators: D. Carleton Gajdusek, M.D., and Clarence J. Gibbs, Jr., Ph.D.

Other Investigators: Paul Brown, M.D., John Hooks, Ph.D., Nancy Rogers, M.S., Mint Basnight, M.S., Robert Cornelius, D.V.M., Raymond Roos, M.D., Francoise Cathala, M.D., Vincent Zigas, M.D., Ronald DiGiacomo, D.V.M., Larry Fry, Ph.D.

Technical Assistants: Michael Sulima, Alfred Bacote, Helena Gilbert, Richard Thomas, Paul Martin, Lloyd Horst, Paul Horst, James Webster, Judith Meyer, Sarah Andersen, Monica Lewis, Galen Miller, Amos Fox, James Noll and Albert Bontrager

Students: By special arrangement this department participates in the co-operative college programs of the University of South Florida, Tampa and Antioch College, Ohio. During the past year the following students have spent 3-6 months in the laboratory: Michael Proctor, Henry Theiss, Jerome Kurent and Mark Littlewood

Administrative: Marion Poms, Juliette Harvey and Sharon Williams

Project Description:

The studies reported in this section are being conducted in the NINDS Laboratory of Slow, Latent and Temperate Virus Infections on the NIH campus and at the Patuxent Wildlife Research Center, Laurel, Maryland. They are in part being conducted in collaboration with the Bureau of Wildlife and Sports Fisheries, U.S. Department of Interior. Additional associated collaborators are the Departments of Neurology and Neurovirology, Johns Hopkins University School of Medicine and the Departments of Epidemiology and Pathobiology, Johns Hopkins School of Public Health and Hygiene. In house collaborators are Jacob Brody, M.D., Epidemiology Branch, John Sever, M.D., Perinatal Research Branch, NINDS; K. Takemoto, S. Baron, H. Levy and W. Hadlow, NIAID; L. Barker, M.D., DBS. Contractural phases of this work are being conducted at Gulf South Research Institute, New Iberia, Louisiana; National Center for Primate Biology, University of California, Davis, California; and Public Health Research Institute of New York, Otisville, New York. This project is part of the Study of Child Growth and Development and Disease Patterns in Primitive Cultures and is under the director of that study. (see Project Report Serial No. NDS (CF)-1282 (I-IX.)

SUB-PROJECT I: Attempts to isolate transmissible agents from subacute and chronic diseases of the nervous system

Principal Investigators: D. Carleton Gajdusek, M.D., and Clarence J. Gibbs, Jr., Ph.D.

Other Investigators: Raymond Roos, M.D., Paul Brown, M.D., David Asher, M.D., John Hooks, Ph.D., Mint Basnight, M.S., Larry Fry, Ph.D., Nancy Rogers, M.S., Vincent Zigas, M.D., Robert Cornelius, D.V.M., Francoise Cathala, M.D., Ronald DiGiacomo, D.V.M., and Richard Sorenson, Ph.D.

Cooperating Investigators: J.D. Mathews and R.W. Hornabrook, Kuru Research Office, Okapa, New Guinea; J.C. Steele, M.D., University of Toronto, Canada; David Poskanzer, M.D., Massachusetts General Hospital, Boston; Richard T. Johnson, M.D., Johns Hopkins University Hospital, Baltimore; J.T. Sever, M.D., NINDS; A.M. Gardashyan, M.D., Mayo Clinic, Rochester, Minnesota; J.A. Morris, Ph.D., and Hope E. Hopps, DBS; B.H. Dessel, M.D., Veterans Hospital, Wood, Wisconsin; Elisabeth Beck and Professor P.M. Daniel, Department of Neuropathology, The Maudsley Institute, London; P.A. Palsson, D.V.M., and M. Gudnadottir, M.D., Institute for Experimental Pathology, Keldur, Iceland; W. Hadlow, D.V.M., Rocky Mountain Laboratory, NIAID; J. Hourrigan, D.V.M., and H.A. McDaniel, D.V.M., Animal Research Section, USDA; E. Dustman, Ph.D., and C.M. Herman, Sc.D., Patuxent Wildlife Research Center, Department of Interior; King Engel, M.D., Medical Neurology, NINDS; J.A. Brody, M.D., Epidemiology, NINDS; C. Colson, M.D., Columbia Presbyterian Hospital, New York; R. Katzman, M.D., Yeshiva University, New York; M. Schaeffer, M.D., City of New York Department of Health, and Dr. Ward, Otisville, New York; G. McKhann, M.D., R.T. Johnson, M.D., Leslie Weiner, M.D., Robert Herndon, M.D., Neurology-Neurovirology, Johns Hopkins University School of Medicine, Baltimore; Dr. Marino, Grady Memorial Hospital, Emory University, Atlanta; I.H. Pattison, D.Haig, D.V.M., and D. Hunter, Ph.D., ARS, Compton, England; J.T. Stamp, D.V.M., Moredun Institute, Edinburgh; E.J. Field, Medical Research Council, Newcastle-upon-Tyne; F. Dixon, M.D., and M. Oldstone, M.D., Scripps Clinic and Research Foundation, La Jolla, California; N. Nathanson, M.D., and F. Bang, M.D., School of Public Health, Johns Hopkins University Hospital, Baltimore; W. Zeman, M.D., Indiana University Medical Center, Indianapolis; G. ZuRhein, M.D., University of Wisconsin, Madison; H. Koprowski, M.D., and M. Katz, M.D., Wistar Institute, Philadelphia; K. Earle, M.D., and R. Heffner, Armed Forces Institute of Pathology; P. Lampert, M.D., University of

California, La Jolla; P. Van Nuis, M.D., Grand Rapids; T. Rasmussen, M.D., University of Montreal, Canada; W. Greer, D.V.M., Gulf South Research Institute, Louisiana; C. Espana, Ph.D., and R.E. Stowell, M.D., National Center for Primate Biology, University of California, Davis; A. Lowenthal, M.D., Foundation Born-Bunge for Research, Antwerp; F. Cathala, M.D., and C. Chany, M.D., Hopital de la Salpetriere, Paris; H. Thormar, Ph.D., and R. Carp, Ph.D., Institute for Basic Research in Mental Retardation, Staten Island

Technical Assistants: Michael Sulima, Alfred Bacote, Helena Gilbert, Paul Martin, Lloyd Horst, Paul Horst, Judith Meyer, Galen Miller, Amos Fox, James Noll, and Albert Bontrager

Student Assistants and Part-time Temporary Employees: Robert Rhorer, Wesley Russell, Henry Theiss, Michael Proctor, Mark Littlewood, and Jerome Kurent

Project Description:

Objectives: The objectives of these long-term studies were established in 1957 with the discovery of kuru in the Eastern Highlands of New Guinea. They were fully promulgated in 1962 with the activation of the Laboratory of Slow, Latent and Temperate Viruses of the Nervous System. As established the objectives remain unchanged as follows: (1) to establish infection as the etiology of chronic and subacute progressive degenerative diseases primarily of the nervous system of man and animals; (2) to characterize and determine the nature of viruses isolated during these studies by eliciting physical, chemical, biological and morphological properties; (3) to study the etiological role of viruses in presenile and senile dementias as well as in normal aging processes; (4) to study the ecology of disease, disease processes, and the nature of their causative agents in primitive and frequently virgin populations; (5) to determine the presence and interactions of "helper" or "hinderer" viruses in the pathogenesis of diseases under study; (6) to determine the nature of viral masking, latency, temperateness, incompleteness, persistence, interference, eclipse and immunopathological processes as they contribute to subacute degenerative processes of the CNS. The major diseases under study are kuru, Creutzfeldt-Jakob disease, multiple sclerosis, Alper's disease, Alzheimer's disease, Schilder's disease, subacute sclerosing panencephalitis, amyotrophic lateral sclerosis, parkinsonism-dementia, Parkinson's disease, systemic lupus erythematosus, dermatomyositis, epilepsy partialis continua, ataxia-telangiectasia, progressive supranuclear palsy, polymyositis, Behcet's disease, post-infectious encephalitis, chronic tick-borne hemorrhagic fever, nephro-nephritis, hemorrhagic fever, Guillain-Barré syndrome, scrapie, mink encephalopathy, visna, progressive pneumonia of sheep in Montana, and diabetes mellitus.

Methods Employed: Basic approaches established in 1962 and which have thusfar proved successful are being pursued. Essentially they consist of standard and classical techniques, supplemented by developmental techniques for the isolation of viruses with certain notable exceptions: (1) the demonstration that the genetic mechanism of the host influences the host

resistance or susceptibility to disease requires inoculation of specimens into an extensive array of animals, including higher apes and many species of old and new-world monkeys, birds and cell and tissue culture lines in vitro; (2) exceedingly long asymptomatic incubation periods require experiments to be held, in isolated facilities to prevent cross infections, for periods of from 5 to 10 years before negative conclusions can be determined; (3) establishment and utilization of cell culture lines in vitro prepared by explantation and trypsinization of biopsy and autopsy tissues from humans and animals with CNS diseases - these lines are maintained under rigid requirements of temperature and CO_2 for unmasking latent and endosymbiotic viruses, with considerable emphasis being placed on viral genome rescue employing co-activation, fusion, Sendai virus medicated fusion; (4) utilization of fluorescent, phase and electron microscopy to elicit intracellular viral genome; and (5) histochemical and clinical chemistry procedures to detect significant changes in humans and animals affected with natural and experimental diseases. An elaborate system for optimally obtaining specimens has been established with scientific collaborators in the United States and throughout the World. In addition to studying diseases in search of viruses this laboratory is investigating the nature of over 100 viruses either known or suspected of inducing fatal human diseases particularly those affecting the brain of man and animals. In these studies attempts are made to develop animal or tissue culture models for determining physical, chemical and biological properties and pathogenesis of the virus.

Major Findings: During this year our studies on neurological diseases of man and animals provided data for the classification of a new group of infectious agents which we have called the Subacute Spongiform Virus Encephalopathies: kuru, Creutzfeldt-Jakob disease, scrapie and mink encephalopathy. Over 200 chimpanzees, 1600 smaller monkeys of 27 species or sub-species, more than 200,000 small laboratory animals including over 25 selectively inbred lines of mice, a variety of pure bred and randomly bred domestic animals and avian hosts as well as more than 50 established tissue and cell culture lines of human, animal and avian origins have, since the beginning of these studies, been inoculated with suspensions of tissues obtained at biopsy or early autopsy from humans and animals affected with neurological and systemic diseases. During the last 6 months alone, more than 450 human tissue specimens for virus isolation have been received into the laboratory; of these approximately 260 are brain specimens and 50% of those submitted were brain biopsy specimens. Significant findings include the continued isolation, characterization and passage of kuru through 4 serial passages in chimpanzees, the isolation and serial passage of the virus of Creutzfeldt-Jakob disease from 10 patients to chimpanzees, successful primary and serial transmission of kuru and Creutzfeldt-Jakob disease to 4 species of new-world monkeys: spider, squirrel, capuchin and woolly monkeys; isolation of adenovirus type 32 from the brain of a patient with subacute encephalitis; isolation of virus from brain and lung of sheep with fatal progressive pneumonia in Montana and demonstration that both these viral isolates are antigenically closely related or identical to visna and maedi viruses of Iceland; thus for the first time establishing these viruses in the United States, and the demonstration that two viruses (Pan 1 and Pan 2) isolated from the brains of many chimpanzees in our colony are RNA viruses associated with an RNA dependent DNA

polymerase, not serologically related to known simian foamy or monkey mammary tumor viruses and which morphologically resemble viruses belonging to the RNA oncogenic group.

Kuru: Kuru virus continues to serve as the prototype virus causing fatal subacute degenerative disease of the CNS of man and is a dramatic model for the study of other spongiform encephalopathies particularly those referred to as the presenile dementias. A major breakthrough occurred during this reporting year with the successful primary transmission of the disease to spider monkeys, squirrel monkeys and a capuchin monkey inoculated with suspensions of brain from fatal human cases. Although the incubation periods were somewhat longer on primary passage in these animals as compared to serial passage in the chimpanzee it is anticipated that serial passage of the virus in the more readily available, easier housed and more economically purchased new-world monkeys will be associated with a reduction in the incubation period. Further, we now have conclusive evidence that neuropathological lesions are present in pre-clinical experimentally inoculated animals even as early as 5 months following inoculation. For the first time we have evidence that by millipore filtration the virus of kuru passes through a membrane with an average pore diameter of 100nm. Details on the characterization of kuru virus are presented in Sub-Project II.

Creutzfeldt-Jakob disease: This disease has now been transmitted from 10 humans to 12 chimpanzees and, like kuru, from humans to two species of new-world monkeys: spider monkeys and squirrel monkeys, and from Creutzfeldt-Jakob disease affected chimpanzees to squirrel monkeys and a brown woolly monkey. Successful transmissions have been obtained with brain tissues that have varied greatly in the degree and intensity of status spongiosis, a finding which lends support to the unitarian hypothesis of the disease. Furthermore, the transmissible cases have been worldwide in their distribution coming from the United States, England, Canada, and Belgium. Although previously thought to be a rare disease, since our first report on the transmissibility we have received human specimens from over 60 cases. A critical review of the case histories revealed that 13 of 39 patients had liver abnormalities and at least two cases, as yet not transmitted, had onset of their neurological diseases during immunosuppressive therapy following organ transplantations. During the past year employing the complement-fixation and hemagglutination-inhibition techniques sera from human patients and experimentally infected chimpanzees were tested for antibodies against 10 major groups of viruses and 58 arboviruses representing Group A, Group B, Bunyamwera group and a number of ungrouped arboviruses to determine whether or not any one or more of the viruses tested were involved in the disease. No etiological relationship was elicited. Details on the characterization of the Creutzfeldt-Jakob disease virus are presented in Sub-Project III.

Other CNS diseases: Although we have been successful in (1) transmitting kuru and Creutzfeldt-Jakob disease to several species of primates, (2) isolating adenovirus type 32 from the brain of a patient with subacute encephalitis and (3) isolating measles virus from the brain of a patient with SSPE we are also accumulating negative data on animals inoculated with the following numbers of cases of specified disease: Alper's disease (4), amyotrophic lateral sclerosis of the sporadic and familial types (15), Alzheimer's disease (15), SSPE (65), chronic focal epilepsy or epilepsia partialis

continua (16), progressive supranuclear palsy (10), Schilder's disease (7), multiple sclerosis (14), Alzheimer's disease of the sporadic and familial types (15), Parkinson's disease (6), parkinsonism-dementia (4), progressive multifocal leucoencephalopathy (5), Reyes syndrome (4), endomyocardial fibrosis (22), hemorrhagic fever nephro-nephritis syndrome (9), Werdnig-Hoffmann disease (1), rheumatoid arthritis (7), dermatomyositis (3), polymyositis (3), leukemia (3), and 134 cases of subacute neurological diseases in which final diagnosis have not yet been established. These are in addition to the 56 cases of kuru and 68 cases of Creutzfeldt-Jakob disease currently under study.

Significance: The results thusfar obtained in these long-term studies have firmly established a new group of infectious filterable viruses - which we have classified as subacute spongiform virus encephalopathies. The success of these transmissions warrant continued and increased efforts to demonstrate infection as the etiology of other spongiform encephalopathies of the gray matter of the brain in human diseases. Isolation of the kuru and Creutzfeldt-Jakob disease viruses now provides for the ultimate development of immunoprophylactic measures for humans at risk, chemotherapeutic measures for treatment of affected humans and definition of the pathogenesis of these diseases in the human host. These studies further are providing the optimal techniques for the successful elucidation of the infectious nature of multiple sclerosis, ALS, ALS-PD, Parkinson's disease, Alzheimer's disease, progressive multifocal leucoencephalopathy, the presenile and senile dementias as well as the role of infectious viruses in aging processes.

Proposed Course: 1) Identification and characterization of the agent causing disease in chimpanzees; 2) continued long-term observation of inoculated animals. Continued serial studies on the fractionation of serum specimens from these animals for the determination of shifts in the electrophoretic patterns, as well as their antibody status which may be indicative of sub-clinical infections; 3) continued effort to develop suitable antigen antibody system for the study of established strains of 'slow' viruses; application of these new techniques to the study of human diseases; 4) intensification of the development and application of fluorescent antibody techniques with the model virus and other chronic viruses, such as LCM and rabies, which may remain latent for many years before clinically apparent disease becomes manifest; 5) greater emphasis on growth, cultivation and establishment of cell culture lines of "target organ", nervous tissue, from humans and animals with degenerative diseases of the nervous system, as well as from cases of "auto-immune" diseases in an effort to isolate an etiological agent in a controlled in vitro environment, detection of abnormal antigenic fractions giving indirect evidence of disease and possible association with known viruses and establishment of new cell lines for the study of viral growth, maturation, and measurement of interferon or interferon-like substances; 6) increased efforts to adapt strains of 'slow' viruses to growth, serial propagation and characterization in tissue and cell culture systems employing viral genome rescue techniques; 7) continued efforts toward the development of procedures for the successful isolation of etiological agents responsible for degenerative diseases of the CNS, such procedures to include cell culture blocking techniques, detection of endosymbiotic relationship of masked, latent, or temperate viruses in the intact host

and host cells grown in vitro and chemotherapeutic and immunosuppressive regimens to lower animal resistance to infection; 8) continued efforts to broaden experimental host range for kuru, Creutzfeldt-Jakob, SSPE, visna, and, at the same time, seek out those experimental hosts whose genetic mechanisms render them susceptible to other human diseases under study. 9) follow-up studies on Pan 1 and Pan 2 viruses (simian foamy virus 6 and 7, respectively) from chimpanzees to determine their distribution in man, primates, and other mammalian species; to further elicit oncogenic and tumorigenic properties they may have; and, to further characterize what role they may have in the pathogenesis of experimental disease.

SUB-PROJECT II: Characterization and pathogenesis of kuru virus

Principal Investigators: D. Carleton Gajdusek, M.D., and
Clarence J. Gibbs, Jr., Ph.D.

Other Investigators: John Hooks, Ph.D., Nancy Rogers, M.S., Paul Brown, M.D., Mint Basnight, M.S., Robert Cornelius, D.V.M., Raymond Roos, M.D., Ronald DiGiacomo, D.V.M., and Larry Fry, Ph.D.

Cooperating Investigators: William Greer, D.V.M., New Iberia, Louisiana; Carlos Espana, Ph.D., University of California, Davis; Peter Lampert, M.D., University of California, La Jolla; Morris Schaeffer, M.D., and Gerald Ward, D.V.M., Public Health Research Institute, New York; Peter Daniels, M.D., and Elisabeth Beck, The Maudsley Institute, London; K. Earle, M.D. and R. Heffner, M.D., Armed Forces Institute of Pathology, Washington, D.C.; Byron Kakulas, M.D., Perth, Australia; Michael Alpers, M.D., Perth, Australia; Charles Chany, M.D., and Francoise Cathala, M.D., Paris, France; Vincent Zigas, M.D., New Guinea; Sam Baron, M.D., and Hilton Levy, Ph.D., NIAID

Technical Assistants: Michael Sulima, Helena Gilbert, Monica Lewis, Alfred Bacote, Judith Meyer, Paul Martin, Lloyd Horst, Richard Thomas, Paul Horst, Amos Fox, Galen Miller, James Webster, James Noll, and Albert Bontrager

Project Description:

Objectives: (1) To elucidate the morphological, biological, chemical, immunological and physical properties of kuru virus in experimentally infected animals; (2) to broaden experimental host range of the virus; (3) to determine the pathogenesis of the disease in the experimental host; and, (4) to develop techniques for the elucidation of the nature of kuru virus.

Methods Employed: (1) Inoculation of chimpanzee adapted kuru virus into chimpanzees, lesser primates, small laboratory animals, embryonated hens' eggs and a variety of commercially available tissue and cell cultures; (2) experiments to determine the size of kuru virus by filtration through millipore filters; (3) effects of temperature, lyophilization procedures and organic solvents on kuru virus; (4) attempts to demonstrate neutralizing antibody to kuru virus in human and animal sera; (5) in vitro growth of explanted and trypsinized tissues from kuru affected chimpanzees to demonstrate viral antigens and virions through FA, EM and techniques to unmask latent agents.

Major Findings: Kuru virus has now undergone 4 serial passages in chimpanzees. Of the 96 animals inoculated in these studies kuru has developed in 26 of 46 inoculated on primary passage with human tissues, and on serial passages in chimpanzees the disease has occurred in 11 of 15 on second passage, 9 of 13 on third passage and 8 of 19 on fourth passage. The virus can

be directly isolated in squirrel, spider and capuchin monkeys inoculated with human brain. In addition to serial passage in the chimpanzee the virus is serially transmissible from chimpanzees to spider monkey, spider monkey to spider and squirrel monkeys and back to chimpanzees. Asymptomatic incubation periods on primary passage to chimpanzees have ranged from 14-39 months whereas in capuchin, squirrel and spider monkeys it has been 45 months, 25 months, and 23 months, respectively. In chimpanzees serial passage is associated with a reduction in incubation period to 10-18 months. A similar reduction was noted on passage in spider monkeys when on second passage it was 16 months. In suspensions of human brain the virus has an infectivity titer of $\geq 10^5$ and in chimpanzee brain the virus titer is $\geq 10^7$. In both preparations the virus is thermostable and remains transmissible following exposure to a temperature of $85^{\circ}C/30$ minutes. Although we earlier demonstrated that the virus passes through millipore filters of 450nm and 220nm we have only recently observed neuropathological lesions of kuru disease in the brain of a chimpanzee inoculated with the filtrate of a 100nm filter. In addition to heat stability, kuru virus can be lyophilized, partially purified by high speed centrifugation and stored for several years without appreciable loss in infectivity. As previously reported, numerous attempts to demonstrate specific NT, CF, HI, or FA antibody in the sera from affected humans and animals have proven unsuccessful. Further, attempts to demonstrate a serological relationship of kuru to one or more conventional viruses have not yielded evidence of an etiological relationship. In vitro growth of brain and visceral tissues from kuru affected animals have resulted in the successful isolation of over 200 strains of viruses representing 6-9 major virus groups. (See Sub-Project VII of this report.)

Significance: Serial passage of kuru virus in chimpanzees confirms the infectious nature of this human brain disease. The successful transmission of the disease from human patients to chimpanzees, squirrel monkeys, spider monkeys and capuchin monkeys and the successful serial passage of the virus in these new-world monkeys provides a more economically feasable host in which to further elicit kuru virus properties. These later findings may well serve to break additional species barriers. The properties of the virus thus-far elicited with the exception of the size by filtration are remarkably similar to the properties of scrapie virus and suggest the viruses of these two diseases are similar.

Proposed Course: Continued serial passage of the virus in chimpanzees, spider monkeys, and other new-world monkeys in an effort to elicit additional properties in the nature of kuru; expanded efforts to adapt the virus to more conventional laboratory hosts; and, attempts to purify and more completely identify kuru virus.

SUB-PROJECT III: Characterization and pathogenesis of Creutzfeldt-Jakob disease virus

Principal Investigators: Clarence J. Gibbs, Jr., Ph.D., and D. Carleton Gajdusek, M.D.

Other Investigators: Raymond Roos, M.D., John Hooks, Ph.D., Paul Brown, M.D., Nancy Rogers, M.S., David Asher, M.D., Vincent Zigas, M.D., Robert Cornelius, D.V.M., Mint Basnight, M.S., Ronald DiGiacomo, D.V.M., and Larry Fry, Ph.D.

Cooperating Investigators: William Greer, D.V.M., Carlos Espana, Ph.D., Peter Lampert, M.D., Morris Schaeffer, M.D., Gerald Ward, D.V.M., Peter Daniels, M.D., Elisabeth Beck, Kenneth Earle, M.D., R. Heffner, M.D., Charles Chany, M.D., Francoise Cathala, M.D., Louis Horta-Barbosa, D.V.M., John Sever, M.D., and Jacob Brody, M.D.

Project Description:

Objectives: To elucidate the morphological, biological, chemical, immunological and physical properties of the virus causing Creutzfeldt-Jakob disease by serial transmissions to chimpanzees; to broaden the experimental host range of the virus; to determine the nature of the virus and the pathogenesis of the disease.

Methods Employed: As described for kuru. (See Sub-Project II of this report.)

Major Findings: Employing the techniques we have developed in our studies on kuru we have successfully transmitted a second subacute degenerative disease of the human brain, Creutzfeldt-Jakob disease, from 10 patients to 12 chimpanzees on primary passage. More recently we have been successful in transmitting this disease from two patients to two species of new-world monkeys, the spider monkey and the squirrel monkey. Further, successful serial passages of the virus from humans to chimpanzees and from chimpanzees to squirrel monkeys and the woolly monkey have been accomplished. Although the asymptomatic incubation periods observed in new-world monkeys (9-25 months) are somewhat more variable and longer than those observed in chimpanzees (11-14 months), these less expensive, more readily available and more easily housed and handled animals are providing greater facility for expanded studies of this disease. During the past year independent confirmation of transmissibility of this disease was obtained with the development of a fatal disease in a chimpanzee inoculated in the laboratories of Drs. Charles Chany and Francoise Cathala in Paris, France. The virus is stable at $-70^{\circ}C$ and is detectable in the supernatant fluids of brain suspensions that have undergone a centrifugation of 5000rpm/30 minutes. Complement-fixation and hemagglutination-inhibition techniques were employed to test sera from humans dying with the disease as well as to test pre-inoculation and serially collected post inoculation sera from chimpanzees in an attempt to elicit serological evidence of relationship of antibody in Creutzfeldt-Jakob disease infected hosts with known viral antigens. No specific patterns suggestive of an etiological relationship were observed when these sera were tested with the following

antigens: influenza types A,B,C; para-influenza types 1, 2, and 3; measles; mumps; respiratory syncytial virus; Newcastle disease virus; echo types 6, 9, 11, 16, 20; coxsackie types A2, A4, A8, A9, and B1 through B6; poliovirus types 1 through 3; Herpes simplex; cytomegalovirus; varicella-zoster; group adenovirus; group reovirus; vaccinia; rabies; lymphocytic choriomeningitis; rubella; SV_{40}; psittacosis; K virus; polyoma; SV_5; and, 58 strains of arboviruses representing groups A, B, Bunyamwera super group, and minor and ungrouped isolates from Europe and the North American continent.

Significance: These studies have demonstrated that a second subacute progressive degenerative disease of the CNS of man is caused by a virus. This strongly supports the continued investigations to attempt to demonstrate a virus etiology for other presenile and senile dementias of man. These studies are providing new and significant data on our understanding of subacute spongiform encephalopathies of man and are opening new vistas in neurovirology. The collection of specimens from 68 cases of Creutzfeldt-Jakob disease within a 2-3 year period suggest the not so rare, as previously thought, occurrence of this disease on a world-wide distribution basis. Successful transmissions have been accomplished with specimens from patients residing in the United States, Canada, England and Belgium.

Proposed Course: Emphasis will continue on the characterization, purification and identification of the virus. Continued efforts are being made to broaden the host range. Human tissues and tissues from affected animals are being extensively studied in an effort to rescue the viral genome and "helper" and/or "hinderer" virus interactions in Creutzfeldt-Jakob disease. Additional characterization of this virus as a member of the group of subacute spongiform encephalopathies will be continued. The epidemiology of the disease is being pursued.

SUB-PROJECT IV: Studies on the characterization and nature of scrapie, mink encephalopathy and visna viruses

Principal Investigators: Clarence J. Gibbs, Jr., Ph.D., and D. Carleton Gajdusek, M.D.

Other Investigators: John Hooks, Ph.D., Raymond Roos, M.D., Paul Brown, M.D., Nancy Rogers, M.S., Mint Basnight, M.S., and David Asher, M.D.

Cooperating Investigators: J.A. Morris, Ph.D., DBS; Jacob Brody, M.D., NINDS; Paul Albrecht, M.D., DBS; Michael Stewart, M.D., Hilton Levy, M.D., Larry Sturman, M.D., William Hadlow, D.V.M., NIAID; Leslie Weiner, M.D., Richard Johnson, M.D., and Robert Herndon, M.D., School of Medicine, Johns Hopkins University; Neal Nathanson, M.D., Gerald Cole, Ph.D., and Don Gilden, M.D., Johns Hopkins School of Public Health and Hygiene; James Hourrigan, D.V.M., and A. Klingsporn, D.V.M., USDA; John Hotchin, M.D., Albany; C. Chany, M.D., and F. Cathala, M.D., Paris; H. Koprowski, Wistar Institute; D. Porter, M.D., University of California; Peter Lampert, M.D., University of California; M. Oldstone, M.D., Scripps Clinic and Research Foundation

Project Description:

Objectives: To study scrapie, and mink encephalopathy as animal models of subacute spongiform virus encephalopathies; to determine the pathogenesis of these diseases in laboratory animals in an effort to elicit the mechanisms of infection, virus-cell-relationships, nature of the virus per se and the role of these viruses in inducing an aging process in infected animals. To study visna, a demyelinating disease of sheep, as a model in the study of demyelinating diseases of man, particularly, multiple sclerosis.

Methods: As previously described in earlier Annual Reports.

Major Findings:

Scrapie and mink encephalopathy: The recent results of our studies on these two diseases places them in the group of viruses which we now call subacute spongiform virus encephalopathies primarily affecting the gray matter of the brain: two of man: kuru and Creutzfeldt-Jakob disease; and, two of animals: scrapie and mink encephalopathy. This classification is based on (1) each is transmissible to an animal host though not necessarily the same host, (2) each is associated with a long incubation period of from several months to several years; (3) each induces a clinical syndrome consisting of progressive cerebellar ataxia, tremors and postural instability which is always fatal in termination; and, (4) each manifest histopathological lesions only in the gray matter of the brain consisting of severe intraneuronal vacuolation and dropout, astrocytosis, fibrous gliosis and moderate to marked status spongiosis without signs of acute inflammatory response.

During the period covered by this report we have continued our studies on the characterization of scrapie and mink encephalopathy. We have con-

tinued to study the effects of altering the immune mechanisms of the host by surgical spleenectomy, thymectomy and through the use of anti-lymphocytic serum, cyclophosphamide, and exposure to X-irradiation. None of the procedures employed had an effect on incubation periods, development or duration of clinical disease or fatal termination of the process. In a series of extensive experiments we were again unable to demonstrate antigen-antibody reactions by complement-fixation procedures utilizing crude or sucrose extracted suspensions of brain, kidney and spleen taken from mice at 30 days intervals after IC inoculation of scrapie. Sera employed in these studies were mouse, hyperimmunized rabbits and roosters, and naturally infected sheep. During the course of these studies we observed that following inoculation of scrapie IC into mice, virus remains detectable in the brain, spreads rapidly to the spleen and to a lesser concentration is detectable in the kidney. Virus in the kidney is associated with a pre-zoning effect. Whether this is due to the presence of specific neutralizing gamma globulin deposits on the basement membrane of glomeruli or whether the effect is due to non-specific interference is under study. Our studies suggest that the reaction is probably not due to the production of interferon since no increased levels of interferon have been detected in scrapie infected animals. Further, prophylactic treatment of mice with high concentrations of potent mouse interferon given before and at intervals after exposure to scrapie have shown no effect on the subsequent disease. Our studies of scrapie and mink encephalopathy have further confirmed the unusual properties of thermostability, resistance to ultraviolet irradiation, formalin and proteolytic enzymes. We have successfully repeated our studies on the size of scrapie as determined by filtration and our findings of 20nm-30nm has been independently confirmed by scientists in NIAID and at Compton, England. Administration of poly I-poly C given in large doses at a variety of times had no effect on incubation period, clinical signs or fatal termination of the disease.

Visna: During the period covered by this report we have developed direct and indirect methods of demonstrating visna virus in vitro and antibody in vivo by fluorescent microscopy. We have developed highly specific techniques for demonstrating neutralizing antibody to visna virus in sheep serum in tests conducted in sheep choroid plexus, lamb kidney cells and sheep testis cell cultures. Consistently negative results have been obtained in studies to adapt visna virus to mice, rats, rabbits, guinea pigs and hamsters by inoculation of high concentrations of virus followed by serial blind passages. The use of immunosuppressants did not alter the non-reactivity of animals to the virus. Multiple inoculations of high concentrations into mice and rabbits have failed to induce detectable levels of NT or FA antibodies.

It is of significance that disease has not occurred in chimpanzees, rhesus monkey, cynomolgus monkeys or African green monkeys in the more than 5 years since they were inoculated intracerebrally only or by multiple routes peripherally with scrapie, mink encephalopathy and visna virus.

Progressive pneumonia of sheep (Montana sheep disease): We had earlier postulated that progressive pneumonia of sheep in Montana was caused by a virus closely related to maedi and visna viruses. In 1967 we obtained brain tissue from a sheep and lung tissue from a second sheep both of which had clinically diagnosed progressive pneumonia. Viruses were isolated from both specimens submitted. We have now demonstrated that both strains of virus are

antigenically related to each other as well as to visna virus by cross neutralization and cross fluorescent antibody tests. This work has been confirmed by investigators in NIAID, NIH and at RML, in Montana. These same investigators have extended these findings to show that the viruses are RNA viruses associated with an RNA-DNA dependent polymerase. This plus their morphological similarity to the RNA oncogenic group of viruses as determined by EM warrants investigation into their potential oncogenic and tumorigenic capacities.

Significance: Studies on scrapie, mink encephalopathy, visna and the two strains of viruses isolated from sheep with progressive pneumonia provide optimal models for the in vivo and in vitro mechanisms that are involved in slow infections of the nervous system. Data can be extrapolated to assist in the design of experiments and interpretation of results of studies of human neurological diseases processes. The self fusing properties of visna virus, even when inactivated, offer a possible mechanism for interpreting the in vivo process of demyelination, and the relationship of the visna-Montana sheep disease virus complex to the oncogenic group of RNA viruses.

Proposed Course: Continued studies into the mechanism of infection, the pathogenesis of disease and the immune mechanisms that may influence slow infections due to scrapie, mink encephalopathy, visna virus, and the virus of progressive pneumonia. To expand these latter studies to include a search for naturally occurring tumors in progressive pneumonia of sheep as well as alveolar carcinoma of man.

SUB-PROJECT V: Studies on Australian antigen and antibody in chimpanzees, monkeys and primate handlers

Principal Investigators: Robert Cornelius, D.V.M., D. Carleton Gajdusek, M.D., and Clarence J. Gibbs, Jr., Ph.D.

Other Investigators: Lewellyn Barker, M.D., and Michael Peterson, M.D., DBS; John Hooks, Ph.D., and Nancy Rogers, M.S.

Project Description:

Objectives: Australian antigen was first associated with hepatitis in 1964, and it was first found in the chimpanzee in 1969. In an attempt to define the possible role of the nonhuman primate as a source of human infection, and to explore the carrier state, if any, in the nonhuman primate, this study was undertaken.

Methods: The NIH laboratory at the Patuxent Wildlife Research Center has approximately 80 chimpanzees and 350 smaller nonhuman primates. These animals are housed in two buildings, both of which contain new and old-world monkeys, and several species of each.

In 1969 serum samples from 95 chimpanzees and approximately 50 other primates were tested for Australian antigen and antibody. Serum samples from 59 chimpanzees and 6 other primates were also tested in 1970. Serum was tested for Australian antigen by the Ochterlony micro technique for ppt. and by complement-fixation, and for antibody using the Ochterlony micro technique.

Those animals possessing antigen were then bled at monthly intervals for 6 months and that serum tested for antigen and antibody, and blood chemistries for liver function. Cagemates of any positive animals were subjected to the same schedule. To attempt to ascertain when the infection occurred, serum stored at $-70^{\circ}C$ from previous serial bleedings, commencing soon after arrival, were also pooled and tested.

All laboratory personnel who came in contact with the animals, their tissues or blood, were placed on a bimonthly bleeding schedule. This serum was screened for antigen and antibody and liver functions.

Major Findings: Ninety-five chimpanzees were sampled in the first series. Two animals showed 4+ antigen by CF or agar gel ppt. These two animals A28 and A62 were still in the colony when the current study was undertaken.

Eighty-eight serum samples from 59 chimpanzees, and 7 serum samples from 6 other primates, were tested more than a year later. Two chimpanzees exhibited antigen titers by agar gel ppt. or CF, and one chimpanzee serum contained antibody. In addition, one stumptail monkey showed antibody titer of 1:20 by CF.

The two chimpanzees that had antigen in 1970, were the two animals that previously had shown antigen in 1969, and were still in the colony (A28 and A62). Chimpanzee A51 that showed antibody, was shown to have had antigen of 1:20 by ppt. in 1968, by the testing of stored serum.

The testing of stored serum from A28 and A62 taken soon after arrival in the colony showed that they had carried the antigen for some time. While the titer was decreasing, A28 had been infected for at least 4 years, and A62 for at least 3.5 years.

While A28 was housed singly, A62 was caged with A51. While this is of

interest, it could not be established how long these two animals had been in close contact.

Eleven laboratory personnel were bled 5 times in 7 months. Antigen was not detected by agar gel ppt. or CF in any sample, nor were there any liver enzyme elevations of significance.

Significance: Two findings were considered to be of value in this study. The first was the demonstration that chimpanzees can show infection with Australian antigen for at least 4 years, and still not have detectable antibody. And the second that transmission between cagemates appears to be possible, or has occurred.

Proposed Course: It is planned to continue with the monitoring procedure to detect infection, titer, persistence and carrier/shedding state. Immunization of primates for the production of antibody will be attempted. The purpose of these procedures will be to study the feasability of the large scale production of antibody for use in screening of donors and blood products as well as to prepare purified gamma globulin for immunoprophylactic procedures.

SUB-PROJECT VI: Fluorescent antibody studies on the intracellular localization and identification of viral antigens in vivo and in vitro in tissues from patients with subacute diseases of the CNS

Principal Investigators: D. Carleton Gajdusek, M.D., and Clarence J. Gibbs, Jr., Ph.D.

Other Investigators: John Hooks, Ph.D., Raymond Roos, M.D., Paul Brown, M.D., Nancy Rogers, M.S., Mint Basnight, M.S., and David Asher, M.D.

Project Description:

All phases of this project remain essentially as reported for FY1969. Notable exceptions are the preparation of several new conjugates, adaptation of FA technique to the in vivo and in vitro study of viral strains isolated from explant cultures and utilization of this technique in studies to detect viral antigens and antibodies in patients with multiple sclerosis, kuru, Creutzfeldt-Jakob disease and other degenerative diseases of man primarily those which cause spongiform changes in the gray matter of the brain.

SUB-PROJECT VII: Tissue and cell culture in vitro studies of viral induced slow infections of man and animals

Principal Investigators: D. Carleton Gajdusek, M.D., Clarence J. Gibbs, Jr., Ph.D., and Nancy Rogers, M.S.

Other Investigators: John Hooks, Ph.D., Raymond Roos, M.D., Paul Brown, M.D., Mint Basnight, M.S., Helena Gilbert, David Asher, M.D., and Robert Cornelius, D.V.M.

Cooperating Investigators: Harish Chopra, M.D., NCI; S. Chou, M.D., University of West Virginia; Peter Lampert, M.D., University of California; V. ter Muelen, M.D., Gottengen, Germany; C. Chany, M.D. and F. Cathala, M.D., Paris, France; S. Baron, M.D., NIAID

Project Description:

Objectives: To grow and maintain in vitro tissue and cell culture lines prepared with specimens of brain, visceral, lymphatic, muscle and blood tissues from human patients and animals succumbing to persistent, chronic, subacute progressive degenerative diseases particularly of the central nervous system, for the purpose of isolating viruses and demonstrating an etiological relationship to the diseases under study.

Methods: Brain, visceral, lymphatic, muscle and blood tissues obtained at surgical biopsy or early autopsy are grown in vitro as explanted organ type cultures, trypsinized dispersed monolayer cultures, and as polykaryons following cocultivation and Sendai virus medicated fused cultures with permissive cell lines for the rescue of viral genome from donor tissues. All cultures are serially sub-cultured and maintained in vitro over long periods during which time they are (1) observed for spontaneously developing CPE, (2) transformation, (3) detection of viral antigens by FA, hemadsorption, CF, and, HA tests, (4) examined by EM for morphology and viral particles. They are blind passaged into a wide variety of permissive cell lines and tested for detectable viral antigens. Attempts to isolate viruses are also being made by classical techniques of inoculating suspensions of various tissues and organ suspensions from humans and animals into a wide variety of primary and stable cell lines. Finally, attempts are being made to incorporate kuru and scrapie into mouse tissue culture cells during the process of cell fusion. Two strains of cell lines are currently being employed: C 11 D(TK) and A9(HGPRT-) which were chosen because they allow through their enzyme deficiencies the selection of hybrid cells which grow in the presence of amniopterin. Hybrid cells are also being investigated for the appearance of new antigens after treatment with virus. Such treated cells combined with Freund's complete adjuvant are used to immunize C3H/Hen mice which are subsequently tested for antibody by means of immune hemadsorption test.

Major Findings: To date, over 1500 explants of brain, visceral, lymphatic and muscle tissues from humans and animals dying with subacute degenerative CNS diseases have been grown and maintained in vitro as primary explants and serial cultures. Over 225 viral isolates have been recovered, the vast majority coming from explant cultures of chimpanzee tissues. Of these isolates

approximately 82% are strains of new foamy viruses Pan 1 and Pan 2, described in Sub-Project VIII. 7% are strains of adenoviruses Pan 5, Pan 6, Pan 7 and Pan 9 described in Sub-Project IX; two have been strains of reovirus and three remain unidentified viruses from chimpanzees. One of these has been designated Pan 8. The very slow appearance of CPE is necessitating a search for a more satisfactory cell line to pursue characterization and identification studies. It is significant that virus isolations have now been made from the brains of spider monkeys (foamy-like virus), squirrel monkeys (herpes-like virus) and in one instance a squirrel monkey had both herpes-like and foamy-like virus in his brains. Human tissues have yielded fewer isolates. We have demonstrated measles virus specific antigen intracellularly in explant cultures of brain from a case of SSPE; a single, as yet unidentified virus-like agent has been recovered from brain explant cultures prepared from a patient dying with ALS. An adenovirus, closely related to type 32 by neutralization tests and to type 27 by hemagglutination-inhibition tests, has been isolated from the brain of a human that died of lymphosarcoma with a subacute encephalitis. Virions morphologically resembling adenovirus were observed by EM in the patient's brain as well as in infected human embryo kidney cell cultures. This is only the second reported instance that adenovirus has been isolated from brain and the first reported instance that adenovirus can cause a subacute encephalitis.

Proposed Course: Continued emphasis will be placed on in vitro growth of human and animal tissues in attempts to isolate latent, persistent, chronic, masked viruses and viruses which share an endosymbiotic relationship with the donor hosts. Studies are being continued to elicit the etiological significance of viral isolates to human and animal diseases. The effects of latent viruses as they relate to presenile and senile dementias and to aging processes in cells is being further studied.

SUB-PROJECT VIII: Characterization and distribution of two new foamy viruses isolated from chimpanzees tissues grown in vitro and their relationship to the RNA oncogenic group of viruses

Principal Investigators: John Hooks, Ph.D., Nancy Rogers, M.S., D. Carleton Gajdusek, M.D., and Clarence J. Gibbs, Jr., Ph.D.

Other Investigators: Mint Basnight, M.S., Robert Cornelius, D.V.M., and Ronald DiGiacomo, D.V.M.

Cooperating Investigators: Peter Lampert, M.D., University of California; Harish Chopra, M.D., NCI; H. Thormar, Ph.D., and F.H. Lin, M.D., Institute for Basic Research in Mental Retardation, Staten Island

Project Description:

Objectives: The purpose of this study is to characterize two new foamy viruses, simian foamy 6 (Pan 1) and simian foamy 7 (Pan 2), isolated from chimpanzees; to elicit their experimental host range, and their physical, chemical and biological properties; to determine their etiological significance in human and animal diseases; and, to determine what etiological role they may have in experimental kuru and Creutzfeldt-Jakob disease in chimpanzees and new-world monkeys. To determine the relationship of Pan 1 and Pan 2 to prototype strains of simian foamy viruses as well as to monkey mammary tumor virus.

Methods: Pan 1 and Pan 2 viruses were isolated from brain, visceral tissues and lymphatic tissues from several chimpanzees affected with kuru, Creutzfeldt-Jakob disease and from chimpanzees dying with intercurrent infections. The viruses were isolated and maintained in primary HEK cultures which were incubated at 35°C - 37°C. Characterization studies of Pan 1 and Pan 2 were conducted using HEK cultures as the biological system of choice. Virus purification and assay for the presence of RNA dependent DNA polymerase were done using the procedure described by Lin and Thormar (Journal of Virology, 6: 1970). Cross neutralization tests employing antibody specific to simian foamy viruses 1 through 7 and mouse mammary tumor virus in HEK cultures were performed to determine antigenic relationships between these viruses.

Major Findings: These studies have shown that Pan 1 and Pan 2 viruses isolated from chimpanzees are myxo-like RNA viruses which induce a foamy, syncytia without inclusion bodies in HEK cultures. The viruses do not share common neutralizing or fluorescent antibody antigens and neither is related to any of the known simian foamy viruses or to monkey mammary tumor virus. They are morphologically indistinguishable from each other by EM and are 125nm in diameter. The virions consist of a central core 45nm, surrounded by an inner capsule of 80nm. Virions are observed only in the cytoplasm and at maturation obtain their outer envelope by budding into intracellular vacuoles or at the plasma membrane. The morphology of these viruses is strikingly similar to prototype simian foamy viruses and those viruses belonging to the oncogenic RNA viruses e.g. mouse mammary tumor, murine leukemia, bovine syn-

cytia and monkey mammary tumor virus. Both viruses are ether, chloroform and pH-sensitive and are inactivated by exposure to 56°C/30 minutes. They pass through 220nm millipore filters but not through 100nm. Homologous antibody occurs in the serum of donor chimpanzees. Antibodies to one or both viruses are also found in chimpanzees not inoculated with experimental kuru or Creutzfeldt-Jakob disease. Following purification procedures two main areas of protein and nucleic acid were demonstrable by absorbance of gradient fraction at 260nm and 280nm. Infected materials show a larger peak at fractions 5 through 15. The density of the area ranges from 1.08g/ml to 1.12g/ml. Enzyme activity, determined by uptake of H^3 thymidine, was observed in fractions 5-15 and to a lesser extent in other samples tested. Preliminary studies on the acid-insoluble product show reaction at 37°C and resistance to RNase. Cross neutralization studies showed that Pan 1 and Pan 2 viruses were not antigenically related to each other nor is either one related to simian foamy viruses types 1-5; monkey mammary tumor virus, visna virus, bovine syncytial virus or the virus which causes progressive pneumonia in sheep in Montana. Finally, although Pan 1 and Pan 2 share the property of RNA-DNA dependent polymerase with monkey mammary tumor virus they differ from this virus in their morphology.

Significance: Identification and characterization of two new foamy viruses from multiple tissues of a high percentage of chimpanzees tested suggest wide distribution of these viruses in nature. The CPE they induce in HEK cultures is strikingly similar to the intracytoplasmic vacuolation of neurons and astroglia observed in the brains of humans and animals affected with kuru and Creutzfeldt-Jakob diseases.

Proposed Course: (1) Continuation of seroepidemiological survey to determine the distribution of these viruses in man and animals; (2) increased emphasis on the pathogenesis of these two viruses in chimpanzees to determine their etiological significance in experimentally induced diseases of the brain, (3) increased emphasis to determine the oncogenic and tumor inducing properties of these viruses and (4) expanded studies to determine the relationships between these viruses and other viruses now classified as oncogenic RNA viruses.

SUB-PROJECT IX: Characterization of newly identified adenoviruses isolated from chimpanzee tissues grown in vitro

Principal Investigators: Mint Basnight, M.S., Nancy Rogers, M.S., and D. Carleton Gajdusek, M.D.

Other Investigators: Clarence J. Gibbs, Jr., Ph.D., Raymond Roos, M.D., John Hooks, Ph.D., and Paul Brown, M.D.

Cooperating Investigator: Wallace Rowe, M.D., NIAID

Project Description:

Objectives: Identification and characterization of heretofore unidentified adenoviruses isolated from chimpanzee tissues explants grown in vitro.

Methods: Standard classical techniques for the isolation and identification of viruses utilizing tissue and cell culture systems.

Major Findings: Four antigenically distinct viruses designated Pan 5, Pan 6, Pan 7 and Pan 9 were isolated in primary HEK inoculated with fluids from explant cultures of lymphoid tissues obtained from three chimpanzees. In HEK and primary African green monkey kidney cells each of the viruses induces a CPE consisting of rounding-up of cells and the formation of grape-like clusters characteristic of adenoviruses. All four are DNA, chloroform resistant, heat sensitive viruses which are stable at pH 3.0. Each passes through a 100nm millipore filter. None of the four viruses is antigenically related to known human or simian adenoviruses by neutralization or complement-fixation procedures. Homologous antibody occurs in the serum of each chimpanzee from whose tissue the virus has been isolated.

Proposed Course: Seroepidemiological studies to determine the distribution of these four newly identified viruses in man and animals. Studies are being conducted to determine the significance of these viruses in animals developing experimentally induced kuru and Creutzfeldt-Jakob disease.

SUB-PROJECT X: Attempts to demonstrate a viral etiology for chronic encephalitis with focal epilepsy

Principal Investigators: D. Carleton Gajdusek, M.D., Clarence J. Gibbs, Jr., Ph.D., and David Asher, M.D.

Other Investigators: John Hooks, Ph.D., Nancy Rogers, M.S., Paul Brown, M.D., Raymond Roos, M.D., Larry Fry, Ph.D., Mint Basnight, M.S., Robert Cornelius, D.V.M., and Ronald DiGiacomo, D.V.M.

Cooperating Investigators: T. Rasmussen, M.D. and S. Carpenter, M.D., Montreal Neurological Institute, Canada; A.A. Smorodintsev, M.D. and V.I. Il'yenko, M.D., Leningrad, U.S.S.R.

Project Description:

Occasional patients with chronic focal epilepsy are found at craniotomy to have active inflammation of the brain months or years after the onset of illness. In the USSR this condition, called Kozhevnikov's epilepsy, seems to follow acute tick-borne encephalitis (TBE). There has been one published report of the isolation of TBE virus from the brain tissues of two such patients, but this has never been confirmed. Il'yenko and colleagues in Leningrad have discovered a promising animal model for this condition; several strains of TBE when inoculated into rhesus monkeys by the intracerebral route produce only mild encephalitis, followed by a month or so later by the appearance of focal choreoathetotic tremors, along with histological evidence of chronic active inflammation of the brain, from which virus has been recovered up to a year after inoculation. We have attempted to confirm Dr. Il'yenko's finding using one of her strains of TBE virus. Four monkeys have been observed for three months without evidence of movement disorder, and eight more have been inoculated more recently. If the Soviet findings are confirmed we will investigate the mechanisms of virus persistence by immunosuppression, and by in vitro cultures of brain cells. Dr. Il'yenko has been invited to participate in our studies through the U.S.-U.S.S.R. Health Exchange Agreement.

Rasmussen and co-workers have observed focal epilepsy with chronic encephalitis in 23 patients, most of them from the United States and Canada. None had preceding acute encephalitis, although many had a variety of other febrile illnesses. In our laboratories there have been several attempts to isolate viruses from the brains of these patients by inoculation of mice, primates and tissue cultures, without success. Brain tissue from a recent patient has been grown in vitro by trypsinization and explant; tissue is now in serial passage and will be fused with several indicator cell lines in an attempt to detect latent viruses.

SUB-PROJECT XI: Isolation, epidemiology and pathogenesis of mourning dove pox

Principal Investigators: David Asher, M.D., D. Carleton Gajdusek, M.D., and Clarence J. Gibbs, Jr., Ph.D.

Cooperating Investigators: E. Dustman, Ph.D., C. Herman, Sc.D., and L. N. Locke, D.V.M., Patuxent Wildlife Research Center, Department of Interior, Laurel, Maryland

Project Description:

Troublesome outbreaks of pox disease have occurred among wild mourning doves (Zenaidura macroura) in many areas of the United States, including Maryland. The agent of this disease was isolated in the chorioallantoic membranes of hens' eggs. Of five orders of birds tested only doves and pigeons were susceptible. The domestic pigeon (Columba livia) developed only a mild form of disease. Dove-pox virus protected domestic pigeons but not mourning doves against challenge with pigeon pox virus. A serological study of the antigenic relationship of dove-pox virus to pigeon-pox virus is in progress.

SUB-PROJECT XII: Infectious and contagious disease management in a primate colony

Principal Investigators: Robert Cornelius, D.V.M., Ronald DiGiacomo, D.V.M., D. Carleton Gajdusek, M.D., and Clarence J. Gibbs, Jr.,Ph.D.

Other Investigators: David Asher, M.D., Paul Brown, M.D., Raymond Roos, M.D., John Hooks, Ph.D., Nancy Rogers, M.S., Mint Basnight, M.S., Luis Melendez, D.V.M., and Keerti Shah, M.D.

Technical Assistants: Alfred Bacote, Michael Sulima, Monica Lewis, Paul Horst, Lloyd Horst, Paul Martin, Amos Fox, James Noll, Galen Miller, James Webster, and Albert Bontrager

Cooperating Institutions: Animal Resources Branch, NIH; Armed Forces Institute of Pathology, Departments of Neuropathology and Veterinary Pathology; Department of Neuropathology, University of California; Department of Neuropathology, The Maudsley Institute; Department of Pathology, University of Western Australia School of Medicine; Gulf South Research Institute, National Center for Primate Biology; Otisville Laboratories of the Public Health Research Institute of the City of New York; Department of Pathobiology, Johns Hopkins University School of Public Health and Hygiene; New England Regional Primate Center

Project Description:

Objectives: To systematically study normal behavior, breeding habits, clinical chemistry and hematological values, clinical and sub-clinical intercurrent infections in program and control animals employed in the study of slow, latent and temperate viruses of the nervous system; to establish a registry of normal and abnormal values observed in a well conditioned and well maintained colony.

Major Findings: 1) Herpes simiae: during the later part of 1970 over 90% of the rhesus monkey population in this program was bled and examined for NT antibody to Herpes simiae. A total of 15 of 97 rhesus tested had NT antibody with titers of 1:100 or greater. 87 of the monkeys were imported from India and 19% of these had antibody. There was no apparent correlation of Ab with age, sex or length of stay in the colony. 8 of 13 monkeys that had Ab were or still are cagemates. 16 of the 97 Ab positive monkeys were colony reared.

2) Spontaneous disease in program animals: None of the program disease problems have been observed in a variety of animal species being employed as experimental hosts. In primates pulmonary vascular sclerosis, pregnancy toxemia, mycotic pneumonitis, gastric ulcer, polynephritis, meningitis and Herpesvirus-T infection have been a problem. During the year newly imported mink developed clinical signs of Aleutian mink disease within 5 days after being received into the quarantine unit. This was later confirmed by histological study. In the ferret colony we have observed spontaneous occurrence of liver tumors of vascular origin and bronchial asthma due to filarids.

3) Collection, tabulation and program analysis of data on normative organ weights and hematological parameters in the chimpanzee is currently under study.

Significance: 1) Herpes simiae infection in a primate colony is of importance because of the high mortality rate this virus causes in man. The ongoing study we have initiated will serve to assess the degree of communicability and exposure hazard for animal caretakers. 2) A general diagnostic pathological workup is being performed on each animal (control and inoculated) in our research program that dies with an intercurrent infection. Such a surveillance provides information on diseases occurring within the colony and provides information for successful management of a large colony which is made up of old world and new-world monkeys. 3) Normative data on organ weights, hematological, CSF, clinical chemistries and serological parameters in the chimpanzee and smaller primates will assist in the rapid diagnosis of disease.

Proposed Course: Continuation of this long-term study and publication of a compendium on normal and diseased animal values.

SUB-PROJECT XIII: Slow Virus Symposia, VIth International Congress of Neuropathology, Paris, France

Principal Investigators: Clarence J. Gibbs, Jr., Ph.D., and E. Osetowska, M.D.

Other Investigators: D. Carleton Gajdusek, M.D.

Cooperating Investigators: Hilary Koprowski, M.D., Wistar Institute, Philadelphia; Jean Lapresle, M.D., and Francoise Cathala, M.D., Hopital de la Salpetrière, Paris; Richard Johnson, M.D., Johns Hopkins University, Baltimore

Project Description:

The development, organization and promulgation of a major theme on "Pathogenesis of Slow Virus Diseases of the Central Nervous System" as part of the VIth International Congress of Neuropathology, Paris, France, August 31 through September 4, 1970.

Results: Publication of Proceedings of VIth International Congress of Neuropathology, Masson & Cie, Paris, 1970.

E. Osetowska and C.J. Gibbs, Jr., Theme IV, "Pathogenesis of Slow Virus Diseases of the Central Nervous System", Proceedings of VIth International Congress of Neuropathology, Masson & Cie, Paris, 1970:

Johnson, R.T.: Virus-Host relationships in acute and chronic encephalopathies. pp. 761-778.

Gibbs, C.J.,Jr. and Gajdusek, D.C.: Characterization and nature of viruses causing subacute spongiform encephalopathies. pp. 779-801.

Hunter, G.D.: The biochemical properties and nature of the scrapie agent. pp. 802-817.

Oldstone, M.B.A.: Pathogenesis of cellular injury associated with persistent viral infection. pp. 818-824.

Simons, M.J., Fitzgerald, M.G., and Alpers, M.P.: Lymphocytic function in kuru. pp. 825-826.

Brody, J.A. and Nemo, G.J.: Response of peripheral blood lymphocytes from multiple sclerosis patients to guinea pig basic protein, and multiple sclerosis patients' cerebrospinal fluid and brain. pp. 827-828.

Hotchin, J.: Immunological aspects of slow virus disease: LCM virus-induced anti-brain antibodies as a cause of neurological damage. pp. 829-830.

Petursson, G.: Studies on viral antibodies in visna. pp. 831-832.

Albrecht, P.: Immune status of the organism during experimental scrapie infection. pp. 833-834.

ter Meulen, V., Muller, D., and Katz, M.: Immunohistological and histochemical studies in subacute sclerosing panencephalitis: An example of an analysis of a slow virus infection of the CNS. pp. 835-836.

Koestner, A., Long, J.F., Jacoby, R.O., Olsen, R.G., and Shadduck, J.A.: Canine distemper as a model of a parainfectious demyelinating encephalopathy. pp. 837-838.

Parry, H.B., and Vince, A.A.: Scrapie disease of sheep: the roles of gene and "slow virus" in pathogenesis. pp. 839-840.

Dickinson, A.G.: Classification of scrapie agents based on histological and incubation period criteria in mice. pp. 841-842.

Barlow, R.M., and Rennie, J.C.: Experience with mink encephalopathy in various experimental animals. pp. 843-844.

Zeman, W.: Subacute sclerosing panencephalitis, a slow measles virus infection. pp. 845-849.

Parker, J.C., Jr., Klintworth, G.K., and Graham, D.G.: Myxovirus-paramyxovirus within lesions of the CNS of two cases following vaccination with attenuated measles virus. pp. 850-851.

Baringer, J.R., and Griffith, J.F.: Experimental measles virus infections of the nervous system. pp. 852-853.

Rorke, L.B., Katz, M., Masland, W.S., and Koprowski, H.: Experimental subacute sclerosing panencephalitis in ferrets. An animal model system for study of the human disease. pp. 854-855.

Haig, D.A.: Propagation of the scrapie agent in cell culture. pp. 856-857.

Beck, E., Daniel, P.M., Gajdusek, D.C., and Gibbs, C.J., Jr.: Subacute degenerations of the brain transmissible to experimental animals: a neuropathological evaluation. pp. 858-873.

Anderson, R. McD.: Histopathology of the brain in kuru. pp. 874-875.

Nathanson, N., Cole, G.A., Weiner, L.P., Gilden, D.H., and Johnson, R.T.: Diversity of pathological lesions produced by acute virus infections of the nervous system. pp. 876-891.

Hirano, A.: Further studies on amyotrophic lateral sclerosis and parkinsonism dementia complex on Guam. pp. 892-893.

van Bogaert, L., and Osetowska, E.: Etude comparée de la maladie de Carré et des encéphalites de la rougeole. pp. 894-896.

Fraser, H.: Comparative morphology of ageing and scrapie. pp. 897-898.

Pattison, I.H.: Detection of the scrapie agent in the tissues of normal mice. pp. 899-900.

Zlotnik, I.: The pathogenesis of scrapie. pp. 901-915.

Lampert, P.W., Earle, K.M., Gibbs, C.J., Jr., and Gajdusek, D.C.: Electron microscopic studies on experimental spongiform encephalopathies (kuru and Creutzfeldt-Jakob disease) in chimpanzees. pp. 916-930.

Sever, J.L., Horta-Barbosa, L., Vernon, M.L., Fuccillo, D.A., Plum, F., and Baringer, J.R.: Creutzfeldt-Jakob disease: Virus-like particles in brain biopsies and tissue cultures. pp. 931-932.

Bignami, A., and Parry, H.B.: Electron microscopic observations in slow virus infections of the CNS. pp. 933-934.

Field, E.J.: Slow virus infection of the nervous system. pp. 935-936.

Ovary, E., Benko, Ch., and Gombi, R.: So-called Gonatas-particles. pp. 937-938.

Zu Rhein, G.M., and Eckroade, R.J.: Experimental transmissible mink encephalopathy (TME). An ultrastructural study. pp. 939-940.

Raine, C.S., and Sheppard, R.D.: Ultrastructural observations of measles virus in nervous tissue. pp. 941-942.

Masters, C.L., Kakulas, B.A., Gajdusek, D.C., and Gibbs, C.J., Jr.: The significance of the recent experimental observations on slow virus infections to neuropathology. pp. 943-951.

Kurtzke, J.F.: Multiple sclerosis as a latent infection of the nervous system. pp. 952-957.

Sever, J.L., Kurtzke, J.F., Alter, M., Schumacher, G.A., Gilkeson, M.R., Ellenberg, J.H., and Brody, J.A.: Virus antibodies and multiple sclerosis. pp. 958-959.

Benko, Ch., Ovary, E., and Gombi, R.: Morphological methods in the cerebrospinal fluid (CSF) testing of subacute sclerosing panencephalitis (SSPE). pp. 960-961.

SUB-PROJECT XIV: Studies on the ecology, epidemiology, and pathogenesis of arbovirus infections of man and animals

Principal Investigators: D. Carleton Gajdusek, M.D., and Clarence J. Gibbs, Jr., Ph.D.

Other Investigators: Nancy Rogers, M.S., John Hooks, Ph.D., Paul Brown, M.D., Mint Basnight, M.S., and David Asher, M.D.

Cooperating Investigators: Jacob Brody, NINDS; K. Shah, M.D., and F. Bang, M.D., Johns Hopkins School of Public Health, Baltimore; A. Smorodintsev, M.D., Institute of Influenza, Leningrad, USSR; A. Shubladze and V. Zhdanov, Ivanovsky Institute, USSR; B. Gavrilyuk, Institute of Biophysics, Serpukhovsky Region, USSR; V. Il'yenko, Research Institute of Influenza, Leningrad, USSR; J. Casals, M.D., R. Shope, M.D., and W. Downs, M.D., Yale University, New Haven; C. Wisseman, M.D., University of Maryland; R. Hornabrook, M.D., Kuru Research Office, Okapa, New Guinea; F. Schofield, M.D., Nairobi; J. Sever, M.D., NINDS

Project Description:

Studies are continuing on the following areas:

1. Epidemic hemorrhagic fevers - chronic and persistent infections
2. Seroepidemiology of arbovirus infections in ecologically isolated primitive indigenous populations:
   a) Seroepidemiology of Alaskan populations
   b) Seroepidemiology of the populations of the Caribbean and Central and South American countries with particular reference to Puerto Rico, Bolivia and Paraguay
   c) Seroepidemiology of Australasian populations
3. Japanese B encephalitis studies on Guam
4. Persistence of arbovirus infections in man and animals
5. Isolation and characterization of the virus causing nephro-nephritis hemorrhagic fever syndrome in Southeast Asia

Significance and Course: These are long-term (5 years and more) studies being conducted on a continuing basis. Seroepidemiological and viral ecology studies are being conducted in primitive cultures and specific patterns of disease determined. These studies will continue until statistically sound data are collected for publication.

Publications:

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Gibbs, C.J., Jr. and Gajdusek, D.C.: Characterization and nature of viruses causing subacute spongiform encephalopathies. In: VIth International Congress of Neuropathology, Masson and Cie, Paris, pp. 779-801,1970.

Gibbs, C.J., Jr. and Gajdusek, D.C.: Transmission and characterization of the agents of spongiform virus encephalopathies: kuru, Creutzfeldt-Jakob disease, scrapie and mink encephalopathy. In: Immunological Disorders of the Nervous System, Res. Publ. Assn. Nerv. Ment. Dis., 49:XXIV: Williams and Wilkins, Baltimore, 1971.

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Masters, C.L., Kakulas, B.A., Gajdusek, D.C., and Gibbs, C.J., Jr.: The significance of the recent experimental observations on slow virus infections to neuropathology. In: VIth International Congress of Neuropathology, Masson and Cie, Paris, pp. 843-951.

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ANNUAL REPORT
JULY 1, 1970 THROUGH JUNE 30, 1971
EPIDEMIOLOGY BRANCH
COLLABORATIVE AND FIELD RESEARCH
NATIONAL INSTITUTE OF NEUROLOGICAL
DISEASES AND STROKE

Introduction

The major areas of activity of the Epidemiology Branch during the period of this report have been:

I. epidemiologic studies of neurologic diseases;

II. laboratory studies related to the epidemiology and immunology of neurologic and perinatal diseases;

III. the continued activities on Guam and the Trust Territories;

IV. genetic studies of neurologic diseases;

The following professional personnel have joined the Epidemiology Branch: Dr. Charles E. Morris, Associate Professor of Neurology at the University of North Carolina, has been our Officer-in-Charge on Guam. Dr. John Stanhope, an epidemiologist who was with my staff here in Bethesda was transferred to Guam for the current fiscal year. Dr. Roger Bobowick, who completed his residency in neurology, joined my staff in Bethesda. Dr. Jeffrey Allen, who completed his internship in Seattle at King County, is in the Genetics Section. Kathy O'Meara, Biologist, is also in the Genetics Section, and Marjorie Matthews, R.N., is working with the Epidemiology Branch. Mr. Otis Turner, Statistician, joined our staff for 6 months in Epidemiology, but subsequently accepted a promotion to Associate Director, Division of Maternal & Child Health Services, and Gary Cooper, Medical Technician, is currently working in the Laboratory. Dr. A.V. Bird, a neurologist from South Africa has joined the Branch for 6 months as a Visiting Scientist to pursue studies in the epidemiology of multiple sclerosis.

The following people have left the Branch: Dr. Roger Detels left to accept an Associate Professorship in Epidemiology at U.C.L.A., Renee Owens, Biologist, left to join her husband in Texas. Jane McNew, R.N., left to get an M.P.H. degree at the Johns Hopkins School of Hygiene and Public Health.

In terms of organization and planning for next year, we are seriously hampered because the position of Deputy Branch Chief is vacant. Dr. E. Michael Holden, who is finishing his second year of neurology residency at the Boston City Hospital, will go to Guam as Officer-in-Charge for two years. Dr. Robert Stern, who is finishing his internship at Mt. Sinai, New York City, will be joining the Branch in Bethesda.

We have pursued the mission of the Epidemiology Branch in studying the epidemiologic aspects of neurologic disease in which, through our approaches we can contribute significantly to the understanding of specific aspects of neurologic disease, which will be of immediate value to physicians and patients. We believe that through the technique of specific hypothesis testing in defined populations rather than through large descriptive population studies we can perform this function most effectively. Our major contributions during this year have been: As an offshoot of our Guam studies we have demonstrated that CNS dopamine metabolism is diminished in amyotrophic lateral sclerosis patients in the United States. This is the first demonstrated chemical abnormality in the CNS of ALS patients. Through studies of SSPE we have shown that this disease apparently results from the combination of an abnormal infection with measles and a subsequent zoonotic trigger. Epidemiologic studies of MS in California and Washington State have produced provocative information suggesting that a protective factor exists among people born in low risk areas and further that the risk of developing MS can be altered by migration after puberty. We also found that rates for Japanese and Chinese for MS are consistently low in California and Washington State. Serologic studies of MS continue to implicate the measles virus and a possible familial factor. Several difficult studies have been established and are proceeding well. These include studies of the natural history of Parkinson's disease and the effect of L-Dopa, twin studies on multiple sclerosis, studies of ALS among veterans and a case/control study of Jakob-Creutzfeldt disease. We have made significant advances in diagnosis and treatment of acoustic neuroma and studies relating to I.Q and retinoblastoma and torsion dystonia continue to bear promise.

I. Epidemiologic studies of neurologic disease.

Multiple Sclerosis

We have completed the first phase of our studies of multiple sclerosis in California and Washington State and have made the following observations. Orientals who have an apparently low rate of multiple sclerosis relative to their latitude of birth have low rates of multiple sclerosis after migration to California and Washington. Further, second generation Orientals persist in having a low rate of MS suggesting a possible genetic factor. People born in Washington State had higher rates of MS than those born in California which would be expected according to the north-south differential of this disease. People migrating from northern states to the West Coast had higher rates when they migrated to Washington than to California, implying that they either acquired protection in California or the causative factors are less common in California than in Washington. The implication of this is that the causative factors persist after puberty, which is contrary to current belief. Those who migrate from low risk southern areas did not develop a high rate of MS either in California or Washington (small numbers). This implies that by the time of migration they have protection from multiple sclerosis. If protection can be acquired as these data would seem to imply, there is some hope of developing a preventive mechanism for MS.

In collaboration with Dr. John Sever, Head, Section on Infectious Diseases, Perinatal Research Branch, C&FR, NINDS, we have pursued our studies of measles serology in patients and family members. Recent data suggest that measles antibody is slightly but consistently elevated in MS patients when compared to controls. This elevation is not consistently encountered among siblings of the same sex of the MS patients, suggesting a possible familial factor in this disease. We are still hoping to extend our studies to the Shetland and Orkney Islands where an ideal population for study exists. A brief trip to these islands confirmed the observation that the rates of MS in these small populations are three times higher than the highest rate reported anywhere else.

We have developed several new studies of the epidemiology of multiple sclerosis. We are investigating some 15 or 20 twins from the Veterans Twin Registry in which one of the twins has MS, in order to determine if there are distinct variations in exposure or disease history prior to onset of MS among the twins. We are also conducting patient, sibling studies of a small group of MS patients in which the mother is alive and available. We hope to detect possible differences in early infections such as we encountered with SSPE or in other factors which have been suggested as having possible relevance in the etiology of MS. Further, through the contract mechanism we are involved in a study of 6,000 veterans with MS in order to further elucidate migratory patterns and predisposing factors in this population and selected controls.

Amyotrophic Lateral Sclerosis (stateside studies)

Following our observation on Guam that ALS patients had diminished CNS dopamine metabolism, we extended our studies to stateside ALS patients and again encountered diminished CNS dopamine metabolism. This finding has now been confirmed by others at NIH, at Columbia, at Harvard and at Montreal. Initial treatment trials of ALS patients with L-Dopa have not yielded impressive results. The observation of a chemical abnormality in the CNS of ALS patients is to our knowledge the first substantial chemical abnormality in the CNS of these patients, and hopefully will lead to greater insights in abnormal neurometabolite patterns among patients with motor neuron disease.

We continue to follow 12,000 statesiders who worked for more than one year on Guam between 1945 and 1955. The final information on approximately 20% of the 2500 people in this group who have died continue to trickle in. We have now encountered two patients who died of ALS which does not seem excessive for a population of adult males.

We are pursuing our epidemiologic study to test the hypothesis that pancreatic insufficiency is causally related to ALS which has been suggested by several investigators. We continue to follow some 3,000 veterans who had duodenal ulcer between 1948 and 1958. In one group only vagotomy and pyloroplasty was performed, while in another group gastrectomy with Billroth II anastomosis was conducted and hence the patients had induced pancreatic insufficiency. The clinical phase of this study has ended with the suggestion that an excess of non-specific neurologic disease occurred in those receiving the Billroth II procedure as opposed to those receiving vagotomy and pyloroplasty. We have no evidence of ALS in these groups. We are now

attempting to follow-up through the Veterans Administration on causes of death in this unique population. We are having difficulty in accumulating the final data but there is a suggestion of an excess of several neurologic diseases including Parkinson's disease and multiple sclerosis.

We have developed a protocol with Dr. Gilbert Beebe, Director, Follow-Up Agency, National Research Council of the National Academy of Sciences and Dr. John Kurtzke, Chief, of the Neurology Service, Vererans Administration, Washington, D.C., to conduct studies of ALS veterans patterned generally on the initial MS study among veterans. We have also developed and field tested a detailed questionnaire to be administered to 200 ALS patients and 200 patients with brain tumor in VA hospitals in order to detect possible predisposing factors to ALS. We will also analyze exposure to Guam as a possible predisposing factor to this disease.

In conjunction with our studies of multiple sclerosis in California and Washington State we have analyzed patterns of ALS among patients born in California and Washington and among migrants. In striking contrast to our findings with MS we have detected no geographic patterns in the incidence of ALS in these populations.

Parkinson's Disease.

In collaboration with the National Parkinson's Foundation, Inc., in Miami, Florida, we are studying the effects on long term administration of L-Dopa and the influence of L-Dopa on the natural history of Parkinson's disease. We are following a cohort of up to 500 Parkinson's disease patients who developed their disease from 1962 to 1965 (pre-L-Dopa) and will match these individuals with a similar number who developed their disease after 1968 and are receiving L-Dopa. Our initial findings suggested a possible cohort phenomenon with patients in the L-Dopa treatment group being 5 to 6 years older than those in the pre-L-Dopa group. We have reviewed several other series of patients and have not been able to confirm this impression. It is important however that an adequate understanding of this observation is achieved because if there is a cohort phenomenon, it would imply the gradual disappearance of Parkinson's disease as has been suggested by some investigators.

We are continuing our study of identical schizophrenic twins receiving phenothiazines in order to determine if phenothiazine-induced extrapyramidal disease is the result of a genetic inability to handle these drugs. Analysis of our first six sets of twins suggests that there is no genetic control over this phenomenon. Further, we have been studying the appearance of Parkinson's disease among blacks and whites and have the impression that the disease occurs less frequently among blacks. In our studies of drug-induced Parkinson's disease however, the rate of disease is similar in blacks and whites. In collaboration with Dr. Thomas Chase, Chief, Unit on Neurology, Laboratory of Clinical Sciences, NIMH, we have shown that schizophrenics who receive phenothiazine derivatives and who do not develop extrapyramidal signs have significantly elevated dopamine and seratonin end products in their CSF. Those who do develop extrapyramidal signs have significantly depressed levels, particularly of dopamine end products in the CSF. Thus, a possible mechanism

can be postulated that over-stimulation of dopaminergic cells from phenothiazine utilization resulted in exhaustion of these cells in a certain number of cases and subsequent development of parkinsonian features.

Subacute Sclerosing Panencephalitis.

It is established that measles in some way is causally related to SSPE. As a result of our case/control studies we have determined that SSPE develops in individuals with very unusual measles histories. In up to 2/3 of the patients (P=.001) there was either no history of measles, measles under age one, or measles approximately 2 months after exposure to chicken pox. Further, we have confirmed the observation that SSPE occurs with a strong male preponderance and essentially in rural areas. Since measles even under age one does not follow this male, rural pattern, we believe some form of zoonotic triggering mechanism, perhaps an animal virus, is also involved in the pathogenesis of SSPE. Our patients had a significantly higher rate of exposure to sick animals including dogs and fowl, but not cats, than our controls.

Serum samples collected from patients and their families and controls and their families are now being tested for measles antibody and will be available for studies of other appropriate agents.

Stroke

As the result of a field trip to Panama, a potentially valuable study of stroke among blacks has been developed and awaits funding. We wish to explore the relative effect of genetic factors and environmental factors on the extraordinarily high rate of essential hypertension among blacks.

Jakob-Creutzfeldt Disease

We are conducting a case/control study of 40 patients with J-C disease. Approximately 15 patients and control have been examined and interviewed. Analysis of these data are not yet available.

II. Studies on Cellular Immunity in MS

Our studies on lymphocyte function in MS have continued in an attempt to further define the role of cellular immunity in the pathogenesis of demyelinating diseases. These have consistently failed to show any significant differences between MS and normal persons. However, our results demonstrate that suspensions of brain material (especially white matter) are definitely stimulatory to lymphocytes in culture. This has been true regardless of the health of the donor. The nature of this effect is uncertain but must be considered in all investigations on the response of lymphocyte from patients with neurologic illnesses to CNS antigens.

During the year we continued to investigate the effects on circulating lymphocytes following measles vaccine. Results have been inconsistent; in some experiments a depression in lymphocyte transformation was seen while in others no effect was apparent. Attempts to demonstrate a lymphocyte-

depressing serum factor following vaccination have been equally negative with multiple sclerosis patients and normal control persons. These findings, although involving limited numbers of patients, are in contradistinction to reports by other investigators of an anti-lymphocyte factor in the serum of patients with MS.

Studies on Cellular Immunity in Neurological Diseases other Than Multiple Sclerosis

In order to investigate the possible role of lymphocyte-mediated immunity in the Guam ALS and the PD syndromes, we initiated a cooperative effort with our staff on Guam. The lymphocyte transformation technique was carried out with the lymphocytes from selected patients on Guam; at the end of the tissue culture period, the lymphocytes were "harvested" and sent to our Bethesda laboratory where the remainder of the DNA extraction procedure was done. The results of these experiments indicate that such studies are feasible despite the limited facilities and laboratory personnel on Guam. Definitive studies are now underway.

Basic Studies on the Lymphocyte Transformation Phenomenon and Related Tests of Cell-Mediated Immunity

Liquid scintillation quantitation of tritium incorporation by dividing cells has been adopted as the best measure of lymphocyte transformation. Using this technique we have completed a series of experiments on the effects of a synthetic double-stranded RNA (poly I-C) on vaccinia virus induced transformation. The results of these experiments have substantiated and extended the observations described in last year's report. Complementary studies using a double-stranded RNA virus (Reo-1) have not shown any effect on lymphocyte transformation. Other laboratory studies carried out by Dr. Jeffrey Allen have extended our knowledge on the effects of storage of lymphocytes for hours or days prior to initiation of culture; we anticipate that these will prove useful in future investigations using cells from patients with diseases such as subacute sclerosing panencephalitis (SSPE) and Jakob-Creutzfeldt disease. In addition, Dr. Allen has initiated a series of experiments to study the effect of the measles virus on lymphocyte transformation induced by non-specific mitogens and specific antigenic stimulants.

Because of reports that cellular immunity to CNS antigens in MS patients can be demonstrated by a different technique (human macrophage migration inhibition), we are now initiating studies using this method to complement our lymphocyte transformation investigations. To learn this technique, Dr. Nemo spent one week in Dr. Bartfeld's laboratory at New York University and is currently setting up the method in our laboratory.

Studies on Chronic and Congenital Viral Infections in Experimental Animals

Approximately 50 mice whose mothers received injections of the minute virus of mice (MVM) late in gestation have been observed for one year. The only abnormalities observed to date has been the occurrence of solid tumors in two animals. The first such animal was lost; the second was found to

have a large tumor in the area of the right uterine horn with metastasis to the liver. Preliminary results suggest the presence of MVM in both the tumor and the liver and raise important questions as to the role of chronic, congenital MVM infection in the etiology of solid tumors of the mouse. Further studies are in progress to evaluate the oncogenicity of MVM and cell-free extracts of the uterine tumor.

Other experiments with MVM have suggested that maternal infection early in gestation may result in reabsorption of the infected embryo. This problem will be studied further during the next year.

Our studies on the epidemiology of SSPE led us to hypothesize that a fundamental step in the etiology of that disease may be an initial exposure to measles virus at a time when persistent maternal antibodies might interfere with the normal development of cellular immunity and hence might allow for an unusual persistence of the viral genome in certain long-lived cells such as neurones. Dr. Nemo and Mr. Cooper are currently attempting to develop an animal model for such a system and are investigating the course of infection with measles virus in young mice whose mothers were inoculated with measles a few weeks prior to mating. No results are available at this time.

Studies on the Minute Virus of Mice (MVM)

This agent was selected for use in the development of a model system for the study of chronic and congenital viral infections. It is hoped that an elucidation of mechanisms involved with establishment of chronic MVM infections will contribute to our understanding of certain chronic neurologic diseases whose etiologies may also involve a persistent, latent or recrudescent virus infection. MVM has been reported to cause cerebellar hypoplasia and congenital ataxia but has been the subject of limited investigation because of technical difficulties involved with its detection, quantitation and propagation in high concentration. The efforts of Dr. White, Mrs. Sutton and Dr. Nemo during the past year seem to have resolved these difficulties: they have developed methods of propagation yielding virus at concentration 100 to 1000 times higher than previously possible, and have improved their infectivity quantitation techniques from a six week rather unreliable test to a one week procedure with sharp endpoints. In addition, they have demonstrated the growth of MVM in 4 different continuous cell lines, although reports from other laboratories had been confined to only 2 primary cell types. Of particular interest is the development by Mrs. Sutton of chronic MVM infection of one of these cell lines. This system is proving useful as a continuous source of large amounts of virus and may advance our understanding of chronic infections in vivo.

Experiments in two cell types have substantiated the impression that MVM replication requires that the infected cell divide, even though the virus can infect and persist in a non-dividing cell. This observation may be relevant to the viral latency which may be a prerequisite to the pathogenesis of SSPE, J-C disease, and progressive multifocal leukoencephalopathy (PML). Our working hypothesis is that MVM infection, like certain papova virus (SV-40), polyoma and perhaps the papova-like agents involved in PML is associated with an integration of viral DNA into chromosomal DNA, and that this relates to

the tendency for MVM to persist in non-dividing cells. Experiments are underway to test this hypothesis by defining the sequence of molecular events which occur in the course of MVM infections in vitro.

III. The continued activities on Guam and the Trust Territories

The epidemiologic patterns for ALS and PD on Guam have been updated to include the past five years. A slight but apparently not significant decline in rates was noted. The pattern of age-specific attack rates, however, has not changed. This suggests that we are not observing a cohort phenomenon which would have occurred as a result of a massive common exposure at some time in the past and implies that the cause of causes of ALS and PD on Guam are still operative. L-Dopa trials on PD patients are in their 15th month with some observable benefit in the extrapyramidal features of this disease. Drug trials in ALS patients with L-Dopa, isoprinosine and placebo are in their second month. The studies are "blind" and no dramatic improvement or untoward responses have been observed. We have further confirmatory evidence that CNS dopamine production in PD patients is markedly depressed while CNS dopamine production in ALS patients of Guam and in the continental United States are significantly comprised but to a lesser degree than encountered in PD or classical paralysis agitans.

We are not attempting to develop an assay of impaired CNS dopamine metabolism in which we used expired air and urine rather than CSF to conduct our measurements. The technique is now being used on patients and controls. If is is feasible we will conduct studies of CNS dopamine metabolism in family members of ALS and PD patients and also in geographic areas where the diseases seem to concentrate.

We conducted a follow-up study of the congenital blindness among the Pingelapese population on the Eastern Caroline Islands. Six patients have been seen at NINDS and the diagnosis is now believed to be achromatopsia with the single reservation that the high myopia encountered in the affected patients is perhaps a separate entity. The unusual age distribution of patients observed in our previous trip report which had suggested a possible extrinsic factor was not encountered in our most recent survey.

IV. Genetic studies of neurologic diseases

The two main interests of the Genetic Section continue to be movement disorders, especially the torsion dystonias, and hereditary tumors of the nervous system. In addition the Section is embarking on a study of a highly inbred group of Irish Tinkers living in the Southeastern United States who are thought to have several unusual genetic traits.

Our efforts regarding the dystonias are directed toward determining the basic neurochemical defect in the autosomal recessive and autosomal dominant forms. Ten of our patients have participated in a study of catecholamine metabolism on the research ward of Dr. Thomas Chase, NIMH. Results to date, although not pinpointing a specific abnormality do suggest we are dealing with the abnormal pathway. Evaluation of various therapeutic regimens using L-Dopa, peripheral dopa decarboxylase inhibitors and other

drugs is being conducted by collaborators with patients from our series. No single regimen seems effective for all patients so that tailoring of drug and dose for each patient is still necessary. Finally, we continue to see new patients and their families with unusual movement problems. One interesting group which has emerged consists of professional musicians troubled by a mild movement abnormality which, however, is sufficient to impair proper performance. This may be a distinct new entity or else may represent the heterozygous state of autosomal recessive dystonia.

Acoustic neuroma remains our chief interest in the area of hereditary neoplasms. On the basis of our study of the large Pennsylvania kindred with acoustic neuroma and review of the literature we concluded that this seemingly rare trait might actually be fairly common if the appropriate population were sampled. Therefore, we looked for the presence of bilateral disease and positive family history in individuals with onset of symptoms of acoustic neuroma at a young age. To date we have information on over 2100 individuals in 51 families and have found at least 4 with positive family history of acoustic neuroma. Since only 4 families have been reported in the literature we have been able to double the number of reported families by this approach. The interesting question of relationship between neurofibromatosis and acoustic neuroma is being investigated through clinical study, cell culture techniques and chromosome analysis.

The retinoblastoma study, which deals with perception of affected by sighted individuals, is moving into its final stages. Preliminary results show no significant difference in performance between patients and unaffected sibs used as controls. This is at variance with the two reported studies on this question. Chromosome studies in familial cases show no abnormalities unlike sporadic atypical cases.

Our interest in genetic factors in ALS and PD as seen on Guam continues through study of select families on Guam and among migrants living in California.

Groundwork is being laid for a comprehensive genetic, demograph and medical study of the Irish Tinkers of Murphy Village, South Carolina. Since this group is understandably wary of strangers, great care is being paid to establishing rapport with elders and other informants within the community. We are enthusiastic about this project since it is through study of such isolates that many new recessively inherited traits have been described.

A canvass is being made of isolated communities along the coast of Maine for similarly inbred groups.

CONTRACT NARRATIVE
Epidemiology Branch, C&FR, NINDS
Fiscal Year 1971

NATIONAL RESEARCH COUNCIL, FOLLOW-UP AGENCY (PH43-64-44)

Title: New Epidemiologic Study of Multiple Sclerosis in U.S. Military Veteran Population

Contractor's Project Director: Gilbert Beebe, M.D.
John F. Kurtzke, M.D.

Current Annual Level: $34,700

Objectives: The contractor will perform an extensive survey of multiple sclerosis among veterans of the Second World War and the Korean War. This will be an update of an initial survey which included 600 patients and will include approximately 6,000 patients. Patients will be matched with controls to determine geographic patterns, socio-economic status, urban-rural localization and numerous other variables which have been tested for multiple sclerosis. Parallel information will be available for Negro MS patients and controls and white female MS patients and controls. In addition an extensive investigation of migrant and non-migrant populations to and from high and low risk areas for multiple sclerosis will be conducted. Emphasis will also be placed on the relationship between the communicable diseases experienced in childhood and subsequent multiple sclerosis.

Major Findings: The project has just commenced and data is still being accumulated.

Significance to NINDS Program and Biomedical Research: Multiple sclerosis is a major neurologic disease in the United States. Previous epidemiologic studies have indicated that there is in the Northern Hemisphere a north-south gradient in the rates of multiple sclerosis. From a previous study, certain interesting correlations were noted among military personnel who developed multiple sclerosis, such as an excess of people with higher I.Q.s, with urban residence, with higher socio-economic status and with defective eyes. The recent emphasis on risk of multiple sclerosis among migratory populations is of crucial importance since it must be determined if the causative factors of this disease are more prevalent in northern areas or if there are protective factors or mechanisms operating in southern areas. If clear patterns can be delineated by this study, the results could suggest in which populations we must concentrate in the search for the etiology and prevention of multiple sclerosis.

Proposed Course of Project: This project was initiated in March 1971 and will run for three years.

CONTRACT NARRATIVE
Epidemiology Branch, C&FR, NINDS
Fiscal Year 1971

THE JOHNS HOPKINS UNIVERSITY (PH43-67-1347)

Title: The Epidemiology of Parkinson's Disease

Contractor's Project Director: Abraham M. Lilienfeld, M.D.
Irving I. Kessler, M.D.

Current Annual Level: 0

Objectives: The contractor will: (a) Select a group of hospitals to provide sufficiently large groups of patients with Parkinson's disease; (b) Assemble groups of patients with parkinsonism, Parkinson's disease and similar neurological deficits who have been hospitalized for any reason or seen in any hospital clinic; (c) Assemble matching groups of patients without parkinsonism symptoms who have been hospitalized at the same institutions and who are similar to the cases in age, sex, race, and other pertinent demographic characteristics; (d) Select a probability sample of physicians (neurologists, general practitioners, general surgeons, and internal medicine practitioners) and from them secure a cohort of patients with parkinsonian symptoms who have not been hospitalized; (e) Compare the patients and their matched controls with regard to epidemiologic characteristics of possible relevance to the incidence of or morbidity from Parkinson's disease, including familial mortality experience and smoking histories; (f) compare, within the patient groups, the epidemiologic characteristics of those with specific parkinsonian symptom complexes; (g) Compare the hospitalized and nonhospitalized parkinsonian patients in order to test whether there are methodologic biases in studies restricted to hospitalized patients; (h) Assemble a large group of patients known to have had Parkinson's disease, parkinsonism or similar neurological deficits as the basis for an analysis of the overall and cause specific mortality of the decedents; (i) Reach conclusions as to the adaptability of the case-control method proves to be inadequate, design other more suitable methods for the epidemiological study of Parkinson's disease.

Major Findings: The first phase of the analysis of this study concentrated on previously reported lower rates of Parkinson's disease among smokers than non-smokers. In the population sampled in the present study, this difference was not encountered and in general, patients were less likely to have ever smoked, and smokers tended to smoke less than a comparable group of patients without Parkinson's disease. Further, analysis of the data are currently underway.

Significance to NINDS Program and Biomedical Research: At present, understanding of risk factors and natural history in parkinsonism is limited to a few studies primarily in clinic populations. The extension using a case-control matching could provide important information for this extremely important neurologic condition.

Proposed Course of Project: The project has run for two years. The second year was funded at a reduced rate. Therefore, analysis has been more time-consuming and the final report is not yet available.

CONTRACT NARRATIVE
Epidemiology Branch, C&FR, NINDS
Fiscal Year 1971

THE UNIVERSITY OF CALIFORNIA-BERKELEY (PH43-68-36)

Title: Health Survey of Stateside Guamanians

Contractor's Project Director: Reuel Stallones, M.D.
Dwayne M. Reed, M.D.

Current Annual Level: 0

Objectives: The contractor will: (a) Analyze mortality experience of Guamanian residents of California for the past ten years in comparison to that of California residents generally; (b) Contact adult Guamanians residing in California and enlist the voluntary participation in the survey of approximately 1,000 subjects; (c) Secure family and individual health histories from recruited subjects, and administer a screening examination comprised of: neurologic examination, psychological examination to test for dementia and motivational aspects, EKG, BP, blood tests for cholesterol, triglycerides, uric acid, and glucose; (d) Conduct similar examinations of a matched group of Guamanians residing on Rota Island, Saipan Island, and Guam; (e) Code and process data gathered and analyze results, demonstrating whether or not disease patterns vary between Guamanian residents in the U.S. and the general population, and between Guamanian residents in the U.S. and Guamanian residents in the Pacific.

Major Findings: The investigators documented an unusually high rate of hyperuricemia and hyperglycemia among Chamorros living on Guam, in California, or on Rota. The patterns of diabetes and gout differed to some extent. The major conclusion was that there were no important differences in disease patterns as a result of westernization.

Significance to NINDS Program and Biomedical Research: Establishing a baseline on Guam for metabolic disease and cardiovascular disease as well as some knowledge of psychological patterns and patterns of neurological disease will be invaluable to our studies on Guam. They will also provide important information concerning patterns of disease among migrating populations.

Proposed Course of Project: The project as originally proposed has terminated and a report received. A portion of the funds were unspent and the investigators were authorized to pursue their study in another Micronesian population on Palau. The field work is complete and we have received the final report.

CONTRACT NARRATIVE
Epidemiology Branch, C&FR, NINDS
Fiscal Year 1971

THE MASSACHUSETTS GENERAL HOSPITAL (PH43-68-982)

Title: Screening of Blood and Urine for Abnormal Amino Acid Patterns

Contractor's Project Director: Vivian E. Shih, M.D.

Current Annual Level: 0

Objectives: The contractor will perform cromatographic screening of approximately 1,000 samples each of blood and urine for detection of disorders of amino acid metabolism among residents of Guam and the Trust Territories. Testing will be performed by routine methods developed and utilized in the contractor's laboratory conducting repeat screening on tests of blood or urine samples as needed in order to confirm or nullify the significance of unusual patterns.

Major Findings: No major abnormalities in infants, retarded children or ALS or PD patients were encountered in the survey of blood or urine of 435 patients with various diseases and 574 normal infants and 20 normal adults in Guam and other islands of Micronesia. One girl, apparently healthy but with a history of seizure disorder (apparently a febril convulsion), many years ago, was found to excrete an unknown sulphur amino acid. Other non-specific differences in amino acid patterns were encountered in different populations and were apparently the result of dietary factors in the given communities.

Significance to NINDS Program and Biomedical Research: As the study continues on Guam we become more convinced that metabolic diseases play an important role in causing amyotrophic lateral sclerosis and parkinsonism-dementia. In addition, on many islands in the Trust Territories we notice abnormal genetic patterns of disease which suggest that certain inborn errors of metabolism may exist. Should we identify these specific metabolic errors on Guam or in the other islands we may gain important insights into the cause of the neurologic diseases in Guam and by using population isolates gain information concerning metabolic pathways in a given population which would provide information on metabolic processes in human populations anywhere.

Proposed Course of Project: A final report has been received and a joint publication has been prepared for submission to a Pediatric Journal. Further investigation on the family of the girl with the previously undescribed sulphur amino acid is underway.

CONTRACT NARRATIVE
Epidemiology Branch, C&FR, NINDS
Fiscal Year 1971

THE JOHNS HOPKINS UNIVERSITY (NIH71-2026)

Title: The Study of Regional Differences in Stroke Mortality

Contractor's Project Director: Dean M. Nefzger

Current Annual Level: $21,937

Objectives: The contractor will: (a) Analyze death certificates of all veterans dying in 1967 in Georgia (high mortality area) and five Rocky Mountain States (low mortality area). From the certificates approximately 1,000 certified CVA deaths in each area and 100 randomly selected controls will be chosen for further analysis (2,200 cases total). From these basic data, the frequency of reported CVA among veterans will be compared with male populations in similar areas to determine if the geographic variations reported for civilians occur among veterans; (b) By review of available hospital records and when necessary by physician or family interview, the validity of the diagnosis will be established in order to estimate the relative frequency of mistaken diagnosis or failure to make the diagnosis of CVA; (c) By review of the accumulated information on veterans dying of CVA an estimate of the relative frequency of specific types of CVA will be compiled; (d) All verified stroke deaths and all errors in death certification will be analyzed in terms of geography, age, race, place of residence, marital status, from the point of view of sources of information (competence of certifying individual) and other variables; (e) During this investigation the complete Military and Veterans Administration folders will be reviewed for a subgroup of 50 cases and controls per state in order to evaluate the usefulness of these records in subsequent studies. In addition, all other avenues of ascertainment of a valid rate of CVA among veterans will be explored in order that a definitive study of veterans population be conducted in the future when the great bulk of veterans of the Second World War arrive at the age of high risk for CVA.

Major Findings: A total of approximately 1,000 stroke deaths and 2,800 deaths from other causes have been compiled. The data on these cases are ready for final analysis. A preliminary review of the stroke cases in Georgia which has a high mortality rate from stroke versus the Rocky Mountain States which have a low reported mortality from strokes has revealed surprising and potentially important information. It appears that this wide discrepancy may be purely artifactual and the result of different reporting habits by physicians in the two areas. It is uncommon for stroke to be listed as underlying cause of death in the Rocky Mountain States. This finding is at variance with the Johns Hopkins University Epidemiology of Stroke study which we supported by contract (PH43-66-920).

Significance to NINDS Program and Biomedical Research: The epidemiological patterns of stroke are poorly understood, although it is suspected that there are regional differences throughout the U.S. Any information confirming these differences and indicating a cause for these differences could lead to a better understanding of causation and prevention in this important cause of morbidity and mortality.

Proposed Course of Project: The contract has expired, but final processing of data is not complete. The investigators have received a contract of $21,937 to complete this work. We are awaiting a final report.

Serial No. NDS (CF) - 55 E 201
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Studies on amyotrophic lateral sclerosis/parkinsonism-dementia complex of Guam (ALS-PD)

Previous Serial Number: Same

Principal Investigators: Jacob A. Brody, M.D.
Charles E. Morris
NINDS Research Center
John M. Stanhope, M.D.
NINDS Research Center

Other Investigators: Jose Torres
NINDS Research Center
Francisco Leon Guerrero
NINDS Research Center
Manuel T. Cruz
NINDS Research Center
Olivia Cruz, M.D.
NINDS Research Center
Roswell Eldridge, M.D.

Consultants: Kwang-Ming Chen, M.D.
National Taiwan University, Taipei, Taiwan
Yoshiro Yase, M.D.
Wakayama Medical College, Japan
Leonard T. Kurland, M.D.
The Mayo Clinic, Rochester, Minnesota
Donald W. Mulder, M.D.
The Mayo Clinic, Rochester, Minnesota
Haruo Okazaki, M.D.
The Mayo Clinic, Rochester, Minnesota

Cooperating Units: NINDS Research Center, Agana, Guam
Special Chronic Disease Studies, C&FR, NINDS
Laboratory of Slow, Latent, and Temperate Viruses, C&FR, NINDS
Department of Epidemiology, School of Public Health, University of California, Berkeley
The Mayo Clinic, Rochester, Minnesota
Department of Pathology, Massachusetts General Hospital, Boston
Amino Acid Laboratory, Massachusetts General Hospital, Boston

Department of Neurology, Wakayama Medical College, Wakayama, Japan
Trust Territory Health Office
University of California, Los Angeles
School of Public Health, University of Hawaii, Honolulu
Unit on Neurology, NIMH
Section on Neurology, University of Montreal
Department of Neuropathology, Albert Einstein College of Medicine, New York
Department of Neurology, Neurological Institute of Columbia University, New York
Neuropathology Branch, Armed Forces Institute of Pathology, Washington, D.C.

Man Years:

Total:	1
Professional:	3/4
Other:	1/4

Project Description:

Objectives: To determine the cause of ALS and PD, and to determine the epidemiological, clinical, neuropathological and physiological significance of these diseases and to develop therapeutic approaches to the diseases.

Methods employed: Routine methods for epidemiological, clinical, neuropathological, and neurochemical and therapeutic investigations.

Major findings: As of February 1, 1971, we were following a total of 90 patients. Of these there were 30 confirmed ALS, 13 with suspect ALS, 12 patients with definite PD and 35 with suspect PD. During the calendar year 1970 there were 15 deaths from ALS, 3 deaths from PD, 3 deaths among PD suspects and no deaths among ALS suspects. Of these, autopsies were performed on 17.

We have completed the 5 year updating of the NINDS ALS and PD patients. We have analyzed most of the data and have completed early drafts of manuscripts for publication. The rates of ALS and PD have been declining slightly but apparently not significantly. Age-specific death rates for 1950 - 59 and 1960 - 69 are identical indicating that no cohort phenomenon relative to some form of early common exposure or practice is apparent from these data. This indicates once again that the causes of these diseases are still present on Guam. ALS and PD each caused 10% of all deaths among Chamorros on Guam over age 25 through 1970. Thus 1 in 5 adult Chamorros will die of one of these diseases.

Analysis of offspring of 100 ALS and 100 PD patients and matched controls will be completed shortly upon securing further information on a small residual of people. We hope that analysis of this data will furnish

familial and possibly genetic patterns of the diseases.

Once again we have noted several microfoci of ALS and PD patients in various areas on Guam. Prior to my next trip in August our staff will attempt to identify these foci and map them in order that we may conduct the appropriate field surveys during my stay.

Results of our study of patients with ALS, PD and controls to determine the rate of CNS synthesis of dopamine by administering probenecid and sampling the spinal fluid at appropriate intervals are available. A manuscript has been prepared and accepted for publication.

The data clearly indicate that PD patients synthesize very little dopamine in the CNS while in ALS patients dopamine production is compromised but to a lesser degree. Similar findings among stateside ALS patients were encountered. This is perhaps the most significant finding emanating from the Guam studies. It is the first documentation of an altered CNS metabolic pathway in ALS and may lead to a breakthrough in the understanding of the pathogenesis of this disease.

In order to explore the apparent deficit in dopamine production in the CNS of Guamanian PD patients, ALS patients and some controls, we are studying CNS dopamine synthesis by a new method developed by Dr. Chase and his group in which we sample urine and expired air rather than CSF. Results are not yet available.

In addition to routine case finding and documentation we are continuing trials with L-Dopa. At present of the 8 people in the original trial, 5 are still receiving the drug after 15 months. One patient started on L-Dopa died but we were unable to conduct an autopsy. A paper analyzing our first 6 to 8 month experience with PD patients on L-Dopa has been prepared and submitted for publication. In general, extrapyramidal features in most of the patients improved to some degree on L-Dopa while there was no clear evidence of amelioration of the dementia. Some heightened awareness and interest, however, was observed. Rigidity responded best in our series while tremor also appeared improved by L-Dopa. Bradykenesia was not improved to the degree which we expected, suggesting that part of this feature of the disease may be the result of destruction in non-dopaminergic areas of the brain (frontal lobe). We are following all patients carefully in order to determine if L-Dopa actually affects the natural history and life expectancy in this degenerative fatal disease.

We are planning to initiate treatment trials in other PD patients using a peripheral decarboxylating agent (MD 486-Merck) and much lower doses of L-Dopa. A protocol has been developed for this trial in collaboration with Dr. Thomas Chase, Chief, Section on Neurology, Laboratory of Clinical Sciences, NIMH, and the Merck Pharmaceutical Company.

Significance to biomedical research and the program of the Institute: Almost all ALS patients are now in one of our drug trials. At present 7

patients have been receiving L-Dopa since late October 1970, and 12 patients are receiving isoprinosine since December while 8 patients are receiving placebo. The studies are double-blind and we analyzed our early results. Dr. Chen and Yase re-evaluated all patients and scored their results and our medical staff gave subjective evaluations of each patient's status. Dr. Stanhope and I have the code and we compared results. It is clearly too early to make any definitive statement. Patients are tolerating medication extremely well and are enthusiastic and cooperative in our studies. Our staff has worked very hard and diligently and each patient is seen at least once a week. Because of this heightened interest and attention and our insistence that patients eat at the time they take medication we have introduced a rather interesting potential bias. Patients appear subjectively improved and are eating better than before. We should be able to evaluate this factor over time by comparing patients on drugs and on placebo. The studies will remain double-blind and when Dr. Stanhope leaves, the code and pill dispensing will be supervised by Mrs. Hernandez. We believe our isoprinosine series is now sufficient and new patients will be placed in the L-Dopa (with Mk 486 when available) drug trial.

Dr. Haruo Okazaki, Section of Experimental and Anatomic Pathology of the Mayo Clinic continues his systematic study of the occurrence of neurofibrillary changes in the brains of approximately 100 Guamanians who died of causes other than ALS or PD. Dr. Okazaki visited Guam for 4 days in December as a consultant and discussed relevant matters with our staff and with the pathologists of the Guam Memorial Hospital.

In Guam we have perhaps the highest incidence in the world of motor neuron disease and of a primary CNS degeneration. The documentation of the epidemiological, clinical, and neuropathological aspects of ALS and PD, a major neuromuscular disease and an important primary CNS degeneration have added to the world's knowledge concerning these neurologic diseases. In fields in which there are no known causes and no known cures, data such as these provide one of the most likely avenues for development of concepts and facts which lead to causes and cures. We are also exploiting this unique opportunity to test new drugs of potential benefit to patients and through our studies we have discovered a dopamine deficiency in ALS patients on Guam and in the U.S. that is the first promising lead in the understanding of this disease.

Proposed Course: In addition to pursuing the above studies we are planning the following:

Lymphocyte transformation: These studies have been interrupted because of the increased clinical activities related to the drug studies. Mr. George Nemo is preparing a new protocol and material will be sent to Guam to pursue our investigations of possible immune factors in ALS and PD.

Tissue culture: Some work on tissue culture from fresh brain material continues on Guam and in Bethesda under the supervision of Dr. C. Joseph Gibbs, Head, Laboratory of Slow, Latent and Temperate Virus Infections,

C&FR, NINDS. We hope we will be able to pursue these studies more actively perhaps by future visits to Guam by Dr. Gibbs and Mr. Nemo.

We are awaiting results of the chemical analysis of neurofibriles from Dr. Michael L. Shelanski, Assistant Professor of Neuropathology, Albert Einstein College of Medicine. He received two hemispheres of Chamorro PD patients' brains in whom autopsies were performed within three hours after death and material was frozen in liquid nitrogen. Five brains were sent to Dr. Andre Barbeau, Director, Department of Neurobiology, Clinical Research Institute of Montreal. These include ALS and PD patients and controls and Dr. Barbeau is conducting histo-chemical analysis in order to determine the patterns of dopamine and other substances in various areas of the brains. Material from general autopsies conducted previously and stored as tissue blocks in Bethesda were returned to Dr. Loerzel and Dr. Varona for review.

Honors and Awards: Official Citation of Commendation from Legislature of Guam to NINDS Research Center (Resolution #381 of 10th Guam Legislature)

Publications:

Brody, J.A. and Chen, K.M.: Changing epidemiologic patterns of amyotrophic lateral sclerosis and parkinsonism-dementia on Guam. Motor Neuron Diseases, Grune & Stratton, 1969, pp. 61-79.

Chen, K.M., Brody, J.A., Kurland, L.T. and Elizan, T.S.: Patterns of neurologic diseases on Guam. II. Clinical and genetic aspects. Neurology, 20:954-964, 1970.

Brody, J.A., Hussels, I., Brink, E. and Torres, J.M.: A preliminary report on hereditary blindness among the Pingelapese people of the Eastern Caroline Islands. Lancet, I:1253-1257, 1970.

Brody, J.A.: Phenomenal incidence of amyotrophic lateral sclerosis and parkinsonism-dementia on Guam. Presented at the 97th Annual Meeting of the American Public Health Association, Philadelphia, Pa., November 1969.

Brody, J.A.: Studies of the phenomenally high incidence of amyotrophic lateral sclerosis and parkinsonism-dementia on Guam. Clinical Society and Commissioned Officers Association - Fifth Joint Meeting - March 31 - April 3, 1970, Washington, D.C.

Reed, D., LaBarthe, D. and Stallones, R.: Health effects of westernization and migration among Chamorros. Amer. J. Epid. 92:94-112, 1970.

Brody, J.A., Chase, T.N. and Gordon, E.K.: Depressed monamine catabolite levels in cerebrospinal fluid of patients with parkinsonism-dementia of Guam. New Eng. J. Med. 282:947-950, 1970.

Brody, J.A., Hirano, A. and Scott, R.M.: Recent neuropathologic observations in amyotrophic lateral sclerosis and parkinsonism-dementia of Guam. Neurology. In press.

Chase, T.N., Schnur, J.A., Brody, J.A. and Gordon, E.K.: Parkinsonism-dementia and amyotrophic lateral sclerosis of Guam: Effect of probenecid on monamine catabolite levels in cerebrospinal fluid. Arch. Neurol. In press.

Schnur, J.A., Chase, T.N. and Brody, J.A.: Parkinsonism-dementia of Guam: Treatment with L-Dopa. Neurology. In press.

Serial No. NDS (CF) - 63 E 1103
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Neurological diseases other than ALS/PD on Guam

Previous Serial Number: Same

Principal Investigators: Charles E. Morris, M.D.
NINDS Research Center
John M. Stanhope, M.D.
NINDS Research Center

Other Investigators: Kwang-ming Chen, M.D.
Mayo Clinic
Jacob A. Brody, M.D.
Leonard T. Kurland, M.D.
Mayo Clinic

Cooperating Units: NINDS Research Center, Agana, Guam
Mayo Clinic, Rochester, Minnesota

Man Years:

Total:	1/4
Professional:	3/16
Other:	1/16

Project Description:

Objectives: A survey in 1954 by Donald W. Mulder, M.D. and Leonard T. Kurland, M.D. gave the impression that not only ALS, but also other heredo-familial neurologic disorders seemed unusually prevalent while multiple sclerosis and perhaps CNS tumors are uncommon. The objective of this study is to try to determine the validity of this data and to see if it is related to ALS and PD.

Methods employed: Since the establishment of this Center in 1956, we have occupied a unique position on Guam. It is the only neurological consultation service available to all ethnic groups on Guam and sees most neurological patients at Guam Memorial Hospital and Naval Hospital. Therefore, it is expected that most of the significant neurological cases are eventually brought to our attention. Because of this unique position we hope to determine the frequency of various heredo-familial neurological disorders on the island.

Major findings: During the year 188 patients with neurologic diseases other than ALS and PD were seen and 99 EEG's were performed. From 1960

through 1966, in conjunction with ongoing studies of amyotrophic lateral sclerosis and parkinsonism-dementia on Guam, 1,028 Chamorro patients were referred to our neurologic clinic. In comparison with other populations and particularly that of Rochester, Minnesota, the residents of Guam had higher rates of convulsive disorders, myotonic dystrophy, peroneal muscular atrophy, and hereditary ataxias. There was no indication of an unusual incidence of central nervous system neoplasms, and no cases of progressive muscular dystrophy, myasthenia gravis, or indigenous multiple sclerosis were seen. No patient with proved classic paralysis agitans was observed in the Chamorro population. One sibship of 13 was followed in which 4 patients died of various brain tumors and 2 of acute myelogenic leucemia.

Significance to biomedical research and the program of the Institute: This study adds to the general body of knowledge being collected by the Branch regarding the island of Guam and provides information on diseases possibly related to ALS and PD.

Proposed course: We are expanding studies of neurologic diseases on Guam and the Trust Territories using the same techniques.

Honors and Awards: None

Publications: Chen, K.M., Brody, J.A. and Kurland, L.T.: Patterns of neurologic diseases on Guam, Arch. Neurol. 19:573-578, 1968.

Chen, K.M., Brody, J.A., Kurland, L.T. and Elizan, T.S.: Patterns of neurologic diseases on Guam. II. Clinical and genetic aspects. Neurology, 20:954-964, 1970.

Serial No. NDS (CF) - 66 E 1319
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: A search for automimmune mechanisms in the pathogenesis of chronic neurological diseases by the use of peripheral lymphocytes

Previous Serial Number: Same

Principal Investigators: Jacob A. Brody, M.D.
George Nemo, Ph.D.

Other Investigators: Minnie Toure, Biologist
Gary Cooper, Biologist

Cooperating Unit: None

Man Years:

Total:	1/4
Professional:	1/12
Other:	3/12

Project Description:

Objectives: To study the role of the small lymphocyte in the pathogenesis of neurologic disorders suspected to be of autoimmune etiology.

Methods employed: Peripheral lymphocytes from patients with multiple sclerosis (MS) were challenged in vitro with specific antigens. The incorporation of tritiated thymidine into DNA during the synthetic phase of lymphoblast transformation is used as an indicator of lymphocyte responsiveness. In order to quantitate tritiated thymidine incorporation more accurately, our laboratory has converted from autoradiography to liquid scintillation spectrometry. Liquid scintillation is more sensitive, far less time consuming and subject to a minimal degree of human error.

Major findings: Lymphocytes from normal patients and patients with multiple sclerosis were challenged with brain antigens and cerebrospinal fluid from patients with MS. Basic protein extracted from defatted guinea pig brains was also used as a test antigen. The results show that no significant lymphocyte transformation occurred in the samples tested.

Significance to biomedical research and the program of the Institute: Since it is well established that the lymphocyte is the mediator of cellular immunity, the lack of a significant lymphocyte response as demonstrated in our study casts serious doubt on the hypothesis that MS is an autoimmune disorder.

Proposed course: It may well be that only a small proportion of the total lymphocyte population sampled was sensitive to the antigens tested. The total number of reactive cells may have been too few to elicit a measurable response. If this supposition is indeed correct, then experimental manipulations designed to elevate the response to detectable levels might prove fruitful.

Several reagents are currently being tested for their ability to enhance lymphocyte transformation. Preliminary data indicate that Polyriboinosinic-Polyribocytidylic acid (Poly I:C), a synthetic double-stranded polynucleotide, increases the lymphocyte response to vaccinia virus as much as 40%.

Honors and Awards: None

Publications: Brody, J.A., Harlem, M.M., Plank, C.R. and White, L.R.: Freezing human peripheral lymphocytes and a technique for culture in monolayers. Proc. Soc. Exp. Biol. & Med. 129: 968-972, 1968.

Brody, J.A., Harlem, M.M., Kurtzke, J.F. and White, L.R.: Unsuccessful attempt to induce transformation by cerebrospinal fluid in cultured lymphocytes from multiple sclerosis patients. New Eng. J. Med. 279:202-204, 1968.

Serial No. NDS (CF) - 66 E 1320
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Stateside Guamanian study

Previous Serial Number: Same

Principal Investigator: Jacob A. Brody, M.D.

Other Investigator: None

Cooperating Units: NINDS Research Center, Agana, Guam
School of Public Health, University of California, Berkeley

Man Years:

Total:	1/6
Professional:	1/12
Other:	1/12

Project Description:

Objectives: This study was instituted in July 1966 to determine if ALS and PD occur with the same high frequency among Guamanians who have left Guam. Since the bulk of the stateside Guamanians are in California, efforts have been concentrated there.

Methods employed: A household census was completed in the fall of 1967 which included information on neurologic disease. Names of heads of households were obtained from relatives on Guam, the office of the Guamanian representative to Congress, local Guamanians, social organizations, an eight year old NINDS California Guamanian registry and from other Guamanians already living in this country. Household information was obtained by trained Guamanian interviewers living in California and by personnel from the Branch. Follow-up examination of suspect cases of ALS and PD was conducted by specialist physicians.

Major findings: The ALS rate in California is as high as it is on Guam. Two patients with presumed Parkinson's disease and/or dementia have died and autopsy studies in one are consistent with parkinsonism-dementia although the patient's age is unusually advanced and in other changes were more compatible with paralysis agitans without dementia as seen in the United States. These findings are being included in a report by Brody and Hirano.

Significance to biomedical research and the program of the Institute: The results suggest that a genetic factor and/or early exposure to an environmental factor is responsible for ALS and PD on Guam. The PD patient in California is the only PD patient ever encountered off Guam and the Marianas. The disease is not known to occur in non-Chamorros. Since the rate of PD off Guam is lower than that of ALS it suggests that these diseases are not a spectrum of CNS diseases with a single cause. The patient with paralysis agitans is the first documentation of this disease in a Chamorro. He lived in the United States for 8 years which may be a clue as to the incubation period of paralysis agitans.

Proposed course: It is planned to maintain contact with this migrant population over the years because of the valuable clues we may gain regarding etiology of amyotrophic lateral sclerosis and parkinsonism-dementia as seen on Guam, as well as more general medical, epidemiological and social data as the culturation proceeds. Thus far only Guam-born Chamorros in California are old enough to develop ALS or PD. As the population ages we will attempt to determine if these diseases occur at high rates in United States-born Guamanians.

Honors and Awards: None

Publications:

Eldridge, R., Rosario, J. and Brody, J.A.: Amyotrophic lateral sclerosis and parkinsonism dementia in a migrant population from Guam. (A preliminary report.) In Trans. Amer. Neurol. Ass. 93:204-206, 1968.

Eldridge, R., Ryan, E., Rosario, J. and Brody, J.A.: Amyotrophic lateral sclerosis and parkinsonism dementia in a migrant population from Guam. (A full report.) Neurology, 19:1029-1037, 1969.

Reed, D., LaBarthe, D. and Stallones, R.: Health effects of westernization and migration among Chamorros. Amer. J. Epid. 92:94-112, 1970.

Serial No. NDS (CF) - 66 E 1321
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Japanese encephalitis on Guam

Previous Serial Number: Same

Principal Investigators: Roger Detels, M.D.
Jacob A. Brody, M.D.
C. Joseph Gibbs, Ph.D.
Laboratory of Slow, Latent and Temperate Viruses, NINDS

Other Investigators: None

Cooperating Unit: Laboratory of Slow, Latent and Temperate Viruses, NINDS

Man Years:

Total:	1/3
Professional:	1/6
Other:	1/6

Project Description:

Objectives: A Japanese encephalitis epidemic occurred in 1947 on Guam, but, according to serologic studies, involved only 20% of the population before apparently disappearing from the island. It is the objective of this study to determine if Japanese encephalitis virus (JEV) is persisting on Guam at a low level and to determine why it has not established an epidemic pattern as in Japan, Taiwan and Korea or an endemic pattern as in Malaysia, despite the presence of a suitable vector and reservoir hosts.

Methods employed: Sera will be collected from Guamanians born prior to, during and after the occurrence of the 1947 epidemic and will be analyzed for antibody to JEV and other Group B and Group A arboviruses. Sera will also be collected for antibody screening from animals. Mosquitoes will be collected to determine the types of culicenes present on the island which might act as vectors.

Major findings: Eighteen percent of sera from 498 Guamanians born since 1900 contain hemagglutination inhibition antibodies to JEV. Twenty-one percent born prior to 1950 and 8% born since 1950 have HI antibody to JEV suggesting that there has been Group B arbovirus activity on Guam since 1950. Nonetheless, only 1 of 100 pigs bled had HI antibody to JEV. Culex tritaeniorhynchus, but not Culex annulus, has been identified on the island.

Significance to biomedical research and the program of the Institute: JEV was thought to have disappeared from Guam contrary to the usual pattern. However, the finding of HI antibodies to JEV in 8% of Guamanians born since 1950 but not in pigs when all the known necessary ingredients are present for JEV to be either endemic or epidemic invite investigation to determine the uniqueness of Guam and thus contribute to the knowledge of factors important to the epidemiology of JEV.

Proposed course: Tissue culture neutralization tests will be done on all positive HI sera and an aliquot of HI negative sera using several antigens in addition to JEV. Further sera are being collected from Guamanians born since 1950 for neutralization tests. The C. tritaeniorhynchus from Guam will be subclassified, since Barnet has proposed that only C. tritaeniorhynchus summorosus acts as a vector for JEV.

Honors and Awards: None

Publications: None

Serial No. NDS (CF) - 67 E 1325
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: One year experience of all births on Guam with special reference to diabetic complications

Previous Serial Number: Same

Principal Investigator: Jacob A. Brody, M.D.

Other Investigators: John M. Stanhope, M.D.
NINDS Research Center
Anne Kantor
Office of Biometry

Cooperating Units: NINDS Research Center, Agana, Guam
Office of Biometry, OD, NINDS

Man Years:

Total:	1/4
Professional:	1/12
Other:	1/6

Project Description:

Objectives: The present project was designed to expand the scope of this study by analyzing all births for one year. During the year 1965 there were 2,523 births and we compiled data on date of birth, place of birth, birth weight, birth order, length of gestation, birth defects, age and race of parents, maternal complications such as diabetes, etc.

Methods employed: A study by Yen in 1963-64 revealed an unusually high incidence of abnormal carbohydrate metabolism during pregnancy among the native population of Guam. He also found that obesity and large babies appeared to be a constant finding in mothers with abnormal carbohydrate metabolism and were connected with an increased incidence of maternal and perinatal complications.

Major findings: These data revealed an excess of low weight and high weight babies among diabetic mothers. Differences were not statistically significant and in general infant mortality on Guam is similar to that in the United States.

Significance to biomedical research and the program of the Institute: The data will be of value for public health and anthropological studies.

We will also conduct prospective retrospective studies on the use of birth weight as an indication of diabetes in families and communities.

Proposed course: This study has been terminated.

Honors and Awards: None

Publications: None

Serial No. NDS (CF) - 67 E 1485
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Analyses of abnormal urine and blood amino acids metabolism among Guamanians

Previous Serial Number: Same

Principal Investigators: Jacob A. Brody, M.D.
Vivian Shih, M.D.
Massachusetts General Hospital

Other Investigators: Jose M. Torres
NINDS Research Center
Manuel T. Cruz
NINDS Research Center

Cooperating Units: NINDS Research Center, Agana, Guam
Amino Acid Laboratory, Massachusetts General Hospital, Boston, Massachusetts

Man Years:

Total:	1/6
Professional:	1/12
Other:	1/12

Project Description:

Objectives: Earlier observation indicated that indigenous Guamanians have difficulties in handling protein and carbohydrate. However, the relationship between the observed hyperuricemia and hyperglycemia on Guam and the neurologic manifestations is not clear.

Methods employed: A contract with the Amino Acid Laboratory of the Massachusetts General Hospital for the broad testing for inborn errors of metabolism was secured and blood and urine are being sent to the lab from Guam.

Major findings: No major abnormalities in infants, retarded children and ALS or PD patients were encountered in the survey of blood and urine in 435 patients with various diseases and 574 normal infants and 20 normal adults in Guam and other islands of Micronesia. One girl apparently healthy but with a history of seizure disorder (apparently a febril convulsion) many years ago was found to excrete an unknown sulfur amino acid. We are reviewing her family clinically and collecting appropriate urine samples for confirmation of this finding. No specific changes were found in PD or

ALS patients. B-amino isobutyric aciduria was detected in 56.8% of normal infants. Taurine excretion was prevalent in normal infants on the Caroline Islands; it was probably related to breast-feeding. Cystathioninuria was present in 9 normal infants. We have the impression that the infant population in Guam and other areas have relatively low rates of abnormal amino acids when compared with other populations. This cannot be claimed conclusively because sampling methods differed in our series. The Department of Public Health of the Government of Guam has routinely screened all new borns for PKU for the past 6 years and no case has been encountered.

Significance to biomedical research and the program of the Institute: This study contributed to knowledge of metabolic abnormalities of the Chamorro people of Guam.

Proposed course: Collection of specimens is complete. We will follow up on the one unusual patient referred to above.

Honors and Awards: None

Publications: Shih, V.E., Brink, E.W., Peneva, P. and Brody, J.A.: Blood and urinary amino acid patterns in Guamanians and Micronesians. Amer. J. Dis. Child. In press.

Serial No. NDS (CF) - 67E 1486
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Torsion Dystonia - a clinical and genetic study

Previous Serial Number: Same

Principal Investigator: Roswell Eldridge, M.D.

Other Investigators: Irving S. Cooper, M.D.
St. Barnabas Hospital
Morris B. Gross, M.D.
Hunter College in the Bronx
Wolfgang Zeman, M.D.
University of Indiana
Mary Bazelon Coleman, M.D.
Children's Hospital
Kathy O'Meara

Cooperating Units: Department of Neurologic Surgery, St. Barnabas Hospital, New York
Laboratory of Clinical Science, NIMH

Man Years

Total	1
Professional:	2/3
Other:	1/3

Project Description:

Objectives: Torsion dystonia (TD) comprises a heterogeneous group of conditions characterized by disordered movement. TD may be due to either genetic or environmental factors. The present study has already defined further the nosology of these conditions. Further clinical family studies may suggest the basic defect in each.

Methods employed: Initially, probands with a history of TD selected through 180 neurologic and neurosurgical centers provided the families for study. Recently, physicians and affected individuals themselves have contacted us requesting help. A detailed clinical family history is obtained. The latter stresses geographical origin of ancestral couples. The proband and all available relatives were given physical examinations. Patients from all areas of the U. S. treated by various methods are seen to avoid geographic, ethnic, and therapeutic bias.

Major findings: Clinical, genetic, psychometric and therapeutic aspects of dystonia have been evaluated in more than 200 patients in 130 families. The results have appeared in publications indicated below. Among the conclusions are: at least two hereditary forms of dystonia exist; there is variation in clinical features and course of the hereditary types of dystonia; psychotherapy has a limited role as primary treatment; drugs reported to be helpful in the dystonias generally have been ineffective in most patients over a long period; and recent neurosurgical procedures offer hope.

Significance to biomedical research and the program of the Institute: Elucidation of the fundamental defect in these forms of dystonia will be of practical importance. In addition to suggesting specific treatment, it should be possible to distinguish between the recessive and dominant forms chemically. The application to genetic counselling of such a test is obvious. As in other inborn errors of metabolism, such a study could provide basic, new information about central nervous system physiology.

Parkinson's disease shares certain clinical features with dystonia and is relieved by the same operative procedure so that information gained from the dystonia study may bear on this important problem.

Proposed course: Present efforts in this project are directed toward documentation of all abnormalities in the hereditary dystonias and search for appropriate therapy. In the latter connection we are working with other medical centers including the National Institute of Mental Health, Department of Neurology at Children's Hospital, Neurological Institute of New York City, St. Barnabas and Albert Einstein Hospitals, New York City, University Hospital, Cleveland and University of Montreal, Montreal.

We are pleased with the ground work that has been laid in terms of understanding the nosology and clinical course of the dystonias.

Honors and Awards: None

Publications:

Eldridge, R., Ryan, E., Brody, J.A. and Cooper, I.S.: Dystonia musculorum deformans: Evidence for two hereditary forms. Excerpta Medica International Congress Series No. 175, Progress in Neuro-Genetics, Vol. I of the Proceedings of the Second International Congress of Neuro-Genetics and Neuro-Ophthalmology, Montreal, September 1967. pp. 772-788, 1969.

Eldridge, R., Harlan, A., Cooper, I.S., and Riklan, M.: The Hereditary Torsion Dystonias (Dystonia Musculorum Deformans): Geographical distribution and I.Q. in dominant and recessive forms. In Transactions of the American Neurological Association, 94, 1969.

Eldridge, R., Harlan, A., Cooper, I.S. and Riklan, M.: Superior intelligence in recessively inherited torsion dystonia. The Lancet I:7637, pp. 65-67, 1970.

Eldridge, R., Edgar, A., and Cooper, I.S.: Genetics, Geography and Intelligence in the torsion dystonias. Proceedings of the Second Conference on the Clinical Delineation of Birth Defects, May 1969. The National Foundation-March of Dimes (In press)

Eldridge, R.: The Torsion Dystonias: Literature Review: Genetic and Clinical Studies. In The Torsion Dystonias (Dystonia Musculorum Deformans). Editor, Roswell Eldridge, Neurology suppl. 20:11, Part 2, November 1970.

Eldridge, R. and Koerber, T.: The Torsion Dystonias: Some Genetic and Psychiatric Implications. The Psychiatric Forum, April, 1971.

Serial No. NDS(CF) - 67 E 1487
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Genetic analysis of family data on Guam ALS cases

Previous Serial Number: Same

Principal Investigators: John M. Stanhope, M.D.
NINDS Research Center
Jacob A. Brody, M.D.
Charles E. Morris, M.D.
NINDS Research Center
Roswell Eldridge, M.D.

Other Investigator: Manuel T. Cruz
NINDS Research Center

Cooperation Unit: NINDS Research Center, Agana, Guam

Man Years:

Total:	1/4
Professional:	1/6
Other:	1/12

Project Description:

Objectives: To utilize the accumulation of 20 years of experience for an indepth genetic analysis of pedigree information of Guam ALS.

Methods employed: Pedigrees were developed for the 370 definite cases of Guam ALS by Guamanian practitioners aware of actual biologic parents. Of these, 70 were suitable for segregation analysis. Also examination was made of sibs whose parents were both affected by ALS.

Major findings: The initial data indicate that Guam ALS may be inherited as a simple autosomal recessive trait. In 46 families suitable for test of the autosomal recessive hypothesis, 64 cases were observed while 74 cases would be expected from truncate analysis. The 95% confidence limits for such analysis cover the range from 64 cases to 84 cases so the observed number of cases is compatible at this level of significance although barely so.

Two of 13 over 35 years of age whose parents both had ALS were found to have signs compatible with early ALS. If ALS is recessive all such offspring should eventually be affected.

In testing for the autosomal dominant hypothesis in the 16 families suitable for this analysis, 9 cases were observed, 27 would be expected and 19 to 35 cases would be the range at a 95% limit confidence. Therefore, autosomal dominant inheritance is possible only if one postulates the gene is not penetrant (i.e., there is no expressing of the disease) in 50% to 70% of those carrying it.

Troublesome to any simple mode of inheritance postulated is the 2:1 male to female ration of ALS patients. A finding which may answer this discrepancy and possibly shed light on the basic process of the disease is that in these siblings there is an excess of female deaths under one year of age. In addition, 11 of our ALS or PD patients have only one Guamanian parent. We are also following the offspring of the first 100 ALS and PD patients and controls referred to above.

Significance to biomedical research and the program of the Institute: Obviously establishing a genetic basis for Guam ALS and PD would have far-reaching consequences. The elusive question of the cause of Guam ALS and PD would be answered. A new genetic disease would be added to the expanding catalogue of inherited neurologic diseases.

Proposed course: Complete information is being obtained on pedigrees of other Guam ALS and PD cases so as to increase the sample studied. Detailed study of offspring now at risk of specific mating types is underway.

Honors and Awards: None

Publications: None

Serial No. NDS (CF) - 67 E 1488
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Serological studies of common viruses in cases of multiple sclerosis (MS) and controls

Previous Serial Number: Same

Principal Investigators: Jacob A. Brody, M.D.
John L. Sever, M.D.
Perinatal Research Branch, NINDS
Anne H. Edgar
Jane McNew, R.N.

Other Investigators: Mark Dyken, M.D.
Neurology Department, Indiana University Medical Center
A. Donald Merritt, M.D.
Department of Medical Genetics, Indiana University Medical Center

Cooperating Units: Section of Infectious Diseases, PRB, NINDS
Neurology Department, Indiana University Medical Center, Indianapolis
Department of Medical Genetics, Indiana University Medical Center, Indianapolis
The Wistar Institute, Philadelphia, Pennsylvania

Man Years:

Total:	2 1/6
Professional:	1 1/6
Other:	1

Project Description:

Objectives: To test the hypothesis that MS may be caused by an unusual response to a common virus infection. To search for possible distortions of segregation and association among MS patients, siblings and controls.

Methods employed: MS patients known to the Neurology Department, IUMC and to the Indiana Chapter of the National Multiple Sclerosis Society were contacted and asked to participate. In addition, for each case several controls with similar backgrounds and infectious disease experience were selected. Controls are classmate friends of the patient who grew up in the same community. Siblings of MS patients were also tested. A second sample

of MS patients and siblings was taken from the Washington, D.C. area. Patients and controls answered standard questions regarding infectious disease, environment, course of illness and family history, and blood specimens were taken.

Serological analysis was conducted in the Section of Infectious Diseases, PRB, NINDS using a battery of common virus antigens by hemagglutination and complement fixation methods. Differences between the patient, his sibling and his controls were analyzed.

A portion of frozen serum is being banked to test promising hypotheses in the future.

Major findings: In the Indiana series we found that MS patients have higher titers than matched controls for measles, mumps, influenza C, parainfluenza type 3, varicilla and herpes virus hominus. In no case, however, were titers of MS patients higher than their siblings of the same sex. In the Washington series, female siblings had titers as high as female patients to measles while male siblings titer were lower than those of the patients.

Significance to biomedical research and the program of the Institute: The observation that MS patients do have consistently higher titers against many viruses than controls supports an infectious or immune mechanism as being involved in the etiology of MS. The finding that higher titers also occur in siblings suggests that the phenomenon may be related to a common familial exposure or a familial immunologic defect.

Proposed course: We are now testing gamma globulin levels of these sera in collaboration with Dr. Oldrich Kolar, Department of Neurology, Indiana University Medical Center, and testing the rabies titers in collaboration with Dr. Hilary Koprowski, Director, The Wistar Institute. We hope to extend our studies to the Shetland and Orkney Islands where MS occurs at a rate three times higher than elsewhere in the world.

Honors and Awards: None

Publications: Henson, T.E., Brody, J.A. and Sever, J.L.: Elevated measles antibodies in patients with multiple sclerosis and in their siblings. Presented at the 97th Annual Meeting of the American Public Health Association, Philadelphia, Pa., November 1969.

Henson, T.E., Brody, J.A., Sever, J.L., Dyken, M.L. and Cannon, J.M.: Measles antibodies in patients with multiple sclerosis and their siblings and controls. JAMA, 211:1985, 1970.

Brody, J.A.: Virus antibody titers in multiple sclerosis patients, siblings and controls. (An abstract.) (Presented

at the American Epidemiological Society Meeting in Seattle, Washington, April 1970.)

Brody, J.A., Sever, J.L. and Henson, T.E.: Virus antibodies in the serum of multiple sclerosis patients and matched controls. Neurology, 20:389, 1970. (Presented at the American Academy of Neurology, May 1970. Proceedings to be published.)

Brody, J.A., Sever, J.L. and Henson, T.E.: Virus antibodies in MS patients, siblings and controls. JAMA. In Press.

Serial No. NDS (CF) - 67 E 1489
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Neuropathological studies in veterans dying of ALS who served on Guam

Previous Serial Number: Same

Principal Investigators: Jacob A. Brody, M.D.
R. Michael Scott, M.D.

Other Investigators: Kenneth Earle, M.D.
Armed Forces Institute of Pathology
Asao Hirano, M.D.
Montefiore Hospital
Joseph Seggora, M.D.
Veterans Administration Hospital
F.A. Quadfasel, M.D.
Veterans Administration Central Office

Cooperating Units: Neuropathology Branch, Armed Forces Institute of Pathology, Washington, D.C.
Department of Neuropathology, Montefiore Hospital New York
Veterans Administration Hospital, Boston
Neurology Section, Veterans Administration Central Office, Washington, D.C.

Man Years:

Total:	1/3
Professional:	1/3
Other:	0

Project Description:

Objectives: To determine if ALS in veterans who served on Guam is an acquired disease.

Methods employed: Dr. Hirano has reported characteristic neurofibrillary changes in Guamanian ALS patients, but these changes are not seen in classical stateside ALS. Since a large number of U.S. servicemen were stationed on Guam during World War II, it is possible to examine the CNS of U.S. veterans who died of ALS who spent considerable time on Guam. The question to be answered is whether these men show the characteristic Guam-type neuropathological changes or the classical stateside ALS changes.

Major findings: Brains from three veterans serving on Guam have been collected. In two, there were no neurofibrillary changes, while in one these changes were present, but not in the distribution observed in Guam ALS patients.

Significance to biomedical research and the program of the Institute: These findings are evidence that Guamanian ALS does not result from short term exposure to an environmental agent. However, the brain of a Filipino dying of ALS after being on Guam for many years has shown typical neurofibrillary changes, suggesting that length of exposure to an environmental agent may be an important factor in subsequent development of ALS.

Proposed course: This study is terminated and the data have been written up and accepted for publication.

Honors and Awards: None

Publications: Brody, J.A., Hirano, A. and Scott, R.M.: Recent neuropathologic observations in amyotrophic lateral sclerosis and parkinsonism-dementia of Guam. Neurology. In Press.

Serial No. NDS (CF) - 67 E 1490
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: The application of fluorescent antibody methods to the study of chronic neurological disorders

Previous Serial Number: Same

Principal Investigators: Jacob A. Brody, M.D.
Minnie Toure, Biologist
George Nemo, Ph.D.

Other Investigator: None

Cooperating Unit: None

Man Years:

Total:	1/3
Professional:	1/4
Other:	1/12

Project Description:

Objectives: To employ fluorescent antibody techniques using frozen sections of CNS tissue for detection of viral antibody.

Methods employed: The two main staining techniques employed in fluorescent microscopy, the direct and indirect methods will be employed. Frozen CNS tissue sections 4-5u will be prepared using a microtome in a refrigerated cryostat.

Major findings: None as yet.

Significance to biomedical research and the program of the Institute: Fluorescent antibody methods are applicable to the study of chronic neurological disorders thought to be of autoantibodies as well as sites of delayed-type hypersensitivity reactions. A search for viral antigens and sites of viral replication in CNS tissue is also made possible using this technique.

Proposed course: To be continued.

Honors and Awards: None

Publications: None

Serial No. NDS (CF) - 67 E 1496
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Sequelae of CNS diseases in childhood

Previous Serial Number: Same

Principal Investigator: Jacob A. Brody, M.D.

Other Investigators: Estelle Kornhauser, R.N.
Otis D. Turner

Cooperating Units: Office of Biometry, OD, NINDS
Children's Hospital, Washington, D.C.

Man Years:

Total:	2/3
Professional:	1/3
Other:	1/3

Project Description:

Objectives: To determine if infection with viruses capable of penetrating the CNS cause permanent neurologic sequelae, particularly in those cases in which infection occurred under the age of two years.

Methods employed: As the result of previous studies we have decided to improve our methods and techniques by investigating populations in which known infections from encephalogenic viruses occurred.

Major findings: Although we have attempted to secure the necessary populations of children under age 1 in New York, Panama, St. Louis and Chicago, we have not yet encountered a situation suitable for testing our hypothesis.

Significance to biomedical research and the program of the Institute: Although it is widely believed that infections of the CNS early in childhood produce brain damage, there are no definite patterns of brain damage or specific diseases which commonly are associated with brain damage. Documentation of specific viral-tropisms to learning and performance would be a major contribution to understanding and preventing minimal and major brain damage.

Proposed course: Well documented arbovirus outbreaks have been observed by MARU, NIAID and several hundred measles patients who were infected under

age 1 are known in Chicago (Kenrad E. Nelson, M.D., Assistant Professor of Preventive Medicine, University of Illinois College of Medicine, Municipal Contagious Disease Hospital, Chicago). We plan to study these occurrences systematically.

Honors and Awards: None

Publications: Brody, J.A. and Wilner, E.: Measles, minor neurologic signs and intelligence. Developmental Med. and Child. Neurol. 11: 449-454, 1969.

Serial No. NDS (CF) - 67 E 1497
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Guillain-Barre (GB) - Bell's palsy (BP) study

Previous Serial Number: Same

Principal Investigator: Paul M. Hoffman, M.D.

Other Investigators: Lon R. White, M.D.
Jacob A. Brody, M.D.
George Nemo, Ph.D.

Cooperating Unit: Perinatal Branch, NINDS

Man Years:

Total:	1/4
Professional:	1/4
Other:	0

Project Description:

Objectives: To determine if GB and BP are caused by abnormal immunological host responses to common viruses. Since during the immune response the virus may not be recoverable from the patient we are attempting to isolate the virus from materials collected from household contacts.

Methods employed: Personal contact was made with the neurology residents or senior medical residents in a number of local hospitals and letters were sent to the practicing neurologists and neurosurgeons in the local area, so that prompt notification might be obtained whenever a case of GB or BP appeared. When notification of a case was obtained, we contacted the patient to learn of any contacts (preferably children) in the household. If there were contacts we proceeded to collect blood, throat swabs, and rectal swabs from the patient and his contacts. Blood specimens were centrifuged and serum stored at -20°C. Throat swabs were placed in Hank's medium and PPLO medium; rectal swabs were placed in Hank's medium and vials were stored at -70°C.

When sufficient numbers of specimens had been collected it was planned to forward them for serological studies and attempts at virus isolation. Records were kept of patient's history and neurological status and of contact's exposure to infections. Lymphocyte transformation studies were also performed on the subject. The significance of serum factors depressing lymphocyte transformation and the interaction of the isolated virus with the patients lymphocytes as well as the reaction of the lymphocytes to

peripheral nerve will be evaluated. In addition careful case-control studies of epidemiologic factors will be conducted in hope of encountering precipitating factors in these diseases.

Major findings: None

Significance to biomedical research and the program of the Institute: This disease has been associated with several abnormalities that suggest auto-immunity as an etiology. Our work with the hypothesis of a viral infection triggering an abnormal immune response is in keeping with our knowledge of SSPE and possibly MS.

Proposed course: This project will continue.

Honors and Awards: None

Publications: None

Serial No. NDS (CF) - 68 E 1594
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Phenothiazine-induced neurological effects: A study among twins

Previous Serial Number: Same

Principal Investigators: James A. Schnur, M.D.
Jacob A. Brody, M.D.
Dean F. Young, M.D.

Other Investigators: John D. Rainer, M.D.
New York State Psychiatric Institute

Cooperating Units: National Institute of Child Health and Human Development, Children's Diagnostic and Study Branch
National Institute of Mental Health
Section on Twin & Sibling Studies, Adult Psychiatry Branch
National Academy of Science, National Research Council
Columbia University, New York State Psychiatric Institute
Spring Grove State Hospital, Catonsville, Maryland

Man Years:

Total:	1/2
Professional:	1/3
Other:	1/6

Project Description:

Objectives: The objective of this study is to assess whether the specific types of neurological side reaction induced by phenothiazine drugs are influenced by genetic factors.

Methods employed: A preliminary study among 46 female geriatric patients on long-term phenothiazine treatment revealed 33% had dyskinetic reactions and 13%, Parkinson-like reactions. The subjects for this study are twin pairs, concordant for the same psychiatric diagnosis, who have been on chronic phenothiazine therapy. Zygosity of the twin pairs is determined by history, appearance, and extensive blood typing. A single neurological examination was conducted on each pair to determine the patterns of neurological reactions. By comparing the patterns of reactions in monozygotic with those of fraternal twins, we can employ the usual methods of analysis to

determine the relative importance of genetic factors in the manifestation of extrapyramidal signs resulting from phenothiazine induction.

Major findings: Six pairs of twin patients, four monozygotic and two dizygotic, on long time-high dosage phenothiazine treatment have been examined. The results indicated that the specific type of neurological side effect is not primarily determined by genetic factors, since identical twins may develop distinctly different patterns of reaction.

Significance to biomedical research and the program of the Institute: This work may help elucidate the presence or absence of a genetic contribution to the occurrence of the important neurological side reactions to phenothiazine drugs.

Proposed course: We propose to add other twin pairs to the series, and analyze the data.

Honors and Awards: None

Publications: None

Serial No. NDS (CF) - 68 E 1595
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: An evaluation of the effect of successful thalamic surgery on the progress of unilateral Parkinson's disease

Previous Serial Number: Same

Principal Investigators: R. Michael Scott, M.D.
Jacob A. Brody, M.D.
Joyce M. Cannon, R.N.

Other Investigators: Irving S. Cooper, M.D.
St. Barnabas Hospital
Robert S. Schwab, M.D.
Massachusetts General Hospital

Cooperating Units: Department of Neurosurgery, St. Barnabas Hospital, Bronx, New York
Department of Neurology, Massachusetts General Hospital, Boston, Massachusetts

Man Years:

Total:	1
Professional:	11/12
Other:	1/12

Project Description:

Objectives: To evaluate Dr. Cooper's hypothesis that in patients with unilateral Parkinson's disease, thalamic surgery which succeeds in permanently abolishing the tremor and rigidity on the involved side will either stop or markedly delay the appearance of the symptoms on the other side of the body; and to determine the natural history of unilateral Parkinson's disease.

Methods employed: The charts of 1,700 consecutive thalamic surgical cases from January 1963 through September 1964 at St. Barnabas Hospital were reviewed to select 100 patients who came to surgery with symptoms of tremor and rigidity confined to one side of the body. Seventy-two patients were eventually contacted and examined. Two unoperated groups were studied. The first of these consisted of 15 patients who presented to Dr. Cooper with unilateral signs, who were accepted for surgery, but for various reasons were not operated upon. The second control group consisted of 20 patients seen by Dr. Schwab in Boston and subsequently treated medically.

Major findings: Successful thalamic surgery does not affect the progress of tremor and rigidity to the extremities of the opposite side of the body. The rate and frequency of spread in unoperated and operated patients were strikingly similar. Certain of these patients had a form of Parkinson's disease characterized by unilateral tremor and rigidity of long duration. The average age of onset of these patients was earlier than that of "classical" Parkinson's disease patients, and they had a higher frequency of encephalitis or severe febrile illness prior to the onset of their illness. Thalamotomy was often extremely effective in these patients.

Significance to biomedical research and the program of the Institute: This study further defines the role of surgery in Parkinson's disease. It emphasizes that thalamotomy does not alter the course of progressive Parkinson's disease, but can restore to normalcy certain patients with the benign unilateral syndrome. In addition, it suggests ways in which patients with the benign syndrome might be identified.

Proposed course: Publications have been prepared and the project terminated.

Honors and Awards: None

Publications:

Cooper, I.S., and Scott, R.M.: The clinical and physiological implications of 10 year cure of unilateral movement disorders by thalamic surgery. In: Proceedings of the IIIrd Parkinson's Symposium. Edinburgh, Livingstone, (in press).

Scott, R.M., Brody, J.A., and Cooper, I.S.: The effect of thalamotomy on the progress of unilateral Parkinson's disease. J. Neurosurg. 32:286-288, 1970.

Scott, R.M., Brody, J.A., Schwab, R.S., Cooper, I.S.: Progression of unilateral tremor and regidity in Parkinson's disease. Neurology, 20:710-714, 1970.

Scott, R.M., and Brody, J.A.: Benign early-onset Parkinson's disease: A syndrome distinct from classic postencephalitic parkinsonism. (Presented at American Academy of Neurology, May 1970). Neurology, 21:366-368, 1971

Serial No. NDS (CF) - 68 E 1597
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Neurologic diseases in the Trust Territories and other Pacific areas

Previous Serial Number: Same

Principal Investigators: Jacob A. Brody, M.D.
James A. Schnur, M.D.
NINDS Research Center

Other Investigators: Manuel Cruz
NINDS Research Center
Jose Torres
NINDS Research Center
Francisco Leon Guerrero
NINDS Research Center

Cooperating Units: Ophthalmology Branch, NINDS
Department of Public Health, Trust Territory of the Pacific Islands, Saipan, Marianas Islands
Population Genetics Laboratory, University of Hawaii, Honolulu, Hawaii

Man Years:

Total:	2/3
Professional:	1/3
Other:	1/3

Project Description:

Objectives: To investigate neurologic illness occurring in the Trust Territories and the Pacific area. Last year this involved the study of leprosy patients in New Caledonia. This year it involved the study of congenital blindness among the people of Pingelap.

Methods employed: A field trip was made to Ponape to examine the Pingelapese people in order to document the nature of their eye disease and their mode of inheritance and epidemiologic patterns which could suggest both genetic and environmental factors.

Major findings: The congenital eye disease is apparently a form of achromatopsia characterized by nystagmus, blinking, photophobia, blindness, and impairment of color vision. The development of cataracts usually within

the first 5 to 10 years of life and frequent appearance of high myopia. The mode of inheritance appears to be recessive and nonsex-linked. Our most significant finding was that the apparent trend noted in 1969 that children in age group 0 - 4 had a significantly lower rate of the congenital eye disease was not sustained. In a follow-up trip in 1971 we observed that two new patients were born with the disease since 1969 bringing the rate in the current population age 0 - 4 up into the range of those rates for older populations. Six patients were brought to the Ophthalmology Branch, NINDS for study. The diagnosis appears to be achromatopsia with unexplained high myopia.

Significance to biomedical research and the program of the Institute: The group of diseases which are included under tapetoretinal degenerations are poorly understood. By having a population isolate with a phenomenally high rate (10% of the population is blind) will give a unique opportunity for studying the full range of manifestations of this entity as the expression of a single abnormal gene. In addition, hopefully, a mechanism for prevention will develop.

Proposed course: We will continue to follow this population and other populations as they become known which are of potential medical interest.

Honors and Awards: None

Publications: Brody, J.A., Hussels, I., Brink, E. and Torres, J.M.: A preliminary report on hereditary blindness among the Pingelapese people of the Eastern Caroline Islands. Lancet, I:1253-1257, 1970.

Serial No. NDS (CF) - 68 E 1598
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Epilepsy on Guam

Previous Serial Number: Same

Principal Investigator: John M. Stanhope, M.D.
NINDS Research Center

Other Investigators: Jacob A. Brody, M.D.
Manuel Cruz
NINDS Research Center
Jose Torres
NINDS Research Center
Francisco Leon Guerrero
NINDS Research Center

Cooperating Unit: NINDS Research Center, Agana, Guam

Man Years:

Total:	1/2
Professional:	1/4
Other:	1/4

Project Description:

Objectives: To determine the incidence and prevalence of epilepsy on Guam. To investigate methods for field studies of epilepsy. To determine if previous reports of unusually high incidence of convulsive disorders on Guam are accurate.

Methods employed: We are testing four basic approaches: (1) We are following-up a 1962 survey of convulsive disorders in Umatac and Merizo to determine the outcome of those children known to have had febrile convulsions 6 to 8 years ago; (2) to determine the true incidence and prevalence in a sample population we are doing a house to house survey in the villages of Talofofo, Merizo, and Yona; (3) as referral neurologists on Guam we are updating all previous referrals of convulsive disorders to us and establishing a registry. To add to this registry we are contacting all medical and paramedical personnel on Guam to discover new cases. This registry will be permanent and permit us to conduct studies in the Guam population; (4) to further elaborate on methods for acquiring information we are following-up all births on Guam in 1958 and 1963 throughout the entire island to determine the rates of convulsive disorders in these preselected populations.

Major findings: We have already published that the rates of "true" epilepsy and of febrile convulsions are higher on Guam than elsewhere. We are completing the first three phases of the Epilepsy Study outlined in previous reports. We have the information on the rate of "true" epilepsy (the fourth part of our program) and have established a permanent Epilepsy Registry on Guam. There seems little doubt that the rates are high on Guam, but the reasons are not clear. Available data are now being analyzed by Dr. Stanhope.

Significance to biomedical research and the program of the Institute: Further studies of epilepsy in a well-defined and accessible population will add to the understanding of this disease and contribute new information concerning epilepsy in a tropical environment. It will also yield important information on different survey techniques and their relative accuracy.

Proposed course: The analysis will be completed as soon as possible and more detailed studies of epilepsy will develop from our basic information.

Honors and Awards: None

Publications: None

Serial No. NDS (CF) - 68 E 1605
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Phenothiazine-induced parkinsonism in white and Negro patients with nonorganic psychoses

Previous Serial Number: Same

Principal Investigators: James A. Schnur, M.D.
NINDS Research Center
R. Michael Scott, M.D.

Other Investigators: Jacob A. Brody, M.D.

Cooperating Units: Spring Grove State Hospital, Catonsville, Maryland
Crownsville State Hospital, Crownsville, Maryland

Man Years:

Total:	2/3
Professional:	1/2
Other:	1/6

Project Description:

Objectives: To determine whether increased skin pigmentation is associated with decreased prevalence of phenothiazine-induced Parkinson-like syndromes.

Methods employed: This project was originally designed to investigate influence of skin pigmentation on the prevalence of naturally occurring Parkinson's disease by use of a questionnaire which was to be mailed to a sample of American physicians. A pilot study, however, showed this approach to be impractical, and therefore, it was abandoned. In view of the possible relationship between drug-induced and naturally occurring parkinsonism, we then decide to study comparable white and Negro populations who were on treatment with phenothiazines. Thus far, we have examined approximately 75 patients in each group, samples sufficient in number for a comparative analysis.

Major findings: Our initial results suggest that there is no difference in the prevalence of phenothiazine-induced parkinsonism between the two populations surveyed.

Significance to biomedical research and the program of the Institute: To further define the relationship between melanogenesis in the skin and in the pigmented nuclei of the basal ganglia.

Proposed course: Analysis of data is now in progress.

Honors and Awards: None

Publications: Brody, J.A.: Genetic considerations in Parkinson's disease. Presented at the Laurentian L-Dopa Conference, Montreal, November 1969. In: L-Dopa and Parkinsonism, Edited by Andre Barbeau and Fletcher McDowell, F.A. Davis Co., Phila., 1970, pp. 27-30.

Serial No. NDS (CF) - 69 E 1774
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Neurologic signs and symptoms associated with malabsorption

Previous Serial Number: Same

Principal Investigators: Paul M. Hoffman, M.D.
Jacob A. Brody, M.D.
Anne H. Edgar

Other Investigator: None

Cooperating Units: Central Veterans Administration Authority, Washington, D.C.
Veterans Administration Hospital, Atlanta, Georgia
Veterans Administration Hospital, Long Beach, California
Veterans Administration Hospital, Durham, North Carolina
Veterans Administration Hospital, Los Angeles, California

Man Years:

Total:	1 1/2
Professional:	1 1/2
Other:	0

Project Description:

Objectives: To determine if both clinical and subclinical malabsorption is associated with a high incidence of neurologic signs and symptoms.

Methods employed: The Cooperative Veterans Administration Retrospective Study of surgery for peptic ulcer served as the source for patients in this study. A review of abstracts from this study showed that a given hospital was more likely to have done surgical procedure for the majority of its cases then another. For this reason, patients with 3 different surgical procedures performed at 4 hospitals were chosen. A fourth group of patients who had simple closures of perforated ulcers were selected from all 4 hospitals. Patients who had a subtotal gastrectomy with a Billroth II reanastomosis were selected from the Durham Veterans Hospital, patients who had a hemi-gastrectomy and vagotomy were selected from the Atlanta Veterans Hospital, and patients who had a vagotomy and pyloroplasty were selected from the Long Beach and Wadsworth Veterans Hospital. In order to insure that this population would be in the most susceptible age group, patients between the ages of 30 and 80 who had had surgery between 1952 and 1957 were selected from abstracts and hospital records where available. Patients were not

with neurologic complications, any systemic disease with known neurologic complications, or a history of neurologic disease prior to their ulcer surgery. Patients were also excluded from the study if a revision of their original ulcer surgery or subsequent surgery for a recurrent ulcer had been performed. No attempt was made in this study to analyze the causes of death or those who died since surgery. A mortality study of the 2,800 original cases which includes this sample has also been undertaken. All patients included in the study had letters sent to their last known address asking them to report on one of several days to the outpatient clinic of the hospital where their surgery was performed. A complete history with special attention on the neurologic and gastrointestinal systems as well as a complete neurologic examination was performed on all patients by one of us (PMH) and a random sample of all patients as well as all patients with abnormal findings were examined by a staff neurologist.

Major findings: No cases of motor neuron disease have been found in the examined group. A large number of cases of peripheral neuropathy were identified in the vagotomy and hemi-gastrectomy group. Only two cases were seen in the vagotomy and pyloroplasty group. Through evaluation of all patients with unexplained neurologic disorders in the Atlanta group showed that malabsorption, poor nutrition, chronic alcohol intake, and weight loss was all more prevalent in this group than in those without neurologic findings. In reviewing 500 death certificates of the original study group we encountered 2 patients with multiple sclerosis, 2 with Parkinson's disease and 1 with amyotrophic lateral sclerosis.

Significance to biomedical research and the program of the Institute: There have been many clinical reports of cases of malabsorption who have shown signs and symptoms of nervous system disease. There have also been scattered reports in literature of patients who are known to have motor neuron disease, who had a history of having had a Billroth II type of gastric surgery performed many years prior to the onset of their disease. There has never been, however, a matched, controlled population study of the association of these two abnormalities. If this association is valid then further study into the mechanism of absorption of essential nutrients and their incorporation into nervous tissue may be a meaningful approach to the study of chronic neurologic disease.

Proposed course: This project will continue with emphasis on causes of death within this population.

Honors and Awards: None

Publications: None

Serial No. NDS (CF) - 69 E 1775
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Comparison of MS among Irish and Italian immigrants in Boston

Previous Serial Number: Same

Principal Investigators: Roger Detels, M.D.
Jacob A. Brody, M.D.

Other Investigator: None

Cooperating Unit: None

Man Years:

Total:	11/12
Professional:	1/6
Other:	3/4

Project Description:

Objectives: To determine if people migrating from an area of low multiple sclerosis incidence to an area of high multiple sclerosis incidence retain the low rate of the country of origin or acquire the high rate of the area to which they migrated.

Methods employed: Boston, a city of 2 1/2 million, has a large Irish and Italian population, the majority of which migrated about the turn of the century. Thus, individuals in these groups dying of multiple sclerosis would be expected to have died between 1920 and 1940. During these years an estimated 5,000 deaths occurred each year among the Irish and the Italians in Boston. Since these groups would use the same medical facilities it is possible to do a comparative study of the rates of multiple sclerosis between them. Italy is an area of low multiple sclerosis incidence while Ireland and Boston have high rates. Fatality rates for Irish and Italian immigrants and first generation Americans of Italian and Irish background can be determined by a review of death certificates during this period. As a further control the ratio of multiple sclerosis to amyotrophic lateral sclerosis deaths can be compared since amyotrophic lateral sclerosis rates are considered to be independent of latitude.

Death certificates for the period 1920 to 1940 from the city of Boston will be reviewed for all deaths due to multiple sclerosis and amyotrophic lateral sclerosis and for all deaths occurring among Italian and Irish

immigrants and first generation Americans of these descents. Information will also be obtained for years of residence in the United States and in the Boston area.

Major findings: Preliminary analysis revealed that the Boston population is apparently not suitable for this study.

Significance to biomedical research and the program of the Institute: It is known that populations migrating from high incidence areas of multiple sclerosis to low incidence areas, retain the high rate of the country of origin suggesting that events early in youth (possible infectious) cause multiple sclerosis. This study will provide the corollary data about migration from low risk areas to high risk areas and about multiple sclerosis rates among first generation migrants.

Proposed course: This study will be terminated.

Honors and Awards: None

Publications: None

Serial No. NDS (CF) - 69 E 1776
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1969 through June 30, 1970

Project Title: Cerebrospinal fluid amines in drug-induced extrapyramidal parkinsonism-like disorders.

Previous Serial Number: Same

Principal Investigator: James A. Schnur, M.D.

Other Investigator: Thomas N. Chase, M.D.
NIMH

Cooperating Units: Unit on Neurology, NIMH, Spring Grove State Hospital, Catonsville, Maryland
Crownsville State Hospital, Crownsville, Maryland

Man Years:

Total:	2/3
Professional:	1/2
Other:	1/6

Project Description:

Objectives: To study central nervous system amine metabolism in patients with drug-induced extrapyramidal disorders.

Methods employed: Spinal fluids of clinically defined cases of drug-induced extrapyramidal disorders are analyzed by conventional biochemical methods for amine metabolites.

Major findings: Levels of HVA and 5-HIAA in lumbar CSF were measured in 20 female psychiatric patients. Those receiving long-term treatment with antipsychotic drugs who remained free of extrapyramidal dysfunction had substantially higher concentrations of these monoamine catabolites than patients not taking these drugs. Individuals who developed parkinsonian or dyskinetic signs while receiving antipsychotic agents appeared to have significantly lower concentrations of both HVA and 5-HIAA than patients not manifesting these disorders despite similar drug exposure.

Significance to biomedical research and the program of the Institute: The foregoing observations support the hypothesis that the compensatory acceleration of cerebral monoamine metabolism induced by antipsychotic drugs may be impaired in patients who develop extrapyramidal dysfunction and this is the mechanism of acquisition of this side affect.

Proposed course: This phase of the study is completed and project terminated.

Honors and Awards: None

Publications: Chase, T.N., Schnur, J.A. and Gordon, E.K.: Cerebrospinal fluid monoamine catabolites in drug-induced extrapyramidal disorders. Neuropharmacology, 9:265-268, 1970.

Serial No. NDS (CF) - 69 E 1777
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: ALS among non-Chamorros after residence on Guam

Previous Serial Number: Same

Principal Investigator: Jacob A. Brody, M.D.

Other Investigator: Estelle Kornhauser, R.N.

Cooperating Unit: Bureau of Data Processing and Accounts, Social Security Administration, DHEW

Man Years:

Total:	1/2
Professional:	5/12
Others:	1/2

Project Description:

Objectives: This project was developed to determine if prolonged exposure (over one year) to the environment of Guam increases the likelihood of the development of ALS among statesiders.

Methods employed: Through various workers in the Department of Defense we were put in contact with several construction companies which had maintained large staffs of statesiders on the island of Guam after World War II. These companies were asked to supply us with the names, birthdates, and social security numbers of all personnel employed on Guam and from these lists we selected only those workers who had spent more than one year on Guam. This was a group of approximately 12,000 individuals. The Social Security Administration searched its records to determine which of these workers had died and where they had died. We are contacting the individual states to obtain the death certificates of the deceased workers and determine the cause of their death.

Major findings: Of the approximately 12,000 cards submitted to the Social Security Administration, 450 were found to contain incorrect information making follow-up impossible. 7,730 were considered as representing individuals still alive, and the remainder (4,000) were identified as to place of death. The names were submitted to the individual states and death certificates requested. About 80% of the requested certificates have been returned to date. Preliminary analysis indicates that there was no excess of ALS in this group.

Significance to biomedical research and the program of the Institute: Although results on this study are far from complete, the initial trend is that the rate of ALS among statesiders who have spent considerable time on Guam remains the same as that for statesiders who have never spent time in that environment. If this trend is borne out by subsequent findings, it would suggest that the environmental factors on Guam are less likely to be responsible for high rate of ALS among its native population.

Proposed course: The study will be completed by submitting to Social Security approximately 600 punch cards in order to search for death certificates we were unable to locate through the individual states. These cards will then be coded and searched for possible ALS deaths.

Honors and Awards: None

Publications: None

Serial No. NDS (CF) - 69 E 1778
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Familial cortical cerebellar degeneration

Previous Serial Number: Same

Principal Investigators: Paul M. Hoffman, M.D.
William H. Stuart, M.D.
Kenneth Earle, M.D.

Other Investigators: Joyce M. Cannon, R.N.
Anne Harlan Edgar

Cooperating Unit: Armed Forces Institute of Pathology

Man Years:

Total:	1/2
Professional:	1/3
Other:	1/6

Project Description:

Objectives: To determine the genetic pattern and the incidence of cortical cerebellar degeneration in a family in the northern Georgia section of the Blue Ridge Mountains.

Methods employed: A complete family history and a family tree were obtained from the proband and his family who are now living in Hiawasee, Georgia. Affected members were examined as well as those who were at risk but who have not shown the signs and symptoms of the disease. Hospital records and autopsy material, when available, was collected. An autopsy was performed on the index case.

Major findings: The proband is an 80-year-old white male who demonstrated abnormal finger to nose and heel to shin tests as well as inability to maintain his balance, and persistent truncal titubation. He also exhibited scanning-type of cerebellar speech for the last two years. There was no evidence of long-track involvement or of abnormalities in position or vibratory sensation. The reflexes were described as normal. Further study of other affected members showed that the symptoms of ataxia, scanning spastic speech, and mild upper extremity involvement were constant throughout the family. Six affected members in two generations were examined. The neuropathology consisted of atrophy of the superior and anterior cerebellar cortex with secondary olivary degeneration. The disease was transmitted as an autosomal dominant.

Significance to biomedical research and the program of the Institute: The hereditary cerebellar and spinocerebellar degeneration have been described as unique syndromes. Presently, however, it has been observed that symptoms within a family have been very variable. This family has similar symptoms in all affected members. Interest has now been shown in treating various types of spinocerebellar and cerebellar disorders with L-Dopa. The well-defined hereditary syndromes offer the greatest hope of finding an enzyme deficiency that might result in disordered biogenic amine metabolism.

Proposed course: A report has been accepted for publication and this study was terminated.

Honors and Awards: None

Publications: Hoffman, P.M., Stuart, W.H., Earle, K.W.,and Brody, J.A.: Hereditary cerebello-olivary degeneration of late onset. Neurology, 20:400, April 1970. (Presented at the American Academy of Neurology, May 1, 1970.)

Serial No. NDS (CF) - 69 E 1779
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: The epidemiology of motor neuron disease in the United States

Previous Serial Number: Same

Principal Investigators: Paul M. Hoffman, M.D.
Jacob A. Brody, M.D.

Other Investigator: Joyce M. Cannon, R.N.

Cooperating Units: Bureau of Disability Insurance, Social Security Administration
North Carolina State Department of Public Health
University of North Carolina School of Medicine
Duke University School of Medicine
Charlotte Memorial Hospital

Man Years:

Total:	11/12
Professional:	10/12
Other:	1/12

Project Description:

Objectives: To determine if the diagnoses on death certificates are an adequate reflection of the incidence of motor neuron disease in the United States.

Methods employed: Hospital records were reviewed on those patients who had a diagnosis of motor neuron disease in years 1958 through 1962 at the three North Carolina Hospitals. Death certificates were then obtained on those patients who had died in the state of North Carolina. Those patients who are still living were followed-up through the County Public Health Departments within the state of North Carolina and information was obtained on these patients.

Major findings: Of the death certificates obtained, the diagnosis of motor neuron disease appeared on 72 percent. Of these 70% were certified at death as having ALS. MS was certified in 10% of these patients, suggesting a possible systematic error of overdiagnosis of MS at death and underreporting of ALS.

Significance to biomedical research and the program of the Institute: Most of the estimates of the prevalence of motor neuron disease are based on mortality reporting. Our study cast doubt as to the validity of this technique.

Proposed course: A report has been prepared and accepted for publication. The project was terminated.

Honors and Awards: None

Publications: Hoffman, P.M. and Brody, J.A.: The accuracy of mortality statistics in clinically proven amyotrophic lateral sclerosis. Trans. Amer. Neurol. Ass. 95:261-263, 1970.

Hoffman, P.M. and Brody, J.A.: The reliability of mortality statistics for amyotrophic lateral sclerosis. J. Chron. Dis. 23, 1971.

Serial No. NDS (CF) 69 E 1780
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Familial bilateral acoustic neuroma

Previous Serial Number: Same

Principal Investigator: Roswell Eldridge, M. D.

Other Investigators: Dean F. Young, M.D.
New York, New York
Henry Hood, M.D.
Danville, Pennsylvania
W. J. Gardner, M.D.
Cleveland, Ohio
George T. Nager, M.D.
Johns Hopkins Hospital
Frank H. DeLand, M.D.
Johns Hopkins Hospital

Cooperating Units: Department of Otolaryngology,
Department of Radiology,
Department of Neurosurgery,
Geisinger Medical Center
Danville, Pennsylvania
Johns Hopkins Hospital
Baltimore, Maryland

Man Years:

Total:	2
Professional:	1
Other:	1

Project Description:

Objectives: To perform genetic, clinical and physiologic studies of a large family with hereditary bilateral acoustic neuroma.

To clarify the relationship of this trait to other disorders with acoustic neuromas such as neurofibromatosis.

Methods employed: Field studies were conducted in evaluating family members. On those seen personally, physical examinations were performed, stressing neurological and skin examinations. In addition, audiometric examinations including air and bone conduction and caloric examinations were conducted in the field.

Genealogic information was obtained from family members, family records, D.A.R. records, state and military records, census recordings. Medical history was obtained from family members, hospital and physician records, occasionally from school records or military records.

On select patients extensive outpatient studies have included audiometric and vestibular testing, complete ENT and neurologic examinations, skull x-rays and brain scans using radioactive techniques. These were undertaken in cooperation with the Departments of Neurology, Radiology and Otolaryngology of Johns Hopkins Hospital.

Major findings: The study of this, the largest kindred with hereditary neoplasm yet reported, has generated information which has been particularly useful to neurosurgeons and otolaryngologists since it casts considerably different light on prognosis and genetic risks to those individuals who develop such a disease at a young age.

Significance to biomedical research and the program of the Institute: The mode of inheritance to this trait has been confirmed and the place of this syndrome among those associated with neural sheath proliferation is clearer. Of primary importance is the establishment of appropriate diagnostic techniques for early cases and treatment of these cases.

Proposed course: Affected individuals are being followed to evaluate various forms of treatment. Relatives not known to be affected but at risk are being examined periodically to evaluate various diagnostic techniques.

The initial phase of this project has been completed and resulted in the publications listed below.

Honors and Awards: None

Publications:

Young, D.F., McNew, J. and Eldridge, R.: Hereditary Acoustic Neuroma - Clinical and Genetic Aspects. In Transactions of the American Neurological Association. 94, 1969.

Young, D.F., Eldridge, R., Nager, G.T., DeLand, F.H., and McNew, J.: Hereditary bilateral acoustic neuroma (central neurofibromatosis). Proceedings of the Second Conference on The Clinical Delineating of Birth Defects, May 1969. The National Foundation-March of Dimes (In Press)

Young, D., Eldridge, R., and Gardner, W.J.: Bilateral Acoustic Neuroma in a Large Kindred. JAMA. 214, October 1970.

Serial No. NDS (CF) - 69 E 1781
1. Collaborative Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Twin Studies in Parkinson's Disease

Previous Serial Number: Same

Principal Investigators: Roswell Eldridge, M.D.
Zdenek Hrubeck, M.D.
National Academy of Sciences

Other Investigator: Kathy O'Meara

Cooperating Unit: The National Academy of Sciences - National Research Council Twins Registry, Washington, D. C.

U. S. Veterans Administration
Washington, D. C.

Man Years:

Total:	1/4
Professional:	1/8
Other:	1/8

Project Description:

Objectives: Parkinsonism is not a single entity but rather a symptom complex which may be idiopathic, or may be due to cerebral arteriosclerosis or may follow encephalitis. Genetic factors have been considered important by a number of authors but it is not yet established how important such factors are in any one form of Parkinsonism or if there is a form which has a simple genetic basis independent of acquired neurologic disease. The aim of the present proposal would be to use the twin method to evaluate genetic factors in various forms of Parkinsonism and/or to distinguish genetically determined Parkinsonism from other forms.

Methods employed: Survey of the VA twin registry in 1968 has revealed eight cases of Parkinsonism in one of a Veteran twin pair. Each of the pair were apparently discordant for Parkinsonism. Two twin pairs were monozygotic, five were dizygotic and in one the zygosity was unknown. We would contact each of these individuals and his co-twin, and others with

the diagnosis who may be ascertained through updating of the registry, and arrange for an appointment with the individual in his home at a time when available relatives could also be present. During the family interview a pertinent medical history would be obtained and physician examination would be performed. Permission for review of hospital records would be secured and arrangements might be made for additional neurologic studies. To define zygosity, photographs would be taken of the twins, blood would be drawn for genotyping and dermatoglyphics might be recorded. (Personal examination of both twins and available relatives is important in order that mild cases not be missed).

Major Findings: Interest continues in this project although there has been no additional ascertainment of cases since the last report. The National Science Foundation have not yet agree to our contacting patients on their registry but we expect twin pairs will be available to us in the future.

Significance to biomedical research and the program of the Institute: In a chronic condition with late onset it is often difficult to determine the role of genetic factors in causation. The twin method provides a relatively simple method involving small numbers to answer this question.

The nosology of Parkinson's disease is especially important now that the drug L-Dopa has been shown to help some with Parkinsonism. Is this drug most helpful in a specific form of the disease? Is it effective in hereditary Parkinsonism?

Proposed course: See "Methods employed".

Honors and Awards: None

Publications: None

Serial No. NDS (CF) 69 E 1782
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Twin Studies in Torticollis

Previous Serial Number: Same

Principal Investigators: Roswell Eldridge, M. D.
Zdenek Hrubec, M. D.
National Academy of Sciences

Other Investigators: Kathy O'Meara

Cooperating Unit: The National Academy of Sciences-National Research Council Twins' Registry, Washington, D. C.

U. S. Veterans Administration
Washington, D. C.

Man Years:

Total:	1/4
Professional:	1/8
Other:	1/8

Project Description:

Objectives: Torticollis may be broadly divided into infantile and post-infantile forms. The former may be congenital or appear several weeks after birth but in either the cause appears due to events preceding birth. Post-infantile torticollis consists of a heterogeneous group of disorders which have been ascribed to a number of causes including trauma or inflammation of the cervical spine, myositis of nuchal musculature, functional illness, and disease of the peripheral or central nervous system. In addition torticollis on a hereditary basis, either as an isolated symptom or in association with other movement disorders such as torsion dystonia, has been the subject of numerous reports. The aim of the proposed study is to weigh the genetic factors in the post-infantile forms of torticollis and, if possible, to distinguish between discrete hereditary types.

Methods employed: In 1968 a review of the Veterans Administration twin registry disclosed 11 individuals with a diagnosis of torticollis. Each of the 11 pair were said to be discordant. Five were monozygotic, two were dizygotic and in the four the zygosity could not be established. We would contact each of these individuals and his co-twin, arrange for an appointment with the individual in his home at a time when available relatives could

also be present. (We would hope also to ascertain new cases by assisting in the up-dating of the twin registry). During the family interview the pertinent medical history would be obtained for all relatives and physical examination performed on those present. Permission for review of hospital records would be secured and arrangements might be made for additional neurologic studies. To establish zygosity, photographs would be taken of the twins, blood drawn for genotyping, and dermatoglyphics might be recorded.

Major findings: Interest continues in this project although there has been made no additional ascertainment of cases since the last report. The National Science Foundation has not yet agreed to our contacting patients on their registry but we expect twin pairs will be available to us in the future.

Significance to biomedical research and the program of the Institute: Torticollis may be hereditary or acquired but under each of these headings there appear to be a number of discrete entities. The twin method presents a relatively simple means to distinguish genetically determined forms. Concentration on torticollis which is simply inherited is worthwhile since such disorders should have a discrete biochemical basis which might be revealed by study with the neurochemical techniques now available.

Proposed course: See "Methods Employed".

Honors and Awards: None

Publications: None

Serial No. NDS (CF) - 70 E 1832
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Measurement of immune response after antigen stimulation

Previous Serial Number: None

Principal Investigators: George Nemo, Ph.D.
Lon R. White, M.D.

Other Investigators: Harrie Anne Sutton, Biologist
Minnie Toure, Biologist
Gary Cooper, Biologist

Cooperating Unit: Unit on Congenital and Chronic Viral Infections, DB, NICHD

Man Years:

Total:	5/6
Professional:	1/3
Other:	1/2

Project Description:

Objectives: To investigate the changes in mitogenic, cytotoxic and anti-viral capacities of lymphocytes in relation to the acquisition of specific immunity.

Methods employed: Studies will involve both humans (immunized to vaccinia virus as part of routine health care practices) and experimental animals (mice, rats, guinea pigs). The antigens with which the animals will be inoculated include mycobacteria protein, vaccinia, sindbis, reovirus I, and the minute virus of mice. Lymphocyte transformation studies will be done with spleen, blood and lymph node cells. The effects of immune lymphocytes on the course of virus infection in vitro will be studies using monolayer tissue culture cells isogenic with the lymphocyte donor. Cytotoxicity will be assayed visually and by Cr^{51} release. Viruses will be titered using standard techniques. Macrophage-migration inhibition will also be employed as an indicator of cellular immunity.

Major findings: None as yet.

Significance to biomedical research and the program of the Institute: It is widely thought that "autoaggressive" lymphocytes are involved in the etiology of chronic demyelinating diseases. It is further believed that lymphocytes may be important in recovery from viral infection. Experimental animal systems have suggested the possibility that certain chronic viral

infections may owe their chronicity to impaired lymphocyte competence and that as this competence begins to be restored, host cells which bear viral antigen determinants on their surfaces may be injured, giving the appearance of an autoimmune disease. Such theories must be based and examined on the results of investigations such as those described.

Proposed course: To be continued.

Honors and Awards: None

Publications: None

Serial No. NDS (CF) - 70 E 1833
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Immune mechanisms in chronic and congenital viral infections

Previous Serial Number: Same

Principle Investigator: Lon R. White, M.D., DB, NICHD

Other Investigators: George Nemo, Ph.D.
Jacob A. Brody, M.D.
Harrie Anne Sutton, biologist, DB, NICHD

Cooperating Units: None (HD DB - 5)

Man Years:

Total:	0.6
Professional:	0.3
Other:	0.3

Project Description:

Objectives: 1. To establish one or more models of chronic and/or congenital viral infection in experimental animals. 2. To define the development of cellular and humoral immunity to the same and to heterologous antigens.

Methods employed: Neonatal and pregnant mice are injected with either a reovirus type 1 or the minute virus of mice (MVM). The persistence of virus in animals is determined by isolation and flourescent and immuno-flourescent techniques. Serum levels of hemagglutination inhibition antibodies to both agents are followed. Lymphocyte transformation and cytotoxic activity is studied in spleen cell cultures in the presence of phytohemagglutinin on specific antigens. Any tumors which may develop are studied by light and electron microscopy. Extracts of MVM-containing tumor cells will be injected into newborn mice in addition to being studied in tissue culture.

Major findings: Attempts to infect embryonic animals by maternal infection in the first week of pregnancy have failed, presumably because infected embryos are reabsorbed. Late gestation maternal infection of 8 animals has resulted in the live birth of 53 animals, 39 of which survived to adult life. All progeny have appeared to be normal, most having been observed for 1 year. Two such animals have died of solid tumors; one of these was lost to study and the other was found to have a tumor in the area of the right uterine horn with

hepatic metastases. Extracts of both the tumor and liver yielded a hemagglutinating material presumed to be MVM antigen, suggesting that the animal was either reinfected late in life or had been chronically infected since fetal life. Cell-free materials from these tissues are now being studied both in vivo and in vitro in an attempt to define the possible role of MVM, either directly or indirectly, in the etiology of the tumor.

Significance to biomedical research and the program of the Institute: Infection of the human embryo or fetus with rubella or cytomegalovirus is associated not only with developmental abnormalities and mental retardation, but also with infection persisting for months or years after, despite the presence of neutralizing antibody in the serum. There are several examples of related phenomena in experimental animals infected during prenatal or neonatal life. Previous studies have suggested that an impairment of the immune function of lymphocytes may be associated with such persistent infection. These phenomena provide insight into the question of how viral infection is normally terminated, and represent a challenge to older ideas on the role of specific cellular immunity in the natural history of virus infections. The investigation described represents a direct experimental approach to elucidating the cause and course of chronic infection following initial exposure to the agent during immunologic immaturity. The results of this investigation will be of immediate relevance to our understanding of other types of illness known or suspected to be associated with chronic viral infection. In addition, they may suggest new approaches to research in the role of congenital viral infection as a cause of diseases of unknown etiology such as prematurity, idiopathic growth failure, malignancy, mental retardation, developmental malformation, and autoimmune diseases.

Proposed course: To be confined at present level of effort.

Honors and Awards: None

Publications: None

Serial No. NDS (CF) - 70 E 1834
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Immune mechanisms in experimental allergic encephalomyelitis (EAE)

Previous Serial Number: Same

Principal Investigator: Lon R. White, M.D.

Other Investigators: George Nemo, Ph.D.
Marian Kies, Ph.D., Section on Myelin Chemistry, NIMH

Cooperating Unit: Section on Myelin Chemistry, Laboratory of Cerebral Metabolism, DBBR, NIMH (HD DB - 6)

Man Years:

Total:	0.20
Professional:	0.15
Other:	0.05

Project Description:

Objectives: To demonstrate in vitro "activation" of lymphocytes from guinea pigs with EAE by a myelin derived basic protein.

Methods employed: Techniques of lymphocyte cytotoxicity and blastogenic transformation have been employed.

Major findings: No cytotoxicity has been demonstrated. Transformation has been observed in some, but not all, experiments. These results seem to relate to the following variables: a) the "batch" of basic protein used, b) allotypic identity or dissimilarity between lymphocytes and target cells, c) strain of guinea pig utilized, d) interval between inoculation on animals and testing of lymph node lymphocytes.

Significance to biomedical research and the program of the Institute: EAE is generally held to be pathogenically similar to certain human demyelinating diseases, particularly the post-infections encephalitides and multiple sclerosis, and may involve lymphocyte-mediated autoimmunity. The in vitro demonstration of a basic protein "activatable" lymphocyte would be of great value to a more complete understanding of the experimental disease and would suggest new approach to the human diseases. The knowledge gained relating to lymphocyte function would be of immediate relevance to problems of

autoimmunity and chronic infection in certain diseases definitely or possibly due to viral infection during prenatal and neonatal life.

Proposed course: No further experiments planned at this time.

Honors and Awards: None

Publications: None

Serial No. NDS (CF) - 70 E 1835
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Epidemiologic and immunologic study of families of subacute sclerosing panencephalitis patients and families of matched controls

Previous Serial Number: Same

Principal Investigators: Roger Detels, M.D.
John M. Stanhope, M.D.
Jacob A. Brody, M.D.
Jane McNew, R.N.
Anne H. Edgar

Other Investigators: John Sever, M.D.
Head, Section on Infectious Diseases, Perinatal Research Branch, NINDS
Richard L. Parker, D.V.M.
Assistant Chief, Office of Veterinary Public Health, Center for Diseases Control, Atlanta

Cooperating Units: Section on Infectious Diseases, Perinatal Research Branch, NINDS
Veterinary Branch, National Communicable Disease Center, Atlanta

Man Years:

Total:	1 2/3
Professional:	1 1/2
Other:	1/6

Project Description:

Objectives: To determine, through the use of a matched control study, factors related to the etiology of subacute sclerosing panencephalitis.

Methods employed: Cases of SSPE were selected from the areas around St. Louis, Missouri, North Carolina, and California in order to evaluate the occurrence in distinctly different environments. 49 families with matched controls and an additional 8 families for whom a suitable control could not be obtained have been interviewed. Controls were selected on the basis of close friendship and similar background to the proband. A detailed history of events possibly related to the etiology of SSPE including episodes of measles, unusual illnesses, exposure to sick animals, etc. was asked of

probands, controls and their families. A 20cc sample of blood was drawn from each individual in the proband and control families.

Major findings: Preliminary analysis of the 40 initial cases indicates that the median age of measles among probands was 18 1/2 months, 2 years younger than the median age of measles among controls. One-quarter of the probands, but none of the controls, had measles under one year of age and two of the probands were diagnosed by their physicians as having had measles twice. One-quarter of the probands had no history of measles. All of the patients came from middle or lower income families, and only one of the probands came from a truly urban environment. History of warts, herpes virus infection, allergies and neurologic disease were similar in proband and control families. All but 3 of the 40 probands had pets and 2 of these had had intimate exposure with animals. A history of exposure to sick animals was more than twice as frequent among probands (68%) as among controls.

Significance to biomedical research and the program of the Institute: We have shown that the measles infection in cases was unusual and occurred frequently during a time when passive immunity was present. Further SSPE occurs in an unusual epidemiologic pattern with rates among males far exceeding those of females and rates in non-urban areas vastly exceeding those in urban areas much as would be expected with a zoonotic disease. Thus we have postulated that SSPE is caused by an unusual measles infection and a subsequent triggering event which is probably a zoonotic virus.

Proposed course: Sera from 57 proband families and 49 control families will be analyzed for presence and titers of measles antibodies. Additional testing will be made as indicated by the results of the analysis of the questionnaires. Final analysis of the histories obtained from the proband and control families will be completed. Intensive search for the possible "zoonotic trigger" will be carried out.

Honors and Awards: None

Publications: Brody, J.A. and Detels, R.: Subacute sclerosing panencephalitis: A zoonosis following aberrant measles. Lancet, II:500-501, 1970.

Brody, J.A., Detels, R. and McNew, J.: Evidence that subacute sclerosing panencephalitis is caused by an aberrant measles infection followed by a zoonosis. Presented at Annual Meeting of the American Academy of Neurology, April 1971. To be published.

Serial No. NDS (CF) - 70 E 1836
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Epidemiologic factors in Jakob-Creutzfeldt's disease

Previous Serial Number: Same

Principal Investigators: Jacob A. Brody, M.D.
A. Roger Bobowick, M.D.
Raymond P. Roos, M.D.
C&FR, NINDS

Other Investigator: Marjorie Matthews, R.N.

Cooperating Unit: Laboratory of Slow, Latent and Temperate Virus Infections, C&FR, NINDS

Man Years:

Total:	1/4
Professional:	1/6
Other:	1/12

Project Description:

Objectives: Jakob-Creutzfeldt's disease is one of the spongiform encephalopathies which has been transmitted to chimpanzees. Preliminary investigations suggest that two virus particles occur in the brains of patients. There are many forms of Jakob Creutzfeldt's disease which are distinct clinically and to some degree neuropathologically. Nothing is known of the epidemiology of this syndrome. We wish to determine if there are common factors among patients who develop Jakob-Creutzfeldt's disease and if there are specific factors related to distinct forms of this syndrome.

Methods employed: Letters were sent to neuropathologists soliciting pathologically proven cases. With the cooperation of the referring physicians the families of the patients are contacted and asked to participate in the epidemiologic interview. At the time of the interview session a detailed questionnaire is completed on the relative who is the interviewee, on the patient, and on an age and sex matched friend of the patient who serves as a control. Blood samples are also taken for future seriologic studies.

Major findings: From the series of approximately 50 patients, we have interviewed, thus far, 12 families. The methods outlined above have proved workable. The families have been extraordinarily cooperative and in fact grateful.

Significance to biomedical research and the program of the Institute: This neurologic disease is caused by a virus or a combination of viruses. Transmissible diseases must be studied epidemiologically if predisposing factors are to be elicited. The documentation of a predisposing factor to a virus-induced degenerative disease would be a major contribution to the understanding of the disease, its treatment and prevention.

Proposed course: This series will be developed to include between 30 and 50 cases and analysis of the data will begin.

Honors and Awards: None

Publications: None

Serial No. NDS (CF) - 70 E 1837
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Multiple sclerosis among native-born and migrants to California and Washington States

Previous Serial Number: Same

Principal Investigators: Roger Detels, M.D.
Jacob A. Brody, M.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	2/3
Professional:	1/3
Other:	1/3

Project Description:

Objectives: To determine whether rates of multiple sclerosis among native-born in Washington and California are related to latitude, and to determine if migrants from high and low risk areas within and without the United States retain the rate from place of origin or acquire the rate of the place to which they migrated.

Methods employed: Washington and California have a combined population of approximately 30 million of whom about 20 million are migrants. Decedents with multiple sclerosis and amyotrophic lateral sclerosis between 1954 and 1964 can be analyzed by birthplace, race, sex, age and duration of life in California. ALS patients can be used as a partial control, since the ALS rate is thought to be stable regardless of latitude. Death rates can also be ascertained for both MS and ALS using 1960 census data as a baseline.

Major findings: Death rates from MS were studied among 7 million native-born and 8 million migrant white, Japanese and Chinese in California and Washington. The death rate among native-born Washingtonians was higher than among native-born Californians. Low death rates were found among American-born and foreign-born Japanese and Chinese in both states suggesting that racial factors may influence susceptibility to MS. Migrants to both states from areas reported to have low rates of MS had low rates suggesting that they had acquired protection before migration. Migrants to Washington from areas reported to have high rates had higher rates than similar groups

migrating to California. These findings suggest that causative factors may still be operative in the third and fourth decades of life in high risk areas and that a protective factor may also be involved in the etiology of MS.

Significance to biomedical research and the program of the Institute: If these findings are substantiated they would lead to major insights in MS. This is the first large study of people moving from low risk to high risk areas and we have new evidence of a protective factor in MS. Data on possible changes in risk among those migrating from high risk areas suggests that causative factors persist after adolescence. The concept of separate protective and precipitating factors in MS may provide the clue in understanding the genesis of the disease.

Proposed course: This study will be expanded through prevalence studies focusing on various migrant groups.

Honors and Awards: None

Publications: Detels, R., Brody, J.A. and Edgar, A.H.: Multiple sclerosis among American, Japanese and Chinese migrants to California and Washington. New Eng. J. Med. In press.

Serial No. NDS (CF) - 70 E 1838
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Study of the incidence of Parkinson's disease among offspring of Parkinsonians

Previous Serial Number: Same

Principal Investigators: Roger Detels, M.D.
Jacob A. Brody, M.D.

Other Investigators: None

Cooperating Units: Church of Jesus Christ of Latter-day Saints,
Salt Lake City, Utah
The Genealogic Society,
Salt Lake City, Utah

Man Years:

Total:
Professional:
Other:

Project Description:

Objectives: To determine if genetic factors play a part in the etiology of Parkinson's disease by examining the incidence of disease among offspring of probands.

Methods employed: Fifty members of the Church of Jesus Christ of Latter-day Saints who died with the diagnosis of Parkinson's disease will be selected for review of death certificates in Utah between 1930 and 1940 and an additional 50 members who had a hospital diagnosis of Parkinson's disease between 1930 and 1940 selected from a review of hospital records from the major hospitals in the Salt Lake City area. A matched index control will be selected for each proband by taking the next death certificate or hospital record of an individual of the same sex whose age was within 5 years of the proband at the time of death or illness. Offspring of probands and index controls will be identified, a review of hospital records, of obituary notices, and a review of the records of the Genealogic Society and of the Church files of the Church of Jesus Christ of Latter-day Saints. Addresses of offspring will be obtained from the Church and questionnaires will be sent out to the offspring. Cases of Parkinson's disease will be confirmed when possible by physical examination or in the case of death through available medical records.

Major findings: None

Significance to biomedical research and the program of the Institute: In a chronic condition with late onset, such as Parkinson's disease, it is often difficult to determine the role of genetic factors. Members of the Church of Jesus Christ of Latter-day Saints maintain accurate, up-to-date genealogies. Thus, offspring of Parkinsonians can be located and the incidence of Parkinson's disease among them determined, providing a relatively simple method for determining the genetic factor in the etiology of Parkinson's disease.

Proposed course: See "Methods employed."

Honors and Awards: None

Publications: None

Serial No. NDS (CF) - 70 E 1839
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Studies on the relationship of chromosomal abnormalities and certain viral infections in mice. (Alternate title: Effects of viruses on mitosis and meiosis in the mouse.)

Previous Serial Number: Same

Principal Investigators: Beverly J. White, M.D., LEP, NIAMD
Lon R. White, M.D., DB, NICHD

Other Investigators: George Nemo, Ph.D.
Jacob A. Brody, M.D.

Cooperating Units: Laboratory of Experimental Pathology, NIAMD (LEP/NIAMD-17)
Developmental Biology Branch (Unit on Chronic and Congenital Viral Infections), NICHD

Man Years:

Total:	1.5
Professional:	0.9
Other:	0.6

Project Description:

Objectives: a). To localize within dividing mouse spleen cells in vitro, particularly in relation to chromosomes, the site of residence of the infecting viral genome (of the minute virus of mice - MVM) and the MVM template DNA. b). To search for evidence of injury by virus to chromosomes of spermatogonia and somatic cells. c). To determine whether perinatal infection results in infection of the germ cells and, if so, to determine the duration of infection as well as the chromosomal and reproductive consequences of such infection.

Methods employed: The viruses under study are two strains of MVM and two strains of reovirus type 1. The animal used is the Cumberland View Farms C57BL/6 mouse. The viruses are "grown" and quantitated in tissue culture. Intracellular MVM genome (infectious or template DNA) is localized by autoradiography using tritium "tagged" MVM DNA. Chromosome studies are carried out on short term spleen cell cultures as well as on cytologic preparations made directly from the spleen, testes, and bone marrow of infected and control mice. Viral antigen is localized using immunofluorescent and immunohistochemical methods.

Major findings: a). A "fate" study in mouse spleen cells using tritium-labeled virus preparations showed no difference in autoradiographic grain localization between control and virus infected cultures. These were carried out with "tagged" viruses of relatively low infectivity and specific activity (radioactivity). b). Meiotic preparations from the testes of adult mice were studied 4 days, 1 week, 3, 6, and 12 weeks following MVM infection. The only notable observation was of a relative decrease in number of certain cell types, suggesting an effect on the course of spermiogenesis.

Major improvements in methods involved with the propagation and quantitation of MVM have been accomplished. These have been used to produce viral "reagents" to be used in additional experiments.

Significance to biomedical research and the program of the Institute: The role of viruses in the etiologies of chromosomal and developmental diseases is widely discussed, but negligibly investigated. The interaction of noncytolytic, non-oncogenic viruses with chromosomes and the spindle is also essentially undefined. The potential of these investigations is vast; basic information may be gained important to an understanding of human disease pathogenesis as well as to current concepts on the evolution of viruses and the basic nature of their interactions with dividing, differentiating cells.

Proposed course: To be continued at same level of effort.

Honors and Awards: None

Publications: None

Serial No. NDS (CF) - 70 E 1840
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Retinoblastoma, Clinical, Genetic and Psychometric Aspects

Previous Serial Number: Same

Principal Investigator: Roswell Eldridge, M.D.

Other Investigators: Kathy O'Meara
Jeffrey C. Allen, M.D.
David Kitchen, M.D.

Cooperating Units: Johns Hopkins Hospital, Baltimore, Maryland
Children's Hospital of D. C., Washington, D. C.
Department of Psychology, Downstate Medical Center, New York

Man Years:

Total:	4
Professional:	3
Other:	1

Project Description:

Objectives: Only a few traits are said to be associated with increased intelligence. Such a correlation has been documented for torsion dystonia, the recessive type. Retinoblastoma is another condition for which such an association has been reported. Because we have reservations about choice of controls and value of data obtained in a handicapped population, we wish to evaluate this association in retinoblastoma ourselves.

Methods employed: Efforts will be concentrated on testing the psychometric performance of affected individuals with family history who are sighted. The unaffected sibs will form the control group.

Major findings: To date over 20 affected individuals and 20 controls in 12 families have been evaluated by us. In addition, retrospective data from school groups testing is being obtained.

Significance to biomedical research and the program of the Institute: This work is of great potential significance. If there is an association

between the gene for retinoblastoma and higher intelligence, then finding the underlying chemical abnormality should add to our knowledge of intellectual development as well as suggest the cause of neoplasm.

Honors and Awards: None

Publications: None

Serial No. NDS (CF) - 70 E 1842
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Mortality among Japanese-Americans in relocation camps

Previous Serial Number: Same

Principal Investigator: Roger Detels, M.D.

Other Investigator: None

Cooperating Units: None

Man Years:

Total:	1/2
Professional:	1/6
Other:	1/3

Project Description:

Objectives: To determine death rates among American and foreign-born Japanese in relocation camps in 1943 and 1944 from selected neurologic, cardio-vascular and malignant diseases.

Methods employed: During the period 1942-45 there were over 120,000 Japanese-Americans in the U.S., all of whom were under the authority of the War Relocation Board. The majority were in relocation camps. Copies of the death certificates of persons dying in these relocation camps are on file at the Bancroft Library of the University of California at Berkeley. Records, including medical charts for all internees, are on file at the National Records Center in Suitland, Maryland. Thus, it is possible not only to establish death rates from various causes, but also to determine the accuracy of the diagnosis and the course of the disease by referring to the medical charts. Baseline statistics of Japanese-Americans in the U.S. in 1943-45 and of those in relocation camps were accurately maintained at 6 month intervals and are available at the National Archives.

Major findings: Data were reviewed. No patients with MS or ALS were encountered.

Significance to biomedical research and the program of the Institute: The availability of death certificates and medical charts allowed us to determine accurate death rates among American and foreign-born Japanese-Americans and to correlate these rates to length of residence in Japan and the U.S. Thus, we should be able to comment on relative effects of

environment and genetic make-up on such diseases as multiple sclerosis, amyotrophic lateral sclerosis, stroke and other cardio-vascular and malignant diseases.

Proposed course: This study was terminated.

Honors and Awards: None

Publications: None

Serial No. NDS (CF) - 70 E 1843
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Causes of death among siblings of MS and ALS patients

Previous Serial Number: Same

Principal Investigator: Jacob A. Brody, M.D.

Other Investigator: Estelle Kornhauser, R.N.

Cooperating Unit: Department of Neuropathology, Montefiore Hospital, New York

Man Years:

Total:	1/3
Professional:	1/4
Other:	1/12

Project Description:

Objectives: Recently, we have developed serologic evidence that familial patterns of antibodies of MS patients differ significantly from controls. This suggests a familial process may be involved etiologically in MS. We wish to determine, therefore, if siblings of MS patients have unusual illnesses possibly related to immunologic defect. The diseases we are interested in include collagen diseases, thyroid diseases, and rheumatoid arthritis.

Methods employed: We will control the siblings of MS patients with the siblings of ALS patients. We have selected 100 MS patients and 100 ALS patients from the autopsy file of Dr. Harry Zimmerman, Department of Neuropathology, Montefiore Hospital. We will contact surviving siblings to determine major illness in the sibships.

Major findings: At present, we have information on 60 ALS patients and 70 MS patients in whom the diagnosis was confirmed at autopsy. Various attempts to locate survivors has been unsuccessful.

Significance to biomedical research and the program of the Institute: There is no firm evidence that an autoimmune mechanism or a virus is the cause of MS. There is, however, accumulating suggestive evidence that this is the case. There is also evidence of altered serologic responses among siblings of MS patients. If we can document that the siblings of MS patients

have illnesses related to immunological mechanisms we would have valuable evidence that these mechanisms are involved in the etiology of MS.

Proposed course: Attempts using other patient source will be made in hopes of getting better access to follow-up data on siblings.

Honors and Awards: None

Publications: None

Serial No. NDS (CF) - 70 E 1844
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Amyotrophic lateral sclerosis in veterans

Previous Serial Number: Same

Principal Investigators: John Kurtzke, M.D.
Chief, Neurology Service, Veterans Administration Hospital, Washington, D.C.
Gilbert Beebe, M.D.
Director, Follow-up Agency, National Research Council of the National Academy of Sciences
Virginia C. Karl, M.A.
Social Work Service, Veterans Administration Central Office, Washington, D.C.

Other Investigators: Jacob A. Brody, M.D.
A. Roger Bobowick, M.D.

Cooperating Units: Veterans Administration
National Research Council of the National Academy of Sciences

Man Years:

Total:	1/3
Professional:	1/4
Other:	1/12

Project Description:

Objectives: We will determine if there are distinctive epidemiologic features related to amyotrophic lateral sclerosis using the veteran population. We will also determine if service in Guam and the Marianas Islands was a risk factor in developing ALS. Life-table estimates will be constructed to clarify prognosis as to survival with ALS.

Methods employed: The methods paralleling those of the veterans study of multiple sclerosis will be used to obtain the "records sample." In addition, we will survey approximately 200 veterans with ALS and 200 controls with brain tumors using a detailed historical questionnaire. This population will be called the "interview sample." Finally, a prognostic study will be constructed from Veterans Administration Hospital admissions of the 1957 - 1964 period for ALS and other motor neuron diseases.

Major findings: The records sample should number at least 425 World War II and Korean war veterans with ALS. With about 500 discharges annually for ALS, the large Veterans Administration Hospital system will supply sufficient case material to carry out the interview sample and construct the prognostic study. Dr. Franklin O. Meister, Director, Neurology Branch, Veterans Administration Central Office, Washington, D.C. has endorsed the project and suggest that only VA Hospitals with neurologic units be used.

The detailed historical questionnaire for the interview sample has been drafted and preliminary field tests indicate that it will be quite workable.

Significance to biomedical research and the program of the Institute: Regional differences and socio-economic differences have been commented upon for amyotrophic lateral sclerosis in the United States. This study will determine for a large population if there are predisposing factors to this disease. Should we determine such factors, it would provide a valuable clue to the understanding, treatment and prevention of this disease. Prognosis for survival should also be clarified.

Proposed course: With our collaborators, we will collect all known veterans with ALS and try to detect possible predisposing factors.

Honors and Awards: None

Publications: None

Serial No. NDS (CF) - 70 E 1845
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Natural history of Parkinson's disease and the effect of L-Dopa

Previous Serial Number: Same

Principal Investigator: Jacob A. Brody, M.D.

Other Investigator: A. Roger Bobowick, M.D.

Cooperating Unit: National Parkinson Foundation, Inc., Miami, Florida

Man Years:

Total:	5/24
Professional:	1/8
Other:	1/12

Project Description:

Objectives: We wish to determine if L-Dopa affects the natural history of Parkinson's disease or is merely symptomatic treatment.

Methods employed: We will study a cohort with diagnosed Parkinson's disease who had the disease between 1958 and 1964, prior to the advent of L-Dopa. We will then develop life tables to determine the life expectancy and specific cause of death of patients with Parkinson's disease before the advent of L-Dopa. We will then follow a matched series of patients who are receiving L-Dopa to determine life expectancy and cause of death.

Major findings: Review of the records at the National Parkinson Foundation, Inc. in Miami revealed that the necessary information had been reliably recorded. A sample of 50 pre- and 50 post-L-Dopa era patients has been processed. Medical record data retrival forms and follow-up forms to physicians have been completed. The medical record data is remarkably complete. The follow-up information will require the usual multiple avenues of pursuit. The staff at the National Parkinson Foundation, Inc. is now working on the medical record data retrival forms for the remaining 450 patients in the pre-L-Dopa cohort.

Preliminary analysis of the first 100 patients suggest that in the population of Miami the mean age increased about 3 years from the early to the late 1960's. This is consistent with the cohort theory of parkinsonism which supposes a common etiology of sub-clinical encephalitis lethargica

around 1920 and predicts the disappearance of parkinsonism as a major clinical entity around 1980. Because of the profound implications, verification of this phenomenon is being sought in age data of Parkinson patients at other centers.

Significance to biomedical research and the program of the Institute: It is not known whether L-Dopa provides only symptomatic relief of Parkinson's disease or whether it actually affects the pathologic process which produces this chronic illness. If indeed it does reverse the pathologic process, we would advance the understanding of the genesis of this disease. Since L-Dopa is a drug with known biohazards, we will be able to determine if these hazards shorten the life expectancy of treated patients or if they die of specific diseases such as cardiovascular disease or kidney disease which will permit us to focus medical attention on prevention of these specific complications among treated patients.

Proposed course: The pre-L-Dopa cohort and the cohort receiving L-Dopa will be matched and appropriate follow-up information concerning major illness and death will be secured.

Honors and Awards: None

Publications: None

Serial No. NDS (CF) - 71 E 1917
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Twin study of multiple sclerosis: An epidemiologic inquiry

Previous Serial Number: None

Principal Investigators: A. Roger Bobowick, M.D.
Jacob A. Brody, M.D.

Other Investigators: John F. Kurtzke, M.D.
Veterans Administration Hopsital, Washington, D.C.
Zdenek Hrubec, Sc.D.
Follow-up Agency, NAS-NRC
Marjorie Matthews, R.N.

Cooperating Units: Neurology Service, Veterans Administration Hospital, Washington, D.C.
Follow-up Agency, National Academy of Sciences, National Research Council

Man Years:

Total:	1/4
Professional:	1/6
Other:	1/12

Project Description:

Objectives: The abundant epidemiological literature of multiple sclerosis has indicated beyond reasonable doubt that critical environmental factors influence the rate of disease. Prevelance and migration studies strongly suggest that the environmental factor(s) is operant about the time of puberty. In order to examine the nature of the etiologic exposure in multiple sclerosis then, it is most prudent to concentrate on events in patients and controls with comparable early life histories in whom host factors are equalized. Twins discordant for multiple sclerosis at an age beyond prime risk of acquiring MS offer this opportunity for study.

Methods employed: The NAS-NRC twin registry has identified 23 pairs of twins one or both of whom has multiple sclerosis. This group has come from their population of 16,000 pairs of white male twins who are veterans of military service in World War II and born during the years 1917 to 1927. Initial contact with the twins will be a letter from the registry explaining the study. If the twins express an interest in the study, they will be contacted by phone by the NIH investigators to arrange an interview date. The information collected will be: 1) pertinent medical history and

neurological exam, 2) an indepth epidemiologic interview concentrating on events prior to age 20, and 3) blood samples.

Major findings: Initial contact has been made with most of the twin pairs and thus far five pairs have agreed to participate. The first twin pair will be visited shortly.

Significance to biomedical research and the program of the Institute: Although exhaustive studies have been conducted to invoke specific etiologic factors in MS, no definitive precipitative circumstances have been identified perhaps because of the subtlety of these factors or the difficulties of suitably controlling for numerous unrelated circumstances. This novel application of the twin method of study offers an efficient technique for identifying these precipitating circumstances. Resolution of the cause and prevention of MS would be greatly enhanced by the identification of these precipitating factors.

Proposed course: The twins will be visited over the course of the next year.

Honors and Awards: None

Publications: None

Serial No. NDS (CF) - 71 E 1918
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Follow-up of the Cooperative Measles Vaccine Field Trial in four communities

Previous Serial Number: None

Principal Investigators: Jacob A. Brody, M.D.
Anne H. Edgar

Other Investigators: Marion Dressler, M.D.
DeKalb County Health Department, Decatur, Georgia
Margaret I. Rathbun, M.D.
Monroe County Health Department, Rochester, New York
Edwin P. Isacson, M.D.
State University of New York School of Medicine
Russell E. Alexander, M.D.
University of Washington School of Medicine, Seattle
Philip S. Brachman, M.D..
National Communicable Disease Center, Atlanta

Cooperating Units: DeKalb County Health Department, Decatur, Georgia
Monroe County Health Department, Rochester, New York
State University of New York School of Medicine
University of Washington School of Medicine, Seattle
National Communicable Disease Center, Atlanta

Man Years:

Total:	1/4
Professional:	1/2
Other:	3/12

Project Description:

Objectives: To determine through contact with the participants in the killed measles vaccine field trial of the early 1960s, any possible sequelae to receipt of the vaccine.

Methods employed: Review of the addresses of the study population in four communities (DeKalb County, Rochester, Buffalo and Seattle) provided definite addresses for 46% of the 2091 participants plus possible location of an additional 17%. In order to determine the likelihood of success of follow-up, questionnaires requesting history of childhood diseases, other infections, non-allergic diseases accompanied by a rash, and hospitalizations subsequent to receipt of the vaccine, were sent to the families of the study

population in DeKalb County. To date, 52% of the definitely located families and 29% of the possibly located have responded. These dame methods are to be extended to the participants in the field trial in the other three cities.

Major findings: To date the response to the study has been good but the small number of participants contacted allows no conclusions to be drawn.

Significance to biomedical research and the program of the Institute: The isolation of a measles-like virus from brains of individuals with subacute sclerosing panencephalitis has suggested a relationship between these two diseases. Epidemiologic study has further indicated that these patients may experience measles at an unusually early age. Evidence of high measles antibody titers in patients with multiple sclerosis has raised the possibility that measles might play an etiologic role in this disorder. In addition reports have been published on an atypical measles illness occurring in children who have received killed measles virus vaccine several years earlier. In light of the increasing interest in measles as an etiologic factor in disease, and the specific sequela reported to occur in those who have received the killed measles vaccine, the medical history of this study population subsequent to vaccination is of particular interest.

Proposed course: The participants for whom possible or definite addresses have been located in the other three communities will be contacted by mail and asked to supply the information requested on the questionnaire. Those not responding to the initial request will be contacted a second time in the hope of increasing the response rate.

Honors and Awards: None

Publications: None

Serial No. NDS (CF) - 71 E 1919
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Viability of white blood cells used for lymphocyte transformation

Previous Serial Number: None

Principal Investigators: Jeffrey C. Allen, M.D.
George Nemo, Ph.D.

Other Investigator: Jacob A. Brody, M.D.

Cooperating Unit: None

Man Years:

Total:	1/6
Professional:	1/12
Other:	1/12

Project Description:

Objectives: To develop a technique for studying lymphocyte transformation on blood specimens transported over extended periods of time so that the technique may be used in field studies.

Methods employed: White blood cells are allowed to stand at room temperature in either minimal essential medium or autologous plasma over a 24-48 hour period. The ability of the white blood cells to undergo lymphocyte transformation when exposed to various antigens is studied by the standard technique of measuring lymphocyte transformation used in this laboratory.

Major findings: There was considerable variability in measurements of lymphocyte transformation to vaccinia over a 24 hour period, however, there was no reduction magnitude of response of the cells in the interval studied. MEM was not as good as incubation mediums as autologous plasma.

Significance to biomedical research and the program of the Institute: If this method can be perfected the technique of lymphocyte transformation may be used in field studies and large numbers of relevent individuals may be examined. It is also hoped that if some of the variability is removed the technique of lymphocyte transformation may become sensitive. In addition, if there is a preferential die-off of granulocytes over time, a relatively pure culture of lymphocytes may be obtained.

Proposed course: It remains to be determined how long white blood cells can remain in autologous plasma at room temperature and still retain their ability to undergo transformation. The variability in response is considerable. This variability should be studied. Such techniques as purifying the lymphocytes, drawing blood at a certain time of the day from the same individual, and more careful standardization of the procedure may control some of the variability.

Honors and Awards: None

Publications: None

Serial No. NDS (CF) - 71 E 1920
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: A study of pregnancies among nurses; high risk pregnancies due to transmissible viruses

Previous Serial Number: None

Principle Investigator: Estelle Kornhauser, R.N.

Other Investigator: Jacob A. Brody, M.D.

Cooperating Unit: None

Man Years:

Total:	1/4
Professional:	1/12
Other:	3/12

Project Description:

Objectives: This project was developed to question the effects on the fetus of maternal virus infection.

Methods employed: A questionnaire was designed using the usual backup history and pertinent questions relating to type of nursing service, year of pregnancy, normal or abnormal births, and all information relating to any type of exposure to a viral type disease. The questionnaire and letter of explanation was pre-tested in 1160 nurses registered in the Maryland and District of Columbia area.

Major findings: Of the 1160 questionnaires sent we have received approximately 800 replies. To date there are 1353 pregnancies with approximately 250 miscarriages, stillbirths, and abortions resulting for many reasons.

Significance to biomedical research and the program of the Institute: This study will determine if pregnant women working with people with infectious disease have an elevated risk of fetal damage.

Proposed course: We plan to follow up with a second mailing and then continue our study to a larger group of respondents and continue to examine the risk factors involved in the care of patients who have infectious diseases.

Honors and Awards: None

Publications: None

Serial No. NDS (CF) - 71 E 1921
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Measles infection of mice: Effect of maternal immunity

Previous Serial Number: None

Principle Investigators: Jacob A. Brody, M.D.
George Nemo, Ph.D.

Other Investigators: Lon R. White, M.D., DB, NICHD
Gary Cooper, Biologist

Cooperating Unit: None

Man Years:

Total:	1/6
Professional:	1/12
Other:	1/12

Project Description:

Objectives: To develop an experimental animal model to study the pathogenesis of subacute sclerosing panencephalitis (SSPE).

Methods employed: Adult female mice previously immunized with measles virus will be bred and their offspring challanged with measles at various times after birth. Measles antibody levels will be determined periodically and a thorough analysis of mouse tissues for measles virus and measles-related antigens will be performed.

Major findings: None

Significance to biomedical research and the program of the Institute: An agent which by most virological criteria resembles measles virus has been isolated from brains of SSPE patients. A papova-like virus has been visualized in electron micrographs of specially treated cell lines inoculated with SSPE brain material suggesting the possibility of a dual infection. The exact role these agents play in the pathogenicity of the disease is unclear. An experimental animal model may aid considerably in elucidating the mechanism.

Proposed course: See "Methods employed."

Honors and Awards: None

Publications: None

Serial No. NDS (CF) - 71 E 1922
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Search for a negative DNA strand in cells infected with the minute virus of mice (MVM)

Previous Serial Number: None

Principle Investigator: Lon R. White, M.D., DB, NICHD

Other Investigators: Harrie Anne Sutton, biologist, DB, NICHD
George Nemo, Ph.D.
Gary Cooper, biologist
Jacob A. Brody, M.D.

Cooperating Unit: None (HD DB - 15)

Man Years:

Total:	0.7
Professional:	0.7
Other:	0.0

Project Description:

Objectives: a). To demonstrate a "template" DNA (negative strand) complementary to the single stranded DNA (positive strand) of the MVM virion. b). To determine if the negative strand is associated with the cell's chromosomal DNA. c). To investigate the kinetics of negative strand synthesis relative to cellular DNA replication, cellular division, virus infection, and virus production.

Methods employed: These studies will utilize H^3 or C^{14} "tagged" viral DNA with annealing demonstrated by: a) liquid scintillation detection of retained isotope on a filter, b) autoradiography with virus infected cells in metaphase. Some experiments will use purified chromosomes and chromosomal DNA from mitotically phased cells in culture.

Major findings: None

Significance to biomedical research and the program of the Institute: Characterization of virus-cell interactions using a single-stranded DNA virus (parvovirus group, of which MVM is representative) is expected to advance our understanding of the mechanisms by which a virus infection might alter the genetic potential of the host cell (either a somatic or germ cell) as well as of mechanisms involved with the establishment of latent and chronic viral

infections. These may be directly relevant to the etiologies of certain reproductive, developmental, and chronic diseases whose causes are now unknown.

Proposed course: To be continued at an increased level of effort.

Honors and Awards: None

Publications: None

Serial No. NDS (CF) - 71 E 1923
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Effect of measles virus on specific and non-specific stimulation of lymphocyte transformation

Previous Serial Number: None

Principal Investigator: Jeffrey C. Allen, M.D.

Other Investigators: George Nemo, Ph.D.
Jacob A. Brody, M.D.

Cooperating Unit: None

Man Years:

Total:	1/6
Professional:	1/12
Other:	1/12

Project Description:

Objectives: To define the effects live and UV-inactivated measles virus have on the ability of human peripheral lymphocytes to respond to apecific and non-specific stimulation in vitro.

Methods employed: Measles virus from both Ostereich and Edmonston strains will be grown to high titer in tissue culture. Lymphocytes will be obtained from healthy volunteers and prepared in the usual manner. A battery of specific antigens will be used including vaccinia, PPD, candida, diptheria and mumps. PHA will be the only non-specific antigen used.

Lymphocytes will be cultured in the usual way and incubated for the number of days appropriate to the particular antigen. Measles virus of varying titers, either live or killed, will be added simultaneously, before or after the particular antigen is added. Tritiated thymidine will be added one day before the harvest and uptake will be measured by liquid scintillation spectrotometry. Virus cultures and antibody measurements will be made from lymphocyte cultures at the time of harvest. In certain experiments fetal calf serum will be used to control for the effects of varying titers of measles antibodies of volunteers.

Major findings: Preliminary experiments suggest that measles virus (Ostereich) will inhibit non-specific lymphocyte PHA stimulation at titers greater than or equal to 10^4 PFU/cc even though there is less than 1 virus per lymphocyte in the original culture.

Significance to biomedical research and the program of the Institute: Measles virus has been known for some time to suppress specific cellular immune responses *in vivo*. Controversy exists with regard to its effect on lymphocytes *in vitro* and current reports suggest that the virus inhibits specific but not non-specific stimulation. The mechanism of this inhibitory effect is unknown. It is not known what effects humoral or cellular immunity have on this inhibitory effect. Children with thymic aplasia and absent cellular immunity are subject to aberrant and severe measles infections. *In vitro* studies may suggest the role cellular immunity plays in lost defense against viruses.

Proposed course: After the basic work is completed, measles antigen will be broken down to its basic protein and RNA components, and the effects of these substances will be evaluated. Other viruses will also be investigated such as rubella and mumps. Various disease states will be investigated such as SSPE and MS. A more complete study of the effects of measles antibody and antigen-antibody complexes is anticipated. Investigation at the cellular level for the site of measles operation will be studied with the use of autoradiography or fluorescent staining.

Finally, the supernatort of the measles-lymphocyte cultures will be investigated for the presence of inhibitory factors.

Honors and Awards: None

Publications: None

Serial No. NDS (CF) 71 E 1924
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Biochemical Studies in Torsion Dystonia

Previous Serial Number: None

Principal Investigators: Roswell Eldridge, M.D.
Thomas Chase, M. D.

Other Investigator: Kathy A. O'Meara

Cooperating Unit: Laboratory of Clinical Science, NIMH

Man Years:

Total:	2
Professional:	1 1/2
Other:	1/2

Project Description:

Objectives: Torsion dystonia has been shown recently to consist of at least two hereditary conditions as well as an acquired form. Through appropriate biochemical investigation it should be possible to determine the precise biochemical defect (or abnormal gene product) in the recessive form of torsion dystonia.

Methods employed: Patients are selected from the nationwide registry of torsion dystonia cases we have compiled and admitted to Dr. Chase's service for one or more weeks of the intensive evaluation. In addition to routine neurologic and chemical study, metabolism of biogenic amines is evaluated as completely as possible. Following this, there is often a period lasting several months in which drug trial is carried out.

Major findings: Initial results on the small series of patients suggest that there may be an increased turnover of catecholamines in those with the recessive form of dystonia. This in in marked contrast to the levels seen in Parkinson's disease and Parkinson's dementia on Guam.

Significance to biomedical research and the program of the Institute: Demonstration of the molecular lesion in one of the torsion dystonias would be a noteworthy achievement since another disease would be added to the small series of inborn errors of metabolism for which the precise lesion is recognized. In addition, undoubtedly important information with the forthcoming regarding biochemistry and physiology of movement control.

In addition, because of the recognized association of increased intelligence and the recessive form of dystonia, it is possible information regarding the development of intelligence would also result.

Proposed course: Continued investigation of patients from the genetics registry. Addition of more sophisticated techniques as they become available.

Honors and Awards: None

Publications: Chase, T.N.: Biochemical and pharmacologic studies of dystonia. In The Torsion Dystonias (Dystonia Musculorum Deformans). Editor, R. Eldridge. Neurology suppl. 20:11, Part 2, November 1970.

Serial No. NDS (CF) 71 E 1925
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Intelligence in Israeli Patients with Torsion Dystonia

Previous Serial Number: None

Principal Investigators: Roswell Eldridge, M.D.

Other Investigators: Morris Gross, PhD.
Richard Goodman, M. D.

Cooperating Unit: Department of Education
Herbert H. Lehman College
Bronx, New York

Man Years:

Total:	1/2
Professional:	3/8
Other:	1/8

Project Description:

Objectives: The recessive form of torsion dystonia has recently been shown to be associated with increased intelligence. However, the series which this information is based was small and individuals were scattered over a large area of the northeastern part of the United States. In Israel, where we suspect there to be over 20 patients with the recessive form of torsion dystonia, a more homogeneous testing situation prevails so that results should be more meaningful. If this association is true the implications are dramatic so that confirmation to this Israeli study is clearly in order.

Methods employed: Bona fide cases of torsion dystonia in Israel would be selected by the principal investigator on the basis of personal examination. Psychometric data would be obtained in a retrospective manner through the Israeli Department of Education and similar data would be obtained for unaffected sibs. A comparison would then be made of the performance in these two groups.

Major findings: Although we have known for several years of the presence of over 20 cases of torsion dystonia in Israel we have not yet been able to arrange for visitation. Neurologists, psychologists, and educators in Israel have been alerted and indicated their interest in participating in this study.

Significance to biomedical research and the program of the Institute: The positive association between torsion dystonia and intelligence would suggest that the chemical abnormality producing dystonia may also enhance intellectual development. Elucidation of this basic chemical abnormality would suggest a method for enhancing intellectual development, particularly those from the retarded population.

Proposed course: Several weeks in Israel would be necessary to make the necessary home visits and arrange for the collection of appropriate psychometric data.

Honors and Awards: None

Publications: None

Serial No. NDS (CF) 71 E 1926
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: The Difficulty of Diagnosing Acoustic Neuroma in Young Patients

Previous Serial Number: None

Principal Investigators: Jeffrey C. Allen, M.D.
Roswell Eldridge, M.D.

Other Investigator: Kathy O'Meara

Cooperating Units: Department of Neurosurgery
Columbia Presbeyterian Hospital
New York

Man Years:

Total:	1
Professional:	1/2
Other:	1/2

Project Description:

Objectives: To document and publicize the difficulty of diagnosis when acoustic neuroma occurs in young patients.

During a course of clinical and genetic study of acoustic neuroma in young individuals we have been impressed with the unusual length of time and unusual number of specialists consulted before diagnosis of acoustic neuroma became a consideration. This period was often one of unusual unnecessary stress and expense to the patient and his family and often resulted in loss of valuable time in many instances.

Methods employed: Patients who have early onset of acoustic neuroma were questioned in detail regarding physicians consulted and diagnostic procedures employed.

Major findings: In general, the younger the individual when symptoms of acoustic neuroma begin, the longer the period before diagnosis and the greater the number of physicians consulted and the more expense to the family.

Significance to biomedical research and the program of the Institute: Such documentation should impress the medical community about the resistance of this condition in young individuals thereby reducing time, expense and turmoil to patient, family, and physician when a new case develops. Through such publicity it is possible that our acoustic neuroma registry will be expanded.

Proposed course: Documentation of similar experience in the remainder of our acoustic neuroma patients. Publication of results.

Honors and Awards: None

Publications: None

Serial No. NDS (CF) 71 E 1927
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Family Studies in Young Patients with Acoustic Neuroma

Previous Serial Number: None

Principal Investigators: Jeffrey C. Allen, M. D.
Roswell Eldridge, M. D.

Other Investigators: Kathy A. O'Meara
George T. Nager, M. D.
Johns Hopkins Hospital

Cooperating Units: Department of Otolaryngology,
Johns Hopkins Hospital
Baltimore, Maryland

Man Years:

Total:	1 1/2
Professional:	1
Other:	1/2

Project Description:

Objectives: To determine the nosology of acoustic neuroma by studying clinical features, the age at onset of symptoms and presence of a family history of the disorder in young patients with unilateral and bilateral acoustic neuroma.

Methods employed: Field studies were conducted in evaluating family members. On those seen personally, physical examinations were performed, stressing neurological and skin examinations. In addition, audiometric examinations including air and bone conduction and caloric examinations were conducted in the field.

Patients were selected through chart review of neurosurgeons' files. All patients selected were alive at the outset of this study and experienced onset of symptoms before age 45.

Genealogic information and medical history was obtained from family members and family and hospital records. (Medical history was obtained from family members, hospital and physician records.)

On select patients extensive outpatient studies have included audiometric and vestibular testing, complete ENT and neurologic examinations and skull x-rays. These were undertaken in cooperation with the Departments of Neurology, Radiology and Otolaryngology of Johns Hopkins Hospital.

Major findings: Patients with bilateral acoustic neuroma are more likely to have earlier onset of symptoms and a positive family history of either central, peripheral or mixed neurofibromatosis. Nine of the 51 propositi seen so far had bilateral acoustic neuroma. Three of these had a definite family history of the trait. Of the 41 propositi with unilateral acoustic neuroma, a definite family history was noted in only one.

Most patients in the study commented on the length of time and number of physicians consulted before a definite diagnosis was made. The average number of years between onset of symptoms and surgical intervention was 7.0 for the bilateral cases and 5.4 for the unilateral cases. The larger number of years for bilateral cases may reflect the failure of physicians to consider this diagnosis in younger individuals.

Significance to biomedical research and the program of the Institute: The syndrome of bilateral acoustic neuroma seems to be distinct for unilateral acoustic neuroma using parameters such as age of onset of symptoms, likelihood of having a positive family history, presence of stigmata of neurofibromatosis and mode of inheritance. Unilateral acoustic neuroma on the other hand probably is not a distinct syndrome and may represent the manifestations of a sporadic dominant mutation or the expression of a recessive trait. Nearly 40 percent of our patients with unilateral acoustic neuroma were of Ashkenazic Jewish extraction.

Proposed course: The project will be completed after 70 kindreds have been examined.

A large body of descriptive information has been collected on these diverse kindreds. These kindreds could provide a fertile source for laboratory investigation. Pathologists have not been able to differentiate between an acoustic neuroma from a patient with unilateral or a patient with bilateral disease. Perhaps there are differences at a biochemical level.

Patients with bilateral disease may have a generalized neoplastic diathesis and laboratory investigation may reveal this in the patient and certain members of their family.

Honors and Awards: Presentation at Middle Atlantic Neurosurgery Society

Publications: None

Serial No. NDS (CF) 71 E 1928
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Chromosome Studies in Retinoblastoma

Previous Serial Number: None

Principal Investigators: David Kitchen, M.D.
Roswell Eldridge, M.D.

Other Investigators: Kathy O'Meara

Cooperating Unit: Cytology Laboratory, Eye Institute,
Columbia Presbyterian Hospital
New York, New York

Man Years:

Total:	2
Professional:	1/2
Other:	1 1/2

Project Description:

Objectives: Retinoblastoma is a malignant tumor of infancy and childhood which fortunately is often amenable to surgery. There appear to be several causes for retinoblastoma. Cases are generally sporadic but in approximately 10 percent there is a family history suggesting genetic basis. In addition, a number of reports have now appeared of retinoblastoma associated with gross chromosomal abnormality, generally of the D group. In these cases there is generally an associated physical abnormality in contrast to the other forms of retinoblastoma. Chromosome studies will be performed on select individuals and families to add further documentation to the role of gross chromosomal change and retinoblastoma.

Methods employed: Blood is drawn on selected patients and their relatives during a home visit for our psychometric evaluation of retinoblastoma. Material is sent to Dr. David Kitchen's laboratory for karyotype analysis.

Major findings: In none of the familial cases of retinoblastoma have karyotype analysis has there been evidence of chromosomal abnormality of a gross nature. In contrast several sporadic cases of retinoblastoma associated with other physical defects have been found with abnormal karyotype by Dr. Kitchen.

Significance to biomedical research and the program of the Institute: Confirmation of the role gross chromosomal change makes to retinoblastoma is vital in pinpointing the precise etiologic event of this form of cancer.

Proposed course: See "Methods employed".

Honors and Awards: None

Publications: None

Serial No. NDS (CF) 71 E 1929
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Genetic Study of an Irish Isolate in South Carolina

Previous Serial Number: None

Principal Investigator: Roswell Eldridge, M. D.

Other Investigators: Jeffrey C. Allen, M.D.
Kathy O'Meara
Charles Still, M.D.

Cooperating Unit: Department of Neurology,
University of South Carolina
Columbia, South Carolina

Man Years:

Total:	2
Professional:	1
Other:	1

Project Description:

Objectives: The Irish Travellers of Murphy Village, South Carolina are a unique Catholic isolate whose exact origins are unknown. The present population is approximately 1,100 of which 420 are under 20 years of age. The men receive income from spraying barns and laying linoleum. They secure their business by travelling in small trucks throughout the eastern United States.

The Village is of particular interest to us because of the apparent high rate of inbreeding which predisposes autosomal recessive traits.

Methods employed: At present the need is for precise demographic data such as population break down by sex and age. Patterns and family size would be of interest.

Genetic information may be best secured through conversation with responsible elders in the Village. Undoubtedly the group traces from a small founding population and precise information about this as well as information about members added to the group in recent years would be important.

The genetic relationship of this population to the present day Irish might be established by noting the gene frequencies with ABO, Rh and other accessible footmarkers.

Medical genetic studies most certainly focus on existing recognized familial problems such as mental retardation and skin disease. In addition, a comprehensive medical evaluation of the community and historical account of disorders in earlier generations would be worthwhile. Screening for newborns looking for recognized metabolic and chromosomal abnormalities might be productive.

Major findings: Preliminary information has already been secured regarding the background, demography, economy, social organization, education, medical problems and genetics. Several familial traits are present among the Villagers suggesting that a comprehensive survey would reveal other genetic traits.

Significance to biomedical research and the program of the Institute: Studies of such isolates have been unusually productive in the delineation and understanding of genetic entities. Examples include Victor McKusick's study of the Amish and Carl Witkop's study of the Wiesorts.

Proposed course: Considerable ground work has been laid for this project and now results await investment of time and personnel.

Honors and Awards: None

Publications: None

Serial No. NDS (CF) 71 E 1930
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Von Hippel-Lindau's Syndrome - Clinical, Genetic and Biochemical Aspects

Previous Serial Number: None

Principal Investigator: Roswell Eldridge, M.D.

Other Investigators: Jeffrey C. Allen, M. D.
Kathy O'Meara

Cooperating Units: Department of Neurology
Johns Hopkins Hospital
Columbia Presbeyterian Medical Center
New York

Man Years:

Total:	2
Professional:	1 1/2
Other:	1/2

Project Description:

Objectives: In 1911 Eugen von Hippel described a number of patients with retinal angiomatosis. In 1926 Arvid Lindau described a number of patients, who in addition to the retinal angiomatosis, had such intracranial lesions as cerebellar cysts and cerebellar medullary angioblastic tumors as well as pancreatic cysts, renal cysts and adrenal tumors. The familial nature of this syndrome has since been noted by numerous authors.

What has impressed us is the infrequency with which these tumors are bilateral. In contrast to other hereditary traits associated with neoplasm of paired structures the involvement is generally bilateral. Is the tendency to unilateral involvement in the Von Hippel-Lindau's syndrome real and,if so, does it reflect a different etiologic mechanism than seen in the other hereditary neoplasms? Or is the unilateral involvement only apparent, reflecting failure to scrutinize the presumably healthy member of the paired organs?

Methods employed: The propositi with Von Hippel-Lindau's syndrome will be ascertained through neurologic departments of selected medical centers. Contact with individuals and their families will be made through their personal physician, and home visits will be arranged for physical examination and detailed history.

Major findings: None

Significance to biomedical research and program of the Institute: The first step will be to document whether or not this syndrome tends to be unilateral in its involvement. Later more sophisticated laboratory studies can be undertaken to determine more closely the mechanism of abnormal gene action which produces the neoplasias.

Proposed course: See "Methods employed".

Honors and Awards: None

Publications: None

Serial No. NDS (CF) 71 E 1931
1. Collaborative & Field Research
2. Epidemiology Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Genetic study of population isolates in Maine

Previous Serial Number: None

Principal Investigators: Jeffrey C. Allen, M. D.
Roswell Eldridge, M. D.

Other Investigators: Morris Lambdin, M. D.
Ellsworth, Maine
Thomas Roderick, M. D.
Jackson Laboratory
Bar Harbor, Maine

Cooperating Units: Division of Public Health Nursing,
State of Maine Dept. of Health & Welfare
Maine Genetics Counselling Center
Ellsworth, Maine
Maine Medical Association; Lincoln, Sagadahoc,
Washington, Waldo and Hancock Counties

Man Years:

Total:	1/2
Professional:	1/4
Other:	1/4

Project Description:

Objectives: To investigate numerous genetic subisolates along the coast of Maine for evidence of hereditary neurological disorders.

Methods employed: A preliminary trip was made to establish contacts and examine the feasibility of the study. Thereafter an introductory letter and brief questionnaire was sent to every registered physician and public health nurse in the five major coastal counties. The questionnaire was designed to provide information on the awareness of any unusual familial disorders; diagnosed and undiagnosed, which are prevalent in remote areas of the state, especially on some of the offshore islands.

If feasible, demographic and historical data will be collected from State, County and local sources to corroborate the prevalence of genetic isolation.

Patients and their families will be visited and an appropriate referral will be made to local diagnostic facilities.

Major findings: So far, 69 of 119 (58 percent) questionnaires have been returned. Several interesting leads exist.

Significance to biomedical research and the program of the Institute: Maine's rugged eastern coast contains innumerable islands of varying sizes. Several of these have been inhabited for several generations and populations vary from 50 to 200 persons. Inbreeding is commonly practiced. On several islands, most of the inhabitants have only two or three different surnames. Because of the remoteness of the islands inhabitants have not availed themselves of the usual medical facilities. They are visited rather infrequently by a public health nurse or physician. The likelihood of finding recessive hereditary disorders is high in these populations.

Proposed course: Personal visits will be made to selected families. Genetic and demographic surveys will be conducted wherever appropriate.

Honors and Awards: None

Publications: None

ANNUAL REPORT

July 1, 1970 through June 30, 1971

Special Projects Branch
Collaborative and Field Research
National Institute of Neurological Diseases and Stroke
National Institutes of Health

Following the previous fiscal year's reorganization of the Special Projects Branch, four professional personnel were added to the staff: a fully trained neurologist and a staff associate to the Branch, a staff associate to the Head Injury Section, and a staff associate to the Epilepsy Section. Broad responsibilities of the staff included anticonvulsant drug program, support of activities of the Secretary's Advisory Committee on the Epilepsies, its sub-committees and task forces, the NINDS Ad Hoc Committee on Cerebral Death, the NINDS Ad Hoc Committee on Anticonvulsant Drugs, and the Head Injury and Epilepsy Information Services.

Section on Epilepsy

Meetings of the NINDS Ad Hoc Committee on Anticonvulsant Drugs were held in October 1970 and February 1971. The Fall meeting was held in Seattle, Washington, to enable the committee members to observe progress on the NINDS sulthiame research contract, and the potential for other related neuropharmacological research there. The Committee has continued to function in a highly efficient and useful manner providing review of research in progress, of proposals for new research contracts, and of the pharmacology of investigational anticonvulsant drugs. They suggested the priorities for proposed studies and other guidance to the section. One of the most beneficial effects of the Committee is the origination of suggestions from individual members--as the background of each member differs, a variety of views and ideas are presented for Institute follow-up.

During fiscal year, the pharmaceutical industry continued to cooperate with the section. Data on drugs from seven companies was provided and discussed by the NINDS Ad Hoc Committee on Anticonvulsant Drugs. Priorities were established for support of clinical trials for two investigational drugs. One will be conducted in a state hospital for patients with generalized epilepsy; the other in university clinics for patients with absence (petit mal) epilepsy. Preliminary studies will be employed to perfect anticonvulsant blood level methodology and determine metabolic information prior to the outset of full scale trials.

Research contracts for the study of ethosuximide in absence epilepsy at the University of Virginia and The Medical College of Wisconsin were completed in fiscal year 71. The study of ethosuximide in absence epilepsy has examined several hypotheses. The hypothesis that ethosuximide is not effective in control of approximately one-half of those patients who suffer from absence (petit mal) epilepsy is apparently correct when one defines

control as complete (100%) control. Ethosuximide has, however, improved virtually every patient in whom the drug has been tolerated. The hypothesis that failure of response to ethosuximide occurs in the presence of therapeutic blood levels has been demonstrated when response is defined as complete control. The hypothesis that patients who respond to ethosuximide will show no evidence of focal brain lesions is apparently true although some patients with brain lesions may also respond. Another hypothesis is that ethosuximide does not impair psychometric performance; evidence so far has shown that indeed ethosuximide does not impair such performance when therapeutic blood levels are not exceeded. There is no evidence to the present time that ethosuximide alters clinical manifestations of absence seizures; there is also evidence that many conventional measurements of seizure frequency do not provide a reliable assessment of seizure control.

An ancillary technique, that of EEG telemetry, has been developed during this study, and found to be the best criterion for measurement of control of paroxysmal abnormal discharge. The reliability of this technique is related to the duration of the individual seizure, the longer ones being more easily recognized. Currently, the telemetry study consists of continuous twelve-hour EEG recordings; the information is taken from FM tapes and evaluated at NIH.

The telemetered EEG has correlated quite well with the best clinical method used in the ethosuximide study to date, that is, observation by a trained observer. In almost every case, however, the number of seizures estimated by the observer is less than that ascertained by telemetered recording, strongly suggesting that the observer, regardless of experience, does not see every seizure. This telemetry technique will be utilized in subsequent studies. Detailed analysis of the data from the ethosuximide research contracts will be made at the conclusion of the study. An investigational anticonvulsant will be evaluated in this model upon the completion of the present phase.

Pursuant to a recommendation of the NINDS Ad Hoc Committee on Anticonvulsant Drugs, a contract was awarded for the clinical evaluation of the investigational anticonvulsant, sulthiame, in June 1970. The pilot study has been completed and a double-blind comparison of sulthiame and diphenylhydantoin is now underway. Sixty patients with a diagnosis of partial epilepsy will take part in the study. Each patient will be evaluated over a fourteen-month period. Important features of this research are accurate reports of seizure incidence, other drug effects, anticonvulsant blood level determinations, and measures of psychological performance and social functioning.

The Institute will establish a pharmacology laboratory within the Section on Epilepsy early in the coming fiscal year. Much planning has taken place to prepare for this activity, following its endorsement by the Ad Hoc Committee on Anticonvulsant Drugs. Harvey J. Kupferberg, Ph.D., has been recruited to head the laboratory. The metabolism and interactions of many anticonvulsant drugs will be studied initially.

Staff members collaborated with officials of the National Institute of Mental Health to plan an epidemiology survey for the prevalence and incidence of

convulsive disorders in seventh grade students in Washington County, Maryland. The children are to be examined in the summer of 1971; data will be evaluated in the coming fiscal year.

Two meetings of the Secretary's Committee on the Epilepsies were held. It was decided to sponsor two small closed workshops to examine in depth two neglected areas of interest to workers in epilepsy. A workshop on laboratory methods for preparing animal models of epilepsy will be held in November 1971. Another workshop to be held in November 1971 will examine many aspects of the epidemiology of epilepsy. A third event jointly encouraged by the Secretary's Committee and the Ad Hoc Committee will be a detailed examination of the pharmacology of antiepileptic drugs. World leaders in the field will present papers to an audience of clinicians so their treatment of patients will be enhanced. Unfortunately, much of the researchers' efforts have not yet been applied to the treatment of epileptic patients. A monograph will be published following the symposium.

The Section issued two publications for workers in the epilepsy field; a review of the literature on the effectiveness of marketed anticonvulsants, by James J. Coatsworth, M.D., a consultant to NINDS, and a review of the literature on anticonvulsant blood level determination methods and clinical importance by members of Branch personnel. Editorial support was provided in both instances by Branch personnel. Dr. Coatsworth's report is of especial interest, for it highlights the meager information available on anticonvulsants in use today, and the paucity of well-documented clinical studies that have been conducted in the past. NINDS-sponsored studies on anticonvulsants have been designed to overcome these shortcomings and will provide useful information on the drugs' efficacy and safety.

Epilepsy Abstracts is now in its fourth year of publication. The Excerpta Medica Foundation has assumed all publication and distribution responsibilities with modest support from the Institute. Epilepsy Abstracts Retrieval System (EARS), a free text search and retrieval system based upon Epilepsy Abstracts was proven a powerful research tool. Every word in the abstract is a key word permitting rapid thorough searches of the literature from any of a variety of terminals. EARS was demonstrated at the meetings of the American Epilepsy Society, American Academy of Neurology, and American Neurological Association. In each case, reception was most favorable. It is planned to have this retrieval system available to research workers throughout the country in the coming year.

A computer-based bibliography on epilepsy research from 1900 to 1959, with some 8,000 index citations, has been developed. It will be issued in the coming fiscal year and will compliment the published Epilepsy Abstracts 1947 to the present time.

The investigational anticonvulsant albutoin was compared with two marketed anticonvulsants, primidone and diphenylhydantoin, in a double-blind study at New Castle State Hospital, New Castle, Indiana, September-December 1970, under a NINDS research contract. The design of the trial was improved, based upon experiences of the 1969 trial, when albutoin was compared with phenobarbital. Accurate seizure records, blood level determination, and

other laboratory and ward data were recorded through the NIH WYLBUR computer system for ease in analysis. Plans are underway for a trial of another investigational anticonvulsant, carbamazepine, using the patient population at New Castle State Hospital, and methodology developed during the albutoin study.

Section on Head Injury

In response to a recommendation of the National Advisory Council, Neurological Diseases and Stroke, the NINDS Collaborative Study of Cerebral Death, a directed study under contract has been established. With guidance and support from the Office of the Associate Director for Collaborative and Field Research, the staff prepared comprehensive bibliographies and reprint collections with translations of the literature on cerebral death. These documents were provided to the NINDS Committee on Cerebral Death, and to prospective principal investigators who would collaborate under the contract mechanisms.

The overall objectives of the collaborative study of cerebral death are to verify or modify the current clinical and electroencephalographic (EEG) criteria for cerebral death in relation to patient age and cause of coma: to determine the minimal time that clinical and EEG criteria must be operative to indicate cerebral death in relation to age and cause of coma; and to assess the neurologic deficit of patients who recover after having fulfilled all criteria for some length of time. In addition to the clinical and EEG evaluations of patients, several contractors will conduct ancillary studies of cerebral death; these will include computer analysis of electroencephalograms, bedside EEG monitor, averaged evoked potential responses, electroretinograms, analysis of cerebrospinal fluid for chemical indicators of brain death, and detailed neuro-ophthalmological examinations.

Experience dictates that prior to embarking on a large-scale collaborative project of this type a pilot or feasibility study is needed to pretest procedural and technical elements of the overall study, to develop the statistical model, and to establish executive capability; specifically, its aims include the following: to determine whether the contemplated neurological, EEG, and other appropriate examinations can be adequately handled in a collaborative study, and to establish a basis for achieving standardization; to determine whether, in a collaborating hospital, any cases of suspected cerebral death would be missed, and if so, why? to develop means of determining when a clinical and EEG picture consistent with cerebral death is the result of sedative drug intoxication; to develop procedures for collecting, reviewing, editing and processing data for the later or definitive study; to evaluate means for guaranteeing uniformity in handling patients, making the necessary observations, and recording data for analysis; to determine the minimal significant set of clinical, EEG, laboratory and demographic observations that will be needed for each patient in the definitive study; to determine the best statistical model for describing patients suspected of cerebral death, so that the risks associated with an operating rule for determining brain death can be estimated most efficiently from data gathered in the study; to determine the sample size needed in the later study in order to obtain desired precision of estimates, with due regard for variability in the

patient population with respect to age and etiology of coma; and to create an executive capability that could be extended to the definitive study.

To date, the project has been in a planning phase and will not become operational until about September 1, 1971. Preliminary findings are expected to accrue in the coming fiscal year and major findings in subsequent years.

A thorough search of available literature in the area of cerebral death has clearly indicated that there is currently much disagreement as to which criteria are necessary and sufficient for the diagnosis of cerebral death and that so far no systematic attempt has been made to refine the existing criteria with respect to patient age, cause of coma, or length of time that the criteria must be satisfied. There are cogent medical, legal, and social reasons for establishing valid and refined criteria for cerebral death, and it is anticipated that these criteria will become widely accepted as a result of the collaborative study. Hence, families could be spared the emotional distress and financial burden of an unnecessary delay in the declaration of death in appropriate circumstances and at the same time be protected against undue haste in cessation of efforts at resuscitation. It is further possible that potential organ donors could be more readily identified and at an earlier time, resulting in more viable organs for transplantation.

Contract negotiations for the pilot phase of the study are scheduled for completion in June 1971. The project will then become operational on or about September 1, 1971, and run for approximately one year. At the end of the pilot phase it should be possible to make a decision concerning the advisability of continuing with the collaborative study. If a decision to continue is made, the investigators will meet and agree to a protocol for the remainder of the study. This later or definitive phase will proceed until a sufficient number of patients are studied to give valid statistical results, all data is collected and analyzed, and a final report is drafted.

Contractor's Project Director:

Dr. A. Earl Walker (University of New Mexico)
Dr. Reginald G. Bickford (University of California-San Diego)
Dr. David C. Poskanzer (Massachusetts General Hospital)
Dr. Francis E. McGee, Jr. (Medical College of Virginia)
Dr. Julius Korein (New York University Medical Center)
Dr. Benjamin Boshes (Chicago Wesley Memorial Hospital)
Dr. Wigbert C. Wiederholt (Ohio State University)
Dr. Lorenzo G. Runk, III (University of Pennsylvania Graduate Hospital)
Dr. Donald R. Bennett (University of Utah)

The head injury model construction program has been phased out. The data collected during the past three years is being reviewed and will provide the basis for a monograph on the mechanical properties of the head and neck.

The final report on the survey of head injured veterans of the Korean campaign has been submitted to the Director of the Institute.

The section is continuing its collection and classification of head injury literature. Its recurring classified bibliographies are being shared with more than one thousand interested investigators in the field.

CONTRACT NARRATIVE
Special Projects Branch--Section on Epilepsy
July 1, 1970--June 30, 1971

NEW CASTLE STATE HOSPITAL (PH-43-68-1310)

Title: Study of the Anticonvulsant Properties of Albutoin

Contractor's Project Director: Joseph T. Brock, M.D.

Current Annual Level: $90,000

Objectives: To study the relative anticonvulsant properties of albutoin, diphenylhydantoin, and primidone administered to patients with seizures refractory to treatment, and to evaluate the possible side effects of the drugs and drug blood levels.

Course of Contract: Patient trials began September 14, 1970. Forty-nine patients began the thirteen-week Latin cube experiment. The investigational drug albutoin was compared with the two marketed drugs, each for three week periods with two week intervals of regular medication. Clinical data was collected and sent to the Section on Epilepsy, NINDS, Bethesda, for review and preparation for computer-aided analysis.

Major Findings: Detailed analysis is now underway. A preliminary review of the clinical data shows when compared to patients' ordinary medications, single drugs are less effective in preventing seizures. There was a greater incidence of seizures and side effects with the investigational drug than with either diphenylhydantoin or primidone.

Significance to NINDS program and Biomedical Research: The pharmaceutical industry has demonstrated a little interest in developing new anticonvulsant agents. Aside from the economic factors involved, one of the industry's major problems is to obtain satisfactory clinical studies of anticonvulsant drugs. Through this contract and others, NINDS has supported clinical studies of anticonvulsant drugs. Well controlled studies will be significant indicators of therapeutic merit of new anticonvulsant drugs. It may encourage industry to develop its promising new agents for clinical trial. It is anticipated that through NINDS sponsored studies anticonvulsant drugs will reach the market more readily.

Proposed Course of Contract: The contract will be amended without additional funds to allow performance in FY72. Additional anticonvulsant trials are planned.

CONTRACT NARRATIVE
Special Projects Branch--Section on Epilepsy
July 1, 1970--June 30, 1971

MEDICAL COLLEGE OF WISCONSIN (NIH-69-2169)

UNIVERSITY OF VIRGINIA SCHOOL OF MEDICINE (NIH-69-2196)

Title: Study of Anticonvulsant Properties of Ethosuximide

Contract Project Directors: Philip T. White, M.D., (MCW)
Fritz E. Dreifuss, M.D., (UV)

Current Annual Level: $ 65,462 (Medical College of Wisconsin)
$ 53,474 (University of Virginia School of Medicine)

Objectives: To study the effect of ethosuximide on the frequency and intensity of petit mal epileptic seizures in patients previously untreated for this disease; to evaluate the effect of ethosuximide therapy on physiologic, psychometric, and other functions in human subjects.

Course of the Contract: Due to the difficulty in acquiring previously untreated petit mal patients, the contracts were extended to June 30, 1971. Each center will be successful, or nearly so, in reaching its goal of 20 patients. Data is collected for each patient during the initial hospitalization period, during an outpatient and during the final hospitalization period, and sent to the Section on Epilepsy, NINDS, Bethesda, for review and entry for computer-aided analysis.

Major Findings: The procedures and protocol established during the collaborative study of epilepsy were proven valuable in conducting an evaluation of an anticonvulsant agent. Detailed analysis of the clinical results will be made at the end of the study. EEG telemetry has been developed as an ancillary technique.

Proposed Course: The study will be extended to permit comparison of ethosuximide with an investigational anticonvulsant.

CONTRACT NARRATIVE
Special Projects Branch--Section on Epilepsy
July 1, 1970--June 30, 1971

UNIVERSITY OF MINNESOTA (NIH 70-2269)

Title: Development of a Gas-Liquid Chromatographic Analysis of Blood

Contract Project Director: Harvey J. Kupferberg, Ph.D.

Current Annual Level: $7,819

Objectives: To develop a specific GLC method for analysis of blood and CSF for levels of sulthiame, and for levels of carbamazepine.

Course of the Contract: The contractor has completed the project, providing methodology for blood level determinations of the anticonvulsants which are or will be studied in research contracts for clinical evaluation of sulthiame and carbamazepine.

Major Findings: The GLC methodology was readily adapted from the contractor's animal studies to preliminary patient studies. Information on absorption characteristics and biological half-lives of the drugs was also obtained.

Proposed Course: The contract has been completed.

CONTRACT NARRATIVE
Special Projects Branch--Section on Epilepsy
July 1, 1970--June 30, 1971

UNIVERSITY OF WASHINGTON (NIH 70-2281)

Title: Study of Sulthiame in the Treatment of Partial Epilepsy

Contractor's Project Director: John R. Green, M.D.

Current Annual Level: $106,597

Objectives: To study the relative anticonvulsant properties in partial epilepsy of sulthiame and diphenylhydantoin; to measure these drugs in patients' blood, and to assess patients' psychological competence and social function.

Course of Contract: A preliminary study of hospitalized patients was completed and enabled the contractor to undertake the long term study on October 20, 1970. More than half of the goal of 60 patients have been acquisitioned; each will be evaluated over a 14-month period.

Major Findings: The contractor will review and analyze the data at the conclusion of this two-year double blind study. The bioavailability of the specially prepared diphenylhydantoin was found greater than the commercial preparation the patients had been receiving. This necessitated a revision in dosage schedules to prevent toxicity.

Proposed Course: The contract will be amended with additional funds to allow completion of the second year planned when the contract was awarded.

CONTRACT NARRATIVE
Special Projects Branch--Section on Epilepsy
July 1, 1970--June 30, 1971

EXCERPTA MEDICA FOUNDATION (NIH-71-2018)

Title: Epilepsy Abstracts, Volume 4

Current Annual Level: $39,220

Objectives: To scan serial publications and periodicals from approximately 3000 of the world's biomedical journals, select appropriate articles to be included in Epilepsy Abstracts in accordance with the guidance of the Project Officer and his editorial advisors; prepare abstracts with appropriate translations into English from foreign languages, classify, index, and store the abstracts in a computer retrievable form; and produce a 9-track computer tape in the NIH-PRS format, to be delivered when Volume 4 is completed. The Excerpta Medica Foundation will produce camera-ready copy for each monthly issue of Epilepsy Abstracts, which includes an Index of subjects and authors, and will print and distribute the journal monthly, including a cumulative index at the end of the volume. In order to pay for the production of the camera-ready copy, the printing, and distribution, the Excerpta Medica Foundation will sell subscriptions to recover the cost of production of camera-ready copy, printing, and distribution.

Course of Contract: Following award of the contract, the Excerpta Medica Foundation promoted the subscription of Epilepsy Abstracts, and acquired 900 subscriptions at $15 each. At the close of this report period, subscriptions are still being acquired. The Excerpta Medica Foundation produced monthly issues as directed and have increased slightly the number of original articles abstracted.

Proposed Course of Contract: It is anticipated that all the remaining issues will be distributed as scheduled and that the computer tape will be delivered in accordance with the contract.

Serial No. NDS (CF)-71 SP 1932
1. Collaborative and Field Research
2. Epilepsy Section
3. Bethesda, Maryland

PHS--NIH
Individual Project Report
July 1, 1970--June 30, 1971

Project Title: Long-term electroencephalographic telemetry of patients with absence (petit mal) seizures.

Principal Investigator: J. Kiffin Penry, M.D.

Other Investigators: Roger J. Porter, M.D.
Fritz E. Dreifuss, M.D.

Cooperating Units: Department of Neurology, University of Virginia School of Medicine, Charlottesville, Virginia.

Man Years:

Total:	1.33
Professional:	0.33
Other:	1.0

Facilities and Equipment:

The telemetry equipment and FM tape recorder were provided by the Section on Epilepsy. An oscilloscope and the clinical research unit were provided by the University of Virginia Hospital at Charlottesville.

Project Description:

Objectives: The purpose of this study is to evaluate patterns of paroxysmal abnormal discharge in patients with absence (petit mal) seizures. Another primary objective is to determine if this method can be established as a primary mode of evaluating any absence drug.

Methods employed: Each patient has a 12 hour telemetered electroencephalographic recording on the clinical research unit of University of Virginia at Charlottesville. The telemeter equipment is placed on the patient's head by an EEG technician who also applies the electrodes. The patient is free to move about the ward and and the signal from the transmitters is taken to a dipole antenna and then to two receivers where two channels of EEG are fed to an FM tape recorder. Quality control is maintained by observation of the incoming data on the oscilloscope. The 12-hour recordings are taken back to the laboratory of the Epilepsy Section in Bethesda where they are played back at four times recording speed: Each 12-hour record is then read by a physician. Patterns of discharge and effects of anti-absence drugs are evident when the data is processed by a 360 IBM computer and the Cal Comp plotter.

Major Findings: The initial 12 patients with absence epilepsy have all shown some improvement on ethosuximide. Many patients have shown complete remission of paroxysmal activity. It was felt that this criterion of seizure control is probably the most objective available, and it is the intent of the Section on Epilepsy to incorporate this method in evaluation of investigational anti-absence drugs.

On further evaluation 16 patients with absence seizures have shown a decline in seizure duration with increasing age. While this is a confirmation of previously suspected phenomena, this is the first time that such information has been collected in other than an anecdotal way.

Significance to Biomedical Research and to the Program of the Institute: This study has applied telemetry techniques to improve the evaluation of clinical research in anti-absence pharmacology. The study has further suggested patterns of paroxysmal discharge in patients with absence seizures and points a way for further investigation of the mechanisms of absence epilepsy.

Honors and Awards: None

Publications:

Penry JK, Porter RJ, Dreifuss FE: Quantitation of paroxysmal abnormal discharge in the EEGs of patients with absence (petit mal) seizures, for evaluation of antiepileptic drugs. Epilepsia (Amst) 12: 1971.

Penry JK, Porter RJ, Dreifuss FE: Patterns of paroxysmal abnormal discharges in twelve-hour telemetered EEGs of untreated children with absence (petit mal) seizures. Neurology (Minneap) 21: 392, 1971.

Serial No. NDS (CF)-71 SP 1933
1. Collaborative & Field Research
2. Epilepsy Section
3. Bethesda, Maryland

PHS--NIH
Individual Project Report
July 1, 1970--June 30, 1971

Project Title: Quantitation of clinical manifestations of spike-wave activity by a reaction time method.

Principal Investigator: Roger J. Porter, M.D.

Other Investigators: J. Kiffin Penry, M.D.
Fritz E. Dreifuss, M.D.

Cooperating Units: Department of Neurology, University of Virginia School of Medicine, Charlottesville, Virginia.

Man Years:

Total:	0.5
Professional:	0.25
Other:	0.25

Facilities and Equipment:

Electroencephalographic equipment and space for testing were provided by the University of Virginia Hospital at Charlottesville. The reaction time equipment including seizure detection device and digital timer were designed and built by the Section on Epilepsy. The video-recording apparatus was provided by the Section on Epilepsy.

Project Description:

Objectives: The purpose of this study is to determine whether reaction time in absence patients is or is not impaired in a gradual fashion from the point of spike-wave initiation as has been suggested by some authors but disputed by others. There is some evidence for a "trough-like" pattern decrease of consciousness. The onset of decrease clinical functions during spike-wave paroxysms is evaluated by the reaction time method.

Methods employed: A device is employed which gives instantaneous recognition by voltage criteria that a spike-wave burst has started. This burst is of much higher than normal background, and this factor alone is used to electronically trigger the reaction timer. On instantaneous recognition the reaction timer is triggered and a tone is delivered to the subject. The subject responds by turning off the high pitch tone with a telegraph key. Between paroxysms the patient is maintained in a state of alertness by a program of approximately 10 random stimuli per minute. All the data is collected

by television, including a portion of the screen reserved for the reaction time from the digital clock. There was no age limits in selecting patients, but they must all have spike-wave paroxysmal discharge.

Major Findings: The study is not yet complete but preliminary findings suggest that patients maintain some ability to respond early during the paroxysmal burst; this responsiveness is frequently not seen 1-2 seconds after onset. Analysis of responsiveness during short bursts suggests that patients may retain a normal reaction time during such paroxysms.

Significance to Biomedical Research and to the Program of the Institute: This study has applied video recording techniques and sophisticated electronic methods to improve the quality of clinical research. Specifically, this study is an analysis of the relation of the patient's behavior to his EEG during paroxysmal electroencephalographic events. An understanding of this relationship is important--not only as a guidepost for further research in the mechanism of epilepsy, but also in determining the day-to-day therapeutics of the epileptic patient.

Proposed Course: The study will be continued with additional patients in the coming fiscal year.

Honors and Awards: None

Publications: None

CONTRACT NARRATIVE
Special Projects Branch--Section on Head Injury
July 1, 1970--June 30, 1971

NATIONAL ACADEMY OF SCIENCES (PH 43-64-44, Task Order 11)

Title: A 15-Year Follow-Up of Head-Injured Veterans of the Korean Campaign

Contractor's Project Director: Seymour Jablon, M.S.

Current Annual Level: No additional funds

Objectives: 1. To provide NINDS with the current addresses of the head-injured veterans participating in the study. 2. To select and locate matched controls. 3. To code the Red Cross interview schedules. 4. To key punch the Red Cross coded forms. 5. To key punch the original coded acute data for the so-called Meirowsky cases. 6. To obtain General Classification Test scores from retired records in St. Louis. 7. To prepare a data tape embodying original (acute) and Red Cross Interview information. 8. To prepare tabulations and analyses from the total material, relating to characteristics of the original wound and its treatment.

Major Findings: All 8 objectives were accomplished. A report has been submitted to the director, NINDS.

Proposed Course of Contract: This project will be concluded in fiscal year 1971.

CONTRACT NARRATIVE
Special Projects Branch--Section on Head Injury
July 1, 1970--June 30, 1971

WEST VIRGINIA UNIVERSITY (PH 43-67-1137)
CASE WESTERN RESERVE UNIVERSITY (NIH-69-2201)
UNIVERSITY OF WASHINGTON (NIH-69-2232)

Title: Determination of the Physical Properties of Tissues

Contractor's Project Director: Dr. Russell R. Haynes (West Virginia Univ.)
Dr. Albert H. Burstein (Case Western Reserve Univ.)
Dr. Colin H. Daly (Univ. of Washington)

Current Annual Level: $41,000 (West Virginia University)
$27,000 (Case Western Reserve University)
$30,000 (University of Washington)

Objectives: To determine certain physical properties of the tissues of the head (scalp, skull, dura, brain, fluids); and to develop mathematical solutions for various simple mechanical force problems relating to effect of force upon the head. These objectives represent Phase I and preliminary work on Phase IV of a program to construct and test accurate physical models of the head. Various physical properties such as bulk modulus, shear strength, tensile strength, compressive strength, etc., are being determined using specimens from human cadavers, autopsies, biopsies, and similar specimens from the Macaca mulatta.

Major Findings: This project period represents the fourth year of this collaborative study. The first year was generally devoted to developing test equipment, determining test parameters, establishing mechanisms for securing test specimens, and designing standardized test procedures. The second year saw the beginning of data collection. During the third year, both the University of Michigan and West Virginia University completed their determination of the relevant properties of the head and made considerable progress in the identification of substitute materials. In order to fill certain vital gaps in the data, two smaller contracts were let last year: to the University of Washington to determine the dynamic material properties of the cerebral vasculature and to Case Western Reserve University to evaluate the mechanical properties of the head-neck junction. During the current year preliminary data on the latter two contracts have been collected, and a final report is due in June 1971. A final contract was awarded to the University of West Virginia for a coordinated analysis of all the data obtained in this program. This data analysis and review is currently being studied by the project officer and will form the basis of a monograph on the mechanical properties of the head and neck.

Significance to NINDS Program and Biomedical Research: The data collected provide an essential basis for the study of cell and tissue reaction to mechanical forces, which is essential for understanding head injury.

Serial No. NDS (CF)-69 SP 1785
1. Collaborative and Field Research
2. Special Projects Branch
3. Bethesda, Maryland

PHS--NIH
Individual Project Report
July 1, 1970--June 30, 1971

Project Title: A 15-Year Follow-Up of Head Injured Veterans of the Korean Campaign

Previous Serial Number: None

Principal Investigators: A. R. Taylor, M.B., F.R.C.S.
S. Jablon, M.S.

Other Investigators: W. F. Caveness, M.D.
A. M. Meirowsky, M.D.
A. C. Dresser, M.S.W.
R. W. Hurt, M.D.
C. Kretschmann

Cooperating Units: National Research Council Follow-Up Agency, Washington, D.C.
American National Red Cross Service to Military Families, Washington, D.C.

Man Years:

Total:	0.4
Professional:	0.3
Other:	0.1

Projection Description:

Objectives:

1. To obtain information on employment patterns and the incidence of posttraumatic symptoms and epilepsy over the past 15 years in a group of head-injured veterans of the Korean Campaign.

2. To relate these findings to the initial data on the severity, therapy, and sequelae of the injuries; and to compare the status of the injured men to that of a group of non-injured control subjects.

3. By determining the incidence of seizures in the parents, siblings and children of the veterans, to see if a genetic factor might be involved in predetermining epilepsy after head trauma.

4. To identify those veterans who would be willing to participate in a comprehensive 15-year follow-up hospital evaluation.

Methods Employed: Acute data were supplied by Dr. Caveness and Dr. Meirowsky. The National Research Council Follow-Up Agency took a 30 percent sample of the injured group and selected non-injured controls who were in Korea in the same units as the injured men at the time the head injuries were sustained. Current addresses, induction AGCT scores and pre-induction employment of the injured and non-injured men were obtained by the National Research Council Follow-Up Agency. The staff of the Section on Head Injury obtained permission of the veterans for interviews. American National Red Cross Service to Military Families workers interviewed the veterans, members of their families, and their employers. The medical data were edited by the staff of the Section on Head Injury. The National Research Council Follow-Up Agency edited the employment information, coded the interview schedules, transferred all acute and follow-up data to a seven-track tape file, and prepared tabulations on which the final report was based.

Major Findings: The final report has been submitted to the director, NINDS. The following deductions were made about the future treatment of combat head injuries:

1. Investigation should be made into the possible ways of treating memory and concentration defects in line with present methods employed in speech therapy.

2. Positional vertigo, the common posttraumatic variety, should be intensively studied to uncover possible lines of treatment.

3. Head injured men should be educated, from the time of their first reception, not to fear the outcome and not to regard the brain as the "master organ".

4. They should not be segregated from limb and trunk injuries.

5. There should be a national organization to continue education in civilian life and be ever present to sustain the veteran when his symptoms occur or recur in response to stress.

Significance to Biomedical Research and the Program of the Institute: This study has provided data on employment and the posttraumatic state fifteen years following head injury.

Proposed Course: With submission of the final report, this project has been concluded.

Honors and Awards: None

Publications: None

ANNUAL REPORT
For Period July 1, 1970 through June 30, 1971
Perinatal Research Branch
National Institute of Neurological
Diseases and Stroke
National Institutes of Health

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ANNUAL REPORT
For Period July 1, 1970 through June 30, 1971
Perinatal Research Branch
National Institute of Neurological
Diseases and Stroke
National Institutes of Health

SUMMARY OF PROFESSIONAL OR SCIENTIFIC ACCOMPLISHMENTS

A. FINDINGS

A number of scientific findings that came to light from COLR data during this fiscal year can be highlighted in reference to the development of mental and motor performance in the growing child:

The proportion of variability in four-year IQ attributed to the genetic component (heritability) is lower among Negroes than among whites.

Any measure of social class is an important predictor of Stanford-Binet IQ at age four and is a significant determinant of mental and motor performance. Within both white and Negro children, those with the higher socio-economic index do better on the Binet at age four than those with the lower index. Higher IQ (Binet at age four) in either race is associated with higher educational level of the mother. Females have a higher IQ (Binet) at age four than males in either race.

The IQ in singletons seems to decline in the interval between ages four and seven years whereas the IQ of twins seems to show an improvement, suggesting that twins who usually perform more poorly than singletons during early life are able to "catch up" during childhood.

The offspring from interracial matings do not differ significantly in birth weight or birth length or in mental or motor performance at eight months from the offspring of intraracial matings of either race. However, by age four months and at one year the interracial offspring were significantly smaller than the controls, and at age four years, the IQ's of interracials were lower than either control. This suggests an adverse environmental rather than an adverse genetic influence on the interracial offspring which is not experienced by intraracial progeny.

Birth weight is better than gestational age in predicting mental and motor performance at eight months of age and of IQ at four years, independent of race, social class, or sex.

Head size at one year of age may be a good predictor of IQ at age four. There is a 50% chance of an IQ being less than 80 at age four for the one year male with a head size less than 43 cm, and for the one year female with a head size less than 42 cm.

A number of additional studies on twins and sibs speak to the physical development of the growing child:

Half sibs compared to full siblings show more RH incompatibility, seizures, congenital heart defects, mental retardation, club foot, and polydactyly. Evidence suggests that genetic factors play an important role in seizures and mental retardation and environmental (prenatal) factors in congenital heart defects and club foot.

Billington's hypothesis that sensitization of the mother during pregnancy against paternal antigens leads to non-pathological placental hypertrophy and increased birth weight in succeeding pregnancies has been supported and confirmed.

Respiratory Distress Syndrome occurs more frequently in twins than in singletons.

The relationship between type of twin placentation and zygosity was studied in 569 sets of twins. The perinatal death rate is 14% in either race. The frequency of congenital malformations was not higher in twin than in singleton deaths. Malformations in monochorionic deaths were multiple and lethal. Heavy infants and twins with large intra-pair birth weight differences were common in separate diamniotic-dichorionic groups; the fused di-di group had light infants and the lowest death rates.

In comparing somatic vs. visceral growth rates, histological study of the kidneys of 514 neonatal deaths and stillbirths revealed that the growth in birth weight and body length is faster in the Negro than in the white during the early phases of pregnancy, but visceral growth, as of the kidneys, proceeds at similar rates in both races, independent of whether the growing fetus is small or large for dates.

A few studies focused on the blood and vascular conditions in the growing child:

High RH antibody titers are not as highly associated with serious morbidity in the Negro as in the white progeny.

Neonatal polycythemia is associated with longer gestation, lower birth and placental weights, less placental pathology, and lower socio-economic status than controls. Polycythemic neonates do not differ from controls in psychological scores at eight months or in neurological findings at age one, but showed, especially among the Negro females, a lower four year Binet IQ.

Chorangioma cases show an increased association with neonatal thrombocytopenic purpura, toxemia, fetal hemangioma, erythroblastosis and single umbilical artery. The condition is commonest in whites and females. The prematurity rate of survivors is not different from that of single live births.

Neonatal Retinal Hemorrhage occurs in approximately 20% of vertex births. The possible relationship of such hemorrhage to birth weight, mental and motor performance at eight months, Binet IQ at four years and birth position is being investigated using matched non-retinal hemorrhage cases as controls.

Generalized skin hemorrhages at birth seem to be associated with subsequent marked hearing loss, speech and behavior problems and possible mental retardation. This apparent association is being investigated to assess whether it is spurious or valid.

Among 306 cases of single umbilical artery, in utero and neonatal deaths occurred in 13.8% of cases. Many of these deaths are due to congenital malformations. Compared to controls matched for race, sex, institution, birth weight, gestational age, and socio-economic index, the single umbilical artery group had six-fold more occurrences of velamentous and marginal insertions of the cord, slightly more neurologically abnormal cases but similar mean mental and motor scores at eight months and four year IQ scores.

The cumulative fertility rate, the perinatal death rate, birth weight of offspring, and gestational age of mothers with sicklemia were the same as in non-sickling mothers. The infant and child death rates, however, were higher in the sickling group.

An important finding with reference to cerebral palsy revealed that spastic and non-spastic prematures of equal immaturity do not differ in their Apgar scores, bilirubin levels, or history of birth trauma. Cerebral hemorrhage, however, is one suspected cause of spastic diplegia. In the effort to elucidate the etiology of cerebral palsy in the prematures, this carefully designed study lends no support either to the genetic or the anoxic or toxic (hyperbilirubenia) or traumatic or nutritional (intrauterine blighting) hypotheses. The only statistically significant difference between spastic and non-spastic prematures was a low post-natal hematocrit level in the spastics, and reason for this difference should be explored.

A few studies on virology and immunology pointed out that patients who had carcinoma of the uterine cervix were found to have increased amounts of antibody against Type II Herpes Virus two years before the clinical emergency of cancer. The Australian Antigen was detected in the blood of 600 cases of infectious hepatitis studied during an epidemic in the United States.

B. STUDIES IN PROGRESS

In addition, a considerable number of studies are currently in progress and are specifically described in the Individual Project Reports. It must be pointed out that all this research activity is separate from and has anteceded the research activity of the Ad Hoc Task Forces that came into being during the last fiscal year. Under the aegis of these Task Forces, the main data analysis activity may be highlighted as follows:

I. TASK FORCES

1) Labor and Delivery--This group is analyzing the characteristics of labor and delivery and its complications in relationship to perinatal loss, child development and neurological and intellectual deficits.

2) Infectious Diseases--The main objective here is the investigation of the role of infectious agents and particularly viruses which occur in pregnancy and during the perinatal period in the fetal deaths of subsequent child development.

3) Genetics and Congenital Malformations--The focus is the pursuit of genetic factors related to human development and especially neurological and intellectual deficits. Further, this group is assimilating information regarding congenital malformations and its epidemiology. The unique population of twins born to mothers in the Study will be followed to at least fifteen years of age so that observations under mental and physical development can be made to at least through puberty. The development of special protocols for the yearly examination of these twins and for the special neurological and psychological examinations at ages twelve and fifteen are currently under way. Furthermore, a chromosomal study of children from five Collaborating Institutions (Boston, Philadelphia, Buffalo, Tennessee, and Oregon) to relate major and minor chromosomal aberrations in these children to the outcome of pregnancy has been launched with the University of Colorado acting as the coordinator. In addition, a comprehensive study of sickle cell anemia to relate the occurrence of sickling in the mother with the outcome of pregnancy and to study the growth and development of children who are themselves sicklers is also under way. A proposal by Dr. J. V. Neel of the University of Michigan has been endorsed to establish genetic markers used in a variety of polymorphic blood loci for correlations with pregnancy outcomes; the necessary blood samples will be drawn and stored to be typed later. A request by Dr. Osborne is currently being considered by more than one NIH Institute to collect dental cast impressions on the seven year olds in the Collaborative Study with a view to assessing the timing of the occurrence of an insult in utero and its correlation with the emergence of specific morbidity in the child; this is part of a larger study of morphological and developmental asymmetries of interest to geneticists and teratologists.

4) Drugs in Pregnancy--This group studies the large number of drugs that are taken by women during pregnancy to explore whether these relate to any observed differences in fetal outcome including congenital malformations or subsequent child development.

5) One Year Task Force--The concern is the analysis of pre- and perinatal factors related to neurological abnormalities identified in one year old children. The one year outcome was classified in

specific categories in a mutually exclusive hierarchical order of disease conditions; these categories along with neurologically normal cases will be compared for frequencies of a large array of pre- and perinatal variables to detect associations. Instances where strong associations exist will be subjected to more definitive analysis.

6) Four and Seven Year Task Force--The objective is the analysis of pre- and perinatal factors related to neurological and intellectual deficits identified from four to seven years of age. The intent is to discover predictors of IQ at four years of age by means of multiple regression analysis. The number of variables will be reduced by obtaining a simple correlation of study variables with four year IQ. Variables lacking specific correlation will be eliminated from the regression analysis or analyzed separately. In addition a few composite indices made up of IQ and other four year performance tests will be subjected to regression analysis against the whole array of antecedent factors.

7) Three and Eight Year Speech, Language and Hearing Task Force This group is concerned with analysis of pre- and perinatal factors related to speech, language and hearing performance as judged in three year olds, and then again in eight year old children.

8) Physical Growth--This task force is concerned with the development of the physical growth profiles based on the physical measurements obtained on Collaborative Study children from birth on through age eight. Further, the group is looking at physical growth characteristics in children who differ by socioeconomic background and other developmental characteristics.

9) Toxemia--This task force is concerned with the evaluation of the utility of the clinical signs and symptoms which are part of the toxemia complex and its significance vis-a-vis perinatal mortality and prematurity. The First International Workshop on Clinical Diagnostic Criteria of Toxemia of Pregnancy scheduled in December 1971 is sponsored by the Perinatal Research Branch and shall present data from the Study as well as a proposed set of guidelines for the development of the theory of toxemia and its implications in clinical practice.

10) Pathology Task Force--This task force is concerned with the analysis of the brain specimens from children dying during the perinatal period to get insight into the kind and frequency of apparent brain injury identified from postmortem examination.

11) Basic Document--Frequency and cross tabulations of continuous, discrete and dichotomous variables are used to reveal the prospective relationship, if any, between selected obstetrical variables and mortality, birth weight and one year neurological outcome by race and institution.

12) Anesthesia-Analgesia--Gravida have been categorized into two groups, "normal" and those that are "not normal" as defined. The effect of anesthesia-analgesia in various combinations, as defined, will be tabulated in relation to selected outcomes from these two populations, to assess the effect, if any, of selected anesthesia-analgesia agents as administered. The protective effect of barbiturate against lowering Apgar by anesthesia will be investigated.

13) Epidemiological and Statistical Advisory Committee--This was established by the Perinatal Research Committee to offer merit review of study proposals as submitted by the various task forces prior to final approval.

II. OTHER STUDIES

1) The relationship between weight gain during pregnancy and the weight of the baby is to be investigated by the Primate Nutrition Study currently being funded jointly by the National Institute of Child Health and Human Development, the National Institute of Neurological Diseases and Stroke, and Johns Hopkins University. The pilot phase is to explore through experimental design the lead that came out from COLR data, confirming previous findings, that increase in maternal weight gain reduces the frequency of low birth weight children.

2) The Collaborating Institutions at Minnesota and at Oregon have been funded by the Office of Education to investigate the relationship, if any, between elementary school performance and antecedent pre-, peri-, and postnatal information available in the Project.

3) Dr. Rosenblith, in Providence, has established a neonatal behavioral scale working on the Providence sample of the Project population and is now validating it against the parameters of data collected at various endpoints in the Study. This behavioral scale, if validated on the population of the Project as a whole, will serve as an additional source of identification of high risk infants, especially in areas where more efficient or more accurate methods are needed than the medical examinations can provide.

4) In cooperation with the Collaborating Institution at Boston and through funds made available through the National Institute of Child Health and Human Development, under the scope of the COLR contract for data collection, a study of dermatoglyphics is under way to find out if normal progeny can be thus distinguished from abnormal progeny.

5) In cooperation with a member of NICHD, the Branch will carry out a multi-factor analysis of the contribution of various precursors to the occurrence of minimal brain dysfunction.

OTHER FUNCTIONS

The Branch, through the Office of the Chief, continues to monitor and overview the flow of activities in the following eight areas:

> Sample maintenance, overdue forms, early monitoring of data collected, quality control (inter- and intrainstitutional), form processing (editing, coding, punching, storage and magnetic tape), study proposals from the task forces as well as from individual investigators, merit review of manuscripts prior to publication, the Project Officer's role in contract negotiations and supervision of data collection in the Collaborating Institutions.

The Branch is initiating and exploring various modalities and patterns of enlisting and involving other Institutes within NIH in design and funding of a number of studies in cooperation with one or more Institutions in the field. The Institutes thus far involved are: National Cancer Institute, National Heart Institute, National Institute of Infectious Diseases and Allergy, National Institute of Dental Health, National Institute of Mental Health.

The Branch is investigating and exploring mechanisms of merit review and funding of studies based on local data analysis by the various Collaborating Institutions. This is needed after the sharp fiscal stringency that has come about during Fiscal Year 1972.

The Branch is making contact with several organizations to carry forward cooperative efforts. A joint Conference on Minimal Brain Dysfunction has been agreed upon among the CIBA Corporation, NICHD, and NINDS and has received approval for presentation under the auspices of the New York Academy of Science in March 1972. At this conference, the present status of theory, research, etiology, management, and treatment will be explored and will receive broad dissemination through the Academy's annals publication.

The Branch has also been negotiating with the Office of Education to carry out a study relating pre- and perinatal variables to learning disability in early grades. It has been agreed by the two units involved that the actual study be carried out during FY 1972. It is now being planned.

The Branch has held discussions with the Bureau of Indian Health, HSMHA, in regard to research on communication research--including audiology, treatment of the deaf, and prediction of stroke by analysis of dysarthria. Interaction and agreement on our prospective areas of interest and capability will continue.

The Branch has received a number of requests for reviews of grant applications by Branch members who are specialists in the area of perinatal research.

The Branch has carried out an analysis of its own and NINDS-wide research on the child aged 0-5, following a request by the Division of Research Grants, which is pursuing the question for the Director of NIH in response to the President's proposed initiative on children's health in that age group.

The Branch has received, and has either satisfied or is in process of accommodating, a number of requests for different kinds of data emerging from the Collaborative Study. Among these are The Bureau of Economic Research; Division of Epidemiology, Columbia University Medical School; Faculty of Law, Tel Aviv University; Vocational Counseling Associates; School of Public Health, North Carolina University; School of Business Administration, University of Washington; and Downstate Medical Center, State University of New York.

The Branch has carried out an advisory function for certain organizations. The Branch Chief was appointed to the Advisory Liaison Committee, American College of Obstetricians and Gynecologists, and to the World Health Organization's Expert Committee on the Prevention of Perinatal Mortality and Morbidity.

PROBLEMS

The imposed arbitrary cut of $2.1 million within the Perinatal Project during the coming fiscal year was at long last negotiated and absorbed. With the help of the Perinatal Research Committee, the Branch was able to adjust to this unwarranted cut both within its own internal operation and throughout the Collaborating Institutions. The collaborators showed an acute sense of responsiveness and loyalty despite the growing sense of anxiety and low morale amongst them. To adjust to this cut was an experience which involved considerable effort and time, and was most painful, to say the least.

PROGRAM DEVELOPMENT

During the fiscal year, the Branch has undertaken an analysis of research needs and capabilities for the purpose of developing a research program to follow the termination of data collection in the Collaborative Study. A five year plan is being prepared in this context to explore leads from the current phase of the Perinatal Program and to pursue new and other leads in the whole area of developmental neurology. This plan is to be reviewed for design and budget prior to Fiscal Year 1972. In its targeted research, the plan utilizes laboratory as well as field studies to explore in depth the effect of genetic, biologic, physical, and psycho-social factors on the developing nervous system. Implementation will not be limited to any one group of institutions or to many institutions at a time, nor will it be necessarily limited to any one funding mechanism.

A. SECTION ON OBSTETRICS

Report for the Period July 1, 1970 through June 30, 1971

I. SUMMARY OF SCIENTIFIC OR PROFESSIONAL ACCOMPLISHMENTS

With the active cooperation of 9 experts in clinical research on toxemia of pregnancy and selected members from the Collaborating Institutions, data from the Perinatal Research Study were analyzed for the association of toxemia of pregnancy as reported in the Study protocol with fetal and neonatal mortality and on the reporting of edema, proteinuria and blood pressures during pregnancy. This information was then used to design a new study, utilizing the original data on the clinical diagnostic criteria of toxemia. A chronological data basis for specified periods of pregnancy was established for blood pressures, proteinuria, edema, and their combinations, in association with the immediate and long-term outcomes of pregnancy. These data, in turn, are being used to define "iso-risk zones" of blood pressures, edema, proteinuria, and combinations of the same in the gravida for specified outcomes of pregnancy: live birth, birthweight, fetal death, neonatal death. From these findings, critical limits will be set for identification of cases with toxemia in the Study pregnancies. Our preliminary findings indicate that the epidemiologic approach to the study of toxemia may contribute to the resolution of some of the complex riddles of toxemia. The methodological problems involved in this study were presented and discussed at the annual meeting of the Swiss Obstetric Society. We are cooperating with Dr. E. Hughes, Chairman of the Committee on Nomenclature of the American College of Obstetricians and Gynecologists, on the definition and nomenclature of the toxemias.

Together with the members of the Task Force on Labor and Delivery, a study has been designed to determine the influence of specific labor and delivery factors on the fetus in terms of immediate outcome and later neurologic and psychologic outcome. In this study, a methodology developed by Dr. E. Friedman for the quantification of uterine activity and its deviations in relation to the phases of labor will be used to measure possible damage to the fetus, dependent on the dyscoordination or changes in the duration of any one or combinations of the phases of labor. The identification of gravidae with specified types of labor will produce several standard cohorts that may be used by other Study sections and task forces as variables for their respective special studies. The design of this study was completed in three workshops of the Task Force on Labor and Delivery.

Through the cooperation of Dr. K. Benirschke, the karyotypes of the surviving children with Down's syndrome in the Perinatal Research Study have been identified through skin and leucocyte cultures.

II. PROBLEMS

No problems except for the shortage of professional and clerical-statistical man power.

III. PROPOSED FUTURE OBJECTIVES

The studies on toxemia and labor and delivery will more than fully occupy the staff of the Section during the next fiscal year.

The experience gained in the collection of obstetric data will be employed in the planning of a comprehensive prenatal care organization.

B. SECTION ON PEDIATRIC NEUROLOGY
Report for Period July 1, 1970 through June 30, 1971

I. SUMMARY OF SCIENTIFIC OR PROFESSIONAL ACCOMPLISHMENTS:

A. Logistics:

Editing of data gathered from examinations of Perinatal Project children continues to occupy most of the effort of the Section on Pediatric Neurology. This data is provided from pediatric and neurologic examinations performed at seven years of age and is recorded on forms (PED-74, PED-75, PED-76). In addition, diagnostic summary forms are prepared (IDC-77). To date, 21,711 IDC-77 forms (the final step in the data recording process) have been completed. There is a small backlog of incomplete forms; 191 forms are in progress and 441 are ready for final, brief review.

Staff members serve resource and recorder functions on the Four- and Seven-Year Outcome Task Force of the Perinatal Research Committee (PRC). This Task Force is engaged in comprehensive analysis of perinatal and postnatal factors as they may affect the child's intelligence at four years of age.

Two staff members are serving resource and secretarial functions on the Physical Growth and Development Task Force of the PRC.

Members of the Section are participating in the One-Year Outcome Task Force. This task force, originating in the Office of the Chief, Perinatal Research Branch, will study associations between prenatal and perinatal events and the neurologic status of the infant at one year of age.

One staff member has been serving on the Basic Document Committee and for other task forces of the PRC.

Attending Quality Control examinations, conducted at the Collaborative Study insitutions, occupies 25% of the time of one staff physician.

The Section continues the task of maintaining a postcard inventory of examinations given in order to prevent cases from being lost to the Study.

B. Research Progress:

Accomplishments in research have been meager in the past year because priority for resources to analyze data has been assigned to task forces of the Perinatal Research Committee, and to the Basic Document effort.

A procedure for definitive analysis of Perinatal Research Branch data, designed in 1969, is still being programmed in the Office of Biometry.

The study of electroencephalograms of Project children was terminated by Perinatal Research Committee action.

II. PROBLEMS:

None can be mentioned.

III. PROPOSED FUTURE OBJECTIVES:

The analyses of the One-Year Outcome Task Force and the Four- and Seven-Year Outcome Task Force will occupy much attention in the coming year.

A research design to study spastic diplegia of premature infants is being developed. This study might be launched after completion of the present commitment to analyze project data which has been accumulated.

C. SECTION ON BEHAVIORAL SCIENCES
Report for the period July 1, 1970 through June 30, 1971

I. SUMMARY OF SCIENTIFIC OR PROFESSIONAL ACCOMPLISHMENTS

A. Research

1. Task Force. The major research effort has been devoted to the planning and execution of a study relating findings during pregnancy, delivery, infancy and early childhood to intellectual and motor performance at four years of age. This research is being carried out in collaboration with the Four and Seven Year Neurological and Psychological Task Force. Two hundred and seventy one precursor variables are being related to: (1) Stanford-Binet IQ; (2) the condition of low IQ (mental retardation); (3) a factor score representing overall performance on the four year battery which includes tests of concept formation and fine and gross motor abilities in addition to IQ; (4) a second score representing overall test performance which is characteristic of "brain damaged" children.

Completion of all analyses related to the first two outcomes is expected by the end of this fiscal year.

Individual research efforts are continuing in the areas of (1) item analyses of the four year battery, (2) heritability of intelligence as estimated from the study of twins and sibs, (3) outcomes in children from interracial and from consanguineous matings, (4) follow-up of children rated as dysfluent at three years of age. A study of the relationships of birthweight and gestational age to Bayley scores at eight months and IQ at four years has been completed and is being prepared for publication.

In the area of methodological studies, test-retest reliabilities have been established for all sub-tests and overall ratings in the four and seven year psychology batteries on two small random samples of children. (Ns = 140 and 228 respectively) The four-year results are now complete. This data is collected through the procedure of Inter-institutional Quality Control. All children are retested after an interval of approximately three months by an examiner from another institution. All trials (15 per year) are supervised by a psychologist from this Section. He observes the re-testing, records the data and discusses the results with the psychologists involved. In the four-year battery, the test-retest reliability for the Stanford-Binet is high and the retest-observer reliability, which reflects scoring agreement among examiners from different institutions, is very high. For the Graham-Ernhart Block Sort Test these reliability coefficients are moderate and very high respectively. For the three gross motor tests many of the relatively small number of children who fail on the first test, pass on the second test. This is also true for the four fine motor tests. For the Overall Behavior Rating, the "suspect" category appears to be quite unstable, (it is also small), with most of these children being rated as normal on the second test. This same tendency is evident in the "suspect" category

for the Overall Test Impression Rating.

In the seven-year battery test-retest reliabilities for the three WISC IQs, the Bender-Gestalt Test, the Auditory-Vocal Association Test, the Goodenough-Harris Draw-A-Person Test and the three sub-tests of the WRAT are high and the retest-observer reliabilities are very high. For the Tactile Finger Recognition Test, the test-retest reliability is moderate and the retest-observer reliability is very high. The same tendency observed in the four year sample for children who were rated initially as "suspect" in Overall Behavior and in Overall Test Impression to be rated the second time as normal appears in the seven year sample.

B. Data Collection

From the period 7-1-70 to 3-1-71, 567 four-year psychology examinations have been received in the Section. Data collection with this instrument has been completed. As of 3-1-71, 854 four-year examinations have been processed (edited). The current backlog of these examinations is three.

From the period 7-1-70 to 3-1-71, 3872 seven-year psychology examinations have been received. Projected to 6-30-71, 5472 such examinations will have been received. As of 3-1-71, 4015 seven-year examinations have been processed (edited). The current backlog of these examinations is 242.

II. Proposed Future Objectives

These are completion of the four-year study described above and planning in collaboration with the Task Force for a second comprehensive study using the results of the seven-year psychological test battery as end points.

III. Miscellaneous

Organizational Changes

The position of research psychologist vacated by Dr. Lee Willerman has been filled by Dr. Paul Nichols.

D. SECTION ON INFECTIOUS DISEASES

Report for period July 1, 1970 through June 30, 1971

I. SUMMARY OF SCIENTIFIC OR PROFESSIONAL ACHIEVEMENTS

The Section is organized into eight independent but integrated Units. The research activities are divided into four broad areas:

A. Large serological surveys of perinatal infections in support of the Perinatal Research Study.

B. Extended perinatal investigations, including both clinical studies and laboratory investigations based on leads from the Perinatal Research Study.

C. Brain tissue culture studies of perinatal infections and chronic neurological diseases.

D. Nutrition and infection in man and primates.

The Section published approximately 38 manuscripts and presented 42 papers during the present fiscal year.

A. Serological Surveys of Perinatal Infections

1. Serological Investigations Using Complement Fixation and Hemagglutination Methods

Emphasis has been centered on selected studies of pregnancies with abnormal outcomes and matched controls. Tests have been completed on patients with abortions, stillbirths, cataracts and microcephaly, along with matched controls, to determine the possible influence of virus infections in relation to these outcomes. Several of these studies have now been published; others are in press. In each case the multiple serum specimens from approximately 100 patients and 200 matched controls are used and more than 20 virus antigens are generally employed.

Similar studies now in progress include cancer of the cervix, repeat abortions and stillbirths.

Studies have included the use of Australia antigen to provide New information on hepatitis related infections. Recent tests have been initiated for Au antibody determinations.

2. Serological Tests Employing Tissue Cultures

Specific tests for cytomegalovirus, rubella, EB virus and other antigens are conducted in the laboratory for tissue culture investigations. This laboratory, under the direction of Dr. Fuccillo,

performs the specific neutralization tests as well as fluorescent antibody determinations for KB virus and has developed specific hemagglutination tests for herpesvirus, types 1 and 2, as well as cytomegalovirus. The laboratory has also been actively involved in the virus isolation studies to confirm the serological observations. This involved the attempted isolation of viruses from over 2500 placental specimens and an equal number of throat swab specimens as well as the laboratory support for the isolation of cytomegalovirus from the pregnant women under study at the Kaiser Hospital in Los Angeles and the children being studied in Baltimore and Frederick, Maryland. We are also actively involved in isolating rubella virus as well as other viruses from the tissue specimens obtained from experimentally infected animals and women immunized for rubella. Reports of these investigations are in press.

3. Immunoglobulin Determinations

To help identify congenitally infected children in the Perinatal Research Study, we are testing the 30,000 cord sera which are available for IgM levels. More than 2/3 of these have been tested and the remaining 1/3 should be completed in the next fiscal year. It would appear that approximately 1000 of the 30,000 will have elevated IgM levels. With these identified we will proceed with more specific testing for viral antibody in the IgM component of the serum. This will require the use of fluorescent antibody techniques and ultracentrifugation.

B. Extended Perinatal Investigations

Clinical studies have been in progress in four areas. These represent an extension of perinatal investigations based on leads obtained in the Collaborative Perinatal Research Study. Two of these investigations are funded by contracts.

1. Cytomegalovirus Infections in Pregnant Women

This is a three year study which is now in its third year of active work. Serial urine specimens are being collected at the Kaiser Hospital in Los Angeles. Pregnant women are studied for antibody as well as virus excretion throughout pregnancy and the children are examined and studied for the presence of infection at birth. Specimens are sent to the Section where the virus isolation and antibody determinations are actually conducted. Virus isolations are being made and the study is proceeding as expected. The investigation is supported under contract and is in collaboration with Dr. Margaret Jones, Professor of Pediatrics, UCLA.

2. Mycoplasma Infections in Pregnant Women

This investigation is now in its third year and is conducted in collaboration with the Naval Medical Center, Dr. Melvin Museles. Serial vaginal samples are obtained during pregnancy and tested in our laboratory for the presence of Mycoplasma and T-Strain infection. In addition, throat swabs and blood samples are obtained from the children at birth. Approximately 15% of babies are found to have Mycoplasm infection as are their mothers. The study also involves the attempted recovery of Mycoplasma from the ovaries of women undergoing hysterectomy. The first phase of work is being prepared for publication.

3. Serial IgM Determinations in High Risk Infants

High risk and low birthweight infants at the University of Tennessee are being studied under a contract arrangement with Dr. Sheldon Korones. Serial determinations of IgM are being evaluated as a means for identifying children with perinatal infections. The study is now in its third year. Reports from this study were published this year.

4. Longitudinal Study of Rubella-Damaged Children

This is an ongoing study of children at the Johns Hopkins Medical Center who are identified as having congenital rubella. The longitudinal observations have permitted the association of early second trimester rubella with deafness and peripheral pulmonic stenosis in the children. Follow-up is being continued to permit further identification of more subtle defects, such as mental retardation in association with perinatal infection. The study is funded under contract and the principal investigator is Dr. Janet Hardy. Several publications from this study appeared this year.

5. Experimental Animals

Investigations using pregnant rabbits with vertically transmitted rubella infection have demonstrated not only the transmission of the infection but the particular predilection of the virus for replication in the cartilaginous growing tissue of the fetus. These studies are being extended and because of the observations particular emphasis is being placed on human clinical material to determine if there is an unusual involvement of the cartilaginous tissue in fetal specimens of man as well as in rabbits. Recent work on the experimental production of vaginal herpesvirus hominis I infection in the Cebus monkey has been published. This animal system appears to be an excellent model for the study of vaginal and congenital herpes infections.

6. Volunteer Studies

Human volunteer studies are being conducted with the use of low temperature adapted vaccine material. The antigenic response and shedding characteristics of this vaccine preparation are being studied.

The effect of mannitol on the excretion of human cytomegalovirus was tested in volunteers. The drug was found to produce no increase in viurea or virus in the nasopharynx.

7. Drug Evaluation Studies

Because of the severe damaging effects of cytomegalovirus and Herpes simplex virus, studies have been conducted in conjunction with the Children's Hospital of the District of Columbia - Dr. Gordon Avery on the possible value of drugs for these diseases. These investigations are being reported. In addition investigations on the use of 5-IDU for congenital herpes infection continue in conjunction with Dr. A. J. Nahmias. We are planning a joint collaborative study with Dr. Nahmias and others.

8. Clinical and Experimental Transmission of Toxoplasmosis

Because of recent evidence on possible importance of the cat in the transmission of toxoplasmosis, an investigation was carried out in Puerto Rico on the possible shedding of toxoplasmosis by children in this incidence population. There was no shedding of T. gondii among these children, however serological studies demonstrated the high rate of infection which occurred primarily between one and three years of age. This report is now in press.

9. Hepatitis

Antigen studies utilizing the Australian antigen with complement fixation and gel diffusion tests demonstrated the high frequency of antigen among children with mongolism at the Pacific State Hospital. Comparative studies in other populations are now in progress as are investigations of the experimental transmission of the virus in monkeys and the development of improved techniques for antigen and antibody determinations. Congenital transmission of Australia antigen has been found to occur. An epidemic of infectious hepatitis involving over 300 patients has been studied. Au antibody has been tested for in mothers of mongoloid children. Reports of these studies are in press.

10. Immune Mechanisms in Perinatal Infections

It has become increasingly apparent that specific studies on the immune mechanisms of the developing fetus and newborn are needed so that we may better understand the deficiencies which permit congenital infection and

chronic infection to occur in the fetus and persist in the child. Experimental studies utilizing the hamster, rat and monkey are in progress in which combined antibody, delayed hypersensitivity and interferon determinations are made serially in infected and non-infected animals as well as in association with antigenic stimulation.

11. Cytomegalovirus - Serotypes Associated with Clinical Disease

At least three strains of cytomegalovirus appeared to be identifiable. The importance of the various strains in clinical disease is unknown. Comparative serological tests are now in progress in our laboratory to try to distinguish these strains and correlate this with the clinical findings in the patients. In addition because of the high frequency of infection and disease with cytomegalovirus in patients receiving immunosuppressive drugs and/or patients with leukemia, collaborative studies with investigators in the National Cancer Institute are in progress in which strains of cytomegalovirus are being isolated and compared to each other and the clinical findings in the patients. Reports are being prepared for publication.

C. Brain Tissue Culture Studies

With the isolation of measles virus from the brains of patients with subacute sclerosing panencephalitis, particular emphasis has been placed on the comparison of various strains of virus isolated from these patients and characterization of these strains in relation to wild measles virus and vaccine virus. In addition immunological studies have proceeded and demonstrate no specific immune deficiency in the patients with the disease. Additional reports on these topics have been published by the laboratory this year. Epidemiologic investigations conducted in conjunction with Dr. Jabbour at the University of Tennessee demonstrated that approximately one-half of the patients identified are in the southeastern part of the United States. Additional investigations are emphasizing Creutzfeldt-Jakob Disease, progressive multifocal leukoencephalopathy, multiple sclerosis and Parkinson's Disease, utilizing both biopsy and autopsy specimens. Work being reported this April demonstrates the presence of chronic suppressed measles infection in the lymph nodes of children with SSPE. This finding significantly changes the concept of the infection from one localized in the central nervous system to a generalized involvement. Immune suppression may be related to this disseminated infection.

II. PROBLEMS

The past year has seen a considerable turnover of personnel. Difficulties arose because of the loss of technicians and statisticians in the face of an increasing demand for testing.

Further, losses of technical personnel, either through resignation, maternity leave, or leave without pay, has slowed down the effort in the exploration of serum samples from the Collaborative Perinatal Program.

E. SECTION ON PATHOLOGY

Report for the Period July 1, 1970 through June 30, 1971

I. SUMMARY OF SCIENTIFIC OR PROFESSIONAL ACCOMPLISHMENTS

A. Project Activities and Material

1. Pathology Task Force

On October 7, 1970 the first meeting of the Pathology Task Force was held in Bethesda under the co-chairmenship of Drs. Stanley Aronson (Providence, Rhode Island) and Lewis E. Lipkin. The following pathologists, in addition to the co-chairmen, agreed to serve as members:

Dr. Shirley G. Driscoll (Boston, Massachusetts)
Dr. Floyd Gilles (Boston, Massachusetts)
Dr. Bernard Klionsky (Pittsburgh, Pennsylvania)
Dr. Haruo Okazaki (Rochester, Minnesota)
Dr. Edward P. Richardson, Jr. (Boston, Massachusetts)

The project neuropathologic material was considered in considerable detail by the Task Force. Its characteristics and the implications for data development from such specimens were explored. It was agreed in general that, in addition to modifications in the then current analysis and reporting procedure, the material warranted several in-depth studies. The latter are under exploration at this writing and will be noted under "III. Proposed Future Objectives."

2. Case Integration and Analysis

Following the last Task Force meeting, considerable effort was devoted to completing the procedural modifications begun last year (c.f. I,A,1. FY 69-70). In designing these changes, particular attention was paid to meeting the needs of the other project sections and the contributing institutions, while at the same time accelerating the initial review process. The danger inherent in such partial workup of an individual case is always the possible necessity for repeating the work at a later date. In reformulating procedures, relaxation of previously established standards was necessary in several areas. In particular:

a. The deliberate maintenance of ignorance of clinical aspects by the examiner was sacrificed.

b. The usual multiple stain examination of the same structure was deferred.

At this time only a limited number of tissue blocks are checked and only the H&E sections examined, rather than as previously all blocks and all four stained slides for each. A procedure for block selection based on gross photographs has been developed as has a new histologic protocol. The latter not only facilitates the examination, but serves to document the scope of the current examination, thus reducing future duplication.

3. Tissue Processing and Preservation

Almost the entire technician effort in this laboratory is devoted for the most part to:

a. The preparation of histologic slides from the paraffin embedded blocks.

b. The preservation of the remaining wet tissues which are regarded as "public documents" in the same sense as project forms or sera in other sections.

For long-term efficiency, we have been preparing a full complement of three special stains and one unstained blank for each block of every case. In order to meet the requirements of the accelerated review, it has been necessary to dispense with all blanks and special stains except in urgent instances, and to prepare only a single H&E slide for each block. During the first phase of this effort more than 100 cases were so processed making nearly 3,200 H&E sections available for examination later in the calendar year.

The remaining technician effort is largely devoted to regular checking and filling of storage containers (specimens are preserved in 80% alcohol when total embedding in paraffin is not feasible). Despite diligent efforts, no fully satisfactory bagging method is available. We therefore are in the process of transferring all specimens to specially selected and sealable polyethylene containers which will reduce the need for continual checking and filling.

B. Special Studies

1. Biologic Pattern Data Processing

This project was subject to two commendatory reviews during the past calendar year. The planned-for quantitative analysis of the parameters of chromatolysis has been begun and data regarding cell size and shape, quantity and dispersion characteristics of Nissl substance, nuclear and nucleolar eccentricity, nuclear size and shape, etc., are being determined on hundreds of chromatolytic and contralateral control neurons which have been automatically scanned. The simultaneous determination of

DNA content and synthesis rate in Feulgen stained radio-autographs is proceeding in tissues of a transplanted lymphoma, and about to be begun in embryonic neural tube. The marrow and blood cell identification component of the grain counting system being specified for NCI is in progress.

Extensive equipment additions and modifications have been accomplished without preventing the scanning microscope from being used for data acquisition on the above mentioned projects. These are detailed in the relevant individual project report, as are the numerous computer programs created, debugged and made available for general image processing use. (Serial No. NDS (CF)-65 PR/P 1278)

2. Twin Placentation in Relation to Zygosity

The relationship between type of twin placentation and zygosity was studied in 569 sets of twins in the Collaborative Study. Perinatal death rates, pathologic findings on deaths, birthweight, gestational age and birthweight differences were evaluated in relation to twin placentation. This paper has been published and the study is completed. (Serial No. NDS (CF)-68 PR/P 1650)

3. Kidney Malformations in Fetuses of AxC Line 9935 Rats

The rate of spontaneous genito-urinary malformations in AxC line 9935 rats is being determined. Randomly selected pregnant rats are killed at or near term and the mothers and fetuses were examined in detail for malformations involving the genito-urinary system. This paper has been published and the study is completed. (Serial No. NDS (CF)-69 PR/P 1763)

4. Placental Study of Abortion Material (Obtained by an Induced Abortion)

A detailed histological review of more than 100 specimens of induced abortion material was undertaken by Dr. Fujikura in cooperation with the Department of Anatomy at Kyoto University in Japan. The material was compared with the products of spontaneous abortions. The paper has been accepted for publication. (Serial No. NDS (CF)-69 PR/P 1764)

5. The Interrelationship Between Selected Congenital Malformations and Major Pathologic Findings

The relationship between selected malformations in autopsied deaths and major pathologic factors in the baby as well as in the mother and placenta are being analyzed. (Serial No. NDS (CF)-69 PR/P 1765)

6. Reproductive Ability of the American Negro with Sickling and its Public Health Implications

The reproductive performance of 654 sicklers and 1,890 nonsicklers in the Collaborative Study was compared. The cumulative fertility rate of mothers with sicklemia was the same as that of nonsickling mothers. Because of evidence that mothers with sickling have normal reproductive abilities, the gene will continue to propagate and a plea is being made that sickling tests be done on all Negroes. (Serial No. NDS (CF)-69 PR/P 1766)

7. The Significance of Chorioangiomas

A review of 81 histologically verified cases of chorioangioma showed an increased association with neonatal thrombocytopenic purpura, toxemia, fetal hemangioma, erythroblastosis, and single umbilical artery. There is a distinct female preponderance and the condition is more common in whites. The prematurity rate in the surviving group is not different from that of single live births in the Collaborative Study. The paper has been published and the study is completed. (Serial No. NDS (CF)-69 PR/P 1769)

8. Pathologic Effects of Ligation of the Anterior Spinal Artery and/or the Great Radicular Artery in Monkeys

Spinal cords of monkeys which had been subject to ischemia by means of surgical ligation of the anterior spinal artery and/or great radicular artery. The ligation of the anterior spinal artery just below its anastomosis with the arteria radicularis magna of Adamkiewicz produced clinical paraplegia and severe necrotic changes in the spinal cord caudal to this level. The ligature of either the arteria radicularis magna or the anterior spinal artery above the anastomosis produced anoxic changes which might be only temporary and were not reflected by serious clinical deficits. These findings are significant and of practical application in case one of these arteries must be sacrificed or is involved in a pathologic process. The publication is in preparation. (Serial No. NDS (CF)-69 PR/P 1772)

9. Thrombocytopenic Purpura and Placental Hemangioma

This combination heretofore unreported and found in two cases in the Collaborative Study is discussed. The paper has been published and the study is completed. (Serial No. NDS (CF)-70 PR/P 1858)

10. Mental and Motor Development in Monozygotic Co-Twins with Dissimilar Birthweights

Eight-month mental and motor series as well as four-year I.Q.'s of 125 sets of identical twins in the Collaborative Study who had unequal intrapair birthweights showed no difference in these parameters between co-twins. Since the lighter of monozygotic twins presumably suffers drastic nutritional setbacks in utero compared to its co-twin, these findings suggest that the human brain is fairly resistant to the effects of intrauterine malnutrition. Submission of this paper for publication is being suspended pending data analysis of the seven-year I.Q. (Serial No. NDS (CF)-70 PR/P 1859)

11. A Follow-up of Children with Single Umbilical Artery

A follow-up study of 355 cases of single umbilical artery is being undertaken with particular emphasis on the somatic, mental and motor development of those who have reached four years of age. (Serial No. NDS (CF)-71 PR/P 1911)

12. Birthweight in Relation to Renal Glomerular Development and Gestational Age in Whites and Negroes

A histologic evaluation of the kidneys of 514 neonatal deaths and stillbirths in the Collaborative Study was made and the findings assessed in relation to birthweight and gestational age. Racial differences are discussed particularly in regard to birthweight and in infants whose birthweights do not match gestational age. (Serial No. NDS (CF)-71 PR/P 1912)

13. The Clinical Signifiance of Generalized Petechiae at Birth

In a pilot study about a quarter of the infants diagnosed as having had generalized skin hemorrhages at birth, had significant changes in the form of marked hearing loss, speech and behavior problems and, one instance of mental retardation accompanied by distinct visual impairment. On the hypothesis that significant skin petechiae could be accompanied by hemorrhages in internal vital structures such as the brain, inner ear, retina, etc., a careful evaluation of the balance of these children is being made and findings compared with those of matched controls. (Serial No. NDS (CF)-71 PR/P 1913)

C. Other Activities

There have been numerous presentations of work done within the section. Presentations at the first Biological Congress, the SPIE, and the New York Microscopical Society have covered various aspects of the work in image processing. The last meeting in May 1971 listed three individual talks by involved personnel.

The Head of the Section has served as contract officer for the collaborative program with the Artifical Intelligence Group of the National Bureau of Standards as described in the individual project report. The Section Head continued his working relationship with the Image Processing Group, DCRT and the National Cancer Institute.

II. PROBLEMS

A. Personnel

The basic problem of a contracting, financial and personnel base and the concurrent desire to evaluate the overall nature of the pathological material has made for a serious problem. The need for additional professional personnel is undoubted, and can only partially be met by developing work-type task forces. Additional professional personnel will require additional filing and clerical help since slides alone are examinable at times at faster rates than they can be refiled even with our present relatively minisucle professional staff.

III. PROPOSED FUTURE OBJECTIVES

A. The accelerated overall case review has and will continue to receive first professional and technical priority within the section. At this writing, the results of the first 35 microscopic examinations completed under this revised procedure are under review, in order to assure ourselves that no information essential for other sections or the Collaborating Institutions is likely to be slighted.

B. Several in-depth studies are in the planning stage. These include:

1. A population study of selected obstetric variables within the autopsy population in order to elicit suggestive structures, lesions or patterns to be specifically searched for within the material. Dr. Rudolf Vollman, Head, Section on Obstetrics, has agreed to collaborate with us in this study.

2. We are hoping to interest members of the Pathology Task Force in participating in studies of such characteristic lesions of the newborn period as the Virchow or Banker lesions of the periventricular white matter. These lesions are of particular interest, and our material represents a unique opportunity for a definitive study of such changes.

3. Collaboration with the Task Force on Genetics in an extensive study of sickling. This will largely be under the direction of Dr. Froehlich who has for several years pointed out the importance of such work in the context of our placental material.

C. We continue our plans to apply the technics developed by the biologic image processing project to the project neuropathologic material. Current work on neurocellular degenerations such as chromatolysis will provide the basis for such future applications.

F. SECTION ON EPIDEMIOLOGY AND GENETICS

Report for the period July 1, 1970 through June 30, 1971

I. SUMMARY OF SCIENTIFIC AND PROFESSIONAL ACCOMPLISHMENTS

During the past fiscal year the major effort of this Section was directed towards organizing the task force on Genetics and Congenital Malformations; implementing the comprehensive plan for analysis of genetic and socioeconomic data which was developed by a panel of genetic experts last year and was adopted and extended by the task force on Genetics and Congenital Malformations; reorganizing and reorienting the previous data analysis efforts; improving the quality of the data and completing our data file.

The Section has continued to receive, edit and code information on the Family Health History Review form (FHH-9). During this period, approximately 6,000 FHH-9 forms were received and approximately 5,000 have been processed and sent to punch. The FHH-9 is being used to derive a new socioeconomic index for comparison with that derived for each family seven years earlier. For this, the family income item is being rescored with a more realistic ceiling of $15,000 rather than $10,000 used on the Socioeconomic Interview form (SE-1). This will require recalling of all schedules already processed but it is anticipated that they can be rescored without undue delay.

The most significant event in the operations of this Section during the last fiscal year has undoubtedly been the creation of task forces to plan and direct all data analysis in particular areas. The task force responsible for the analysis of genetic and socioeconomic data is that of Genetics and Congenital Malformations, which at present is composed of Drs. Richard Osborne, Kurt Hirschhorn, Walter Nance, William J. Schull, and Ntinos C. Myrianthopoulos, assisted by professionals from the Office of Biometry, and the Perinatal Research Branch. The task force idea is not new to this Section. As reported in last year's Annual Report, this Section had already convened a panel of genetic experts to discuss problems of genetic analysis and help develop a comprehensive and workable analysis plan. That group had been most successful in developing such a plan and the newly-created task force adopted it into, and has proceeded to implement it and extend it in special areas outside the protocol.

Thus the efforts of this Section toward data analysis are vigorous and the prospects seem good. Some projects have been completed, some are now in progress, some have been approved for immediate implementation, and some are now being planned. These are described in detail in the individual project reports. One part of the genetic study of intellectual and motor performance which is being done in collaboration with investigators at the University of Minnesota has been completed. Among some of the interesting preliminary results of this study are that the within family environmental variance is larger among Negroes than among whites, which means that the heritability of mental performance is lower among

among Negroes than among whites; and that the comparison of the 4-year to the 7-year IQ scores shows an improvement in twins but a decline in singletons which may mean that the twins who usually perform poorer than singletons during their early period of life, may be able to catch up during childhood and adolescence. Dr. Naylor's study with Dr. Warburton on the effect of parity, maternal age and change of mate on placental weight and birthweight has also been completed and appeared in the January issue of the American Journal of Human Genetics.

The task force, operating always on the premise that the major contribution of genetic analysis will be in the area of special studies, has approved a number of new projects. Among these are, a comprehensive study of congenital malformations, a genetic study of obstetric variables, a study of ABO and Rh incompatibility in pregnancy wastage and infant survival and a study of heritability of twinning in man.

The task force during their meetings and discussions took the position that analysis of Collaborative Study data should not constitute an end in itself but should be used to locate areas in which specific problems lie. These problems should then be pursued as logical extensions of the objectives and scope of the Collaborative Study. In accordance with these guidelines the task force recommended to the Perinatal Research Committee that the unique population of twins born to mothers in the Collaborative Study be followed to at least 15 years of age so that observations on their mental and physical development can be made at least through puberty. The Perinatal Research Committee approved the proposal and the task force is now in the process of developing special protocols for yearly examination of these twins and special neurological and psychological examinations at ages 12 and 15 years.

The task force has approved the participation of this Section in a chromosomal study of children from five collaborating institutions to relate major and minor chromosomal aberrations to abnormalities found in these children. The participating institutions are in Boston, Mass., Philadelphia, Pa., Buffalo, N.Y., Memphis, Tenn., and Portland, Ore. Dr. H. Lubs of the University of Colorado is acting as a coordinator for the study. The task force has approved the participation of this Section in a comprehensive study of sickle-cell anemia to relate the occurrence of sickling in the mother with the outcome of pregnancy and to study the growth and development of children who are themselves sicklers. The task force has also endorsed a proposal by Dr. J.V. Neel of the University of Michigan to establish genetic markers using a variety of polymorphic blood loci for correlations with pregnancy outcomes. Because of difficulties with funding of such an extensive project it was decided to draw and store the necessary blood samples now and type them at a more convenient time later. Since there is a reasonable probability that the effects of these genes as well as that for sickle-cell anemia may be influenced by the degree of black ancestry the task force recommended that a simple estimate be made of skin color as an index of this ancestry for control purposes.

The task force on Genetics and Congenital Malformations has been in

constant contact and collaboration with other task forces with common interests in data analysis. Dr. Myrianthopoulos and Dr. K. French of the University of Oregon are collaborating with the task force on four and seven year examinations to provide the demographic and socioeconomic description of the population; a joint study is planned with the task force on physical growth and development of the physical growth and development of twins; advice and help has been provided with the analysis of some of the speech and hearing data to the appropriate task force; and suggestions have been made to the task force on infectious disease concerning new approaches for analysis of the relationship between vital antibodies and congenital malformations.

Dr. Naylor has been instrumental in completing three files of special genetic interest. One is the consanguinity file whose codes have been exhaustively hand-reviewed. All efforts are now being made to resolve code discrepancies, expecially in second and subsequent pregnancies. Another is the record linkage file which involves over 6,000 women who reported having close relatives participating in the Study. This has now been completed and all the information on the women and their reported relatives has been put on tape. A hand review of another file, that of interracial matings has also been completed and this file now contains 199 cases with known outcomes.

II. PROBLEMS

The major problem which has confronted the Section during the past year has been that of the shortage of trained professionals to direct and participate in data analysis. As in past years, the Section has tried to resolve this problem by inviting qualified professionals from within and without the Collaborative Study to participate in data analysis. This plan has for the most part been successful but is no substitute for the constant presence of the investigator at the Section headquarters and his immediate access to all data.

III. FUTURE OBJECTIVES

The future objectives of the Section are to implement the analysis of the genetic and socioeconomic data of the Collaborative Study according to the plan of genetic analysis which was developed with the help of genetic experts and the task force on Genetics and Congenital Malformations. Although the plan calls for collaboration with investigators and institutions outside the Collaborative Study, there is no doubt that the planning and direction of research must come from this Section. A second objective, therefore, is to strengthen our professional staff in the Section.

At the same time the Section, with the advice and guidance of its task force is continuously trying to identify specific areas of fruitful research from the first round of analysis, which, although not within the protocol, should be pursued as logical extensions of the objectives

of the Collaborative Study. Considerable progress in this area has already been made.

IV. MISCELLANEOUS

A. Personnel

The personnel of the Section on Epidemiology and Genetics consists at present of the following: Professional, Dr. N.C. Myrianthopoulos, Head, Dr. A.F. Naylor, geneticist, Miss T.L. Martin, statistician; one secretary, five statistical assistants. During the last year this Section lost two positions, that of Miss A. Baszynski, fieldworker, and that of one secretary. The Section is now recruiting for a fieldworker whose duties would be to assist in the follow-up of the twins to age 15 years.

B. Activities of the Section Head and the Professional Personnel

The Head of the Section, Dr. Myrianthopoulos, in addition to his formal duties has continued his independent investigations in the genetics of neurological disorders, especially the lipidoses, and has contributed chapters in several textbooks on these topics. Dr. Myrianthopoulos has maintained his affiliation with George Washington University School of Medicine as a Clinical Associate Professor of Neurology and Director of the Genetic Counseling and Research Center. He is a member of the Medical Advisory Board of the Huntington's Chorea Foundation, a private organization which supports research in Huntington's chorea, and of the Committee upon Huntington's Chorea of the World Federation of Neurology. Dr. Myrianthopoulos has been elected a member of the Academy of Medicine of Washington, D.C.

In September, 1970, Dr. Myrianthopoulos attended the Fourth Annual Workshop of the World Federation of Neurology Research Group on Huntington's Chorea in Munich, Germany, where he chaired the session on Genetics and Epidemiology and discussed the current status and future prospects of research in Huntington's chorea. In January, 1971, Dr. Myrianthopoulos also attended a meeting of a special committee of this research group in Louvain, Belgium, during which plans were made for the Huntington Centennial Symposium in Columbus, Ohio for April, 1972 and for the preparation of a complete bibliography on Huntington's chorea. In addition, Dr. Myrianthopoulos helped to set up a special study of Huntington's chorea in the low countries and the north of France. Dr. Myrianthopoulos also attended the annual meeting of the American Society of Human Genetics in Indianapolis in October, 1970, where he presented a paper on respiratory distress syndrome in twins.

Dr. Naylor participated in all of the meetings and discussions of the task force on Genetics and Congenital Malformations and has been instrumental in designing many of the ongoing studies, often doing the programming himself. As mentioned earlier, he also designed and completed three data files of special genetic interest. Dr. Naylor attended the annual meeting of the American Society of Human Genetics in Indianapolis in October, 1970, where he presented a paper based on Collaborative Study

data adducing evidence for a powerful parity effect in spontaneous abortion.

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G. SECTION ON DATA MANAGEMENT AND RETRIEVAL
Report for Period July 1, 1970 through June 30, 1971

I. SUMMARY OF ACCOMPLISHMENTS

This Section is responsible for receiving, coding, filing and storing forms in accordance with a system designed to facilitate form and/or data retrieval. The Acting Head of this Section has continued in this assignment from the Office of Biometry until June 1, 1971, at which time Dr. B. H. Fox Asst. to the Chief, Perinatal Research Branch became the Acting Head. He also supervises the systems analysts and programmers providing services to PRB from the Office of Biometry to aid in data analysis. The current data file consists of 58,806 registrants -- of this number 56,177 were core study cases. Of these core study cases 53,837 have delivered.

Approximately 3.4 million forms will have been submitted by June 30, 1971. The available data from approximately 3.1 million of these forms has been processed into over 5.9 million punch cards in 151 card formats and converted into active computer tape files. 49,637 births have been reviewed at 1 year of age, 43,888 at 4 years of age and 32,194 at 7 years of age.

Personnel staffing, with the expected decrease in volume of study forms received, decreased from 33 to 27. Personnel have been encouraged to take advantage of training being offered through career counselling and self-improvement requests in an effort to permit a continued smooth transition.

During the year, work was continued in extending the development and use of complete program packages to provide an extended Variable File System with update and editing in conjunction with PRB operated master files and data banks, thus reducing future cost of operations. In addition several standard programming packages have been utilized during this fiscal year period for both studies and statistical analysis of data greatly facilitating this type of work previously done by other methods. This included the use of Express III System, Data Check System, Table Maker 2, Inquiry Response System, and Remote Terminal programs. Personnel have been trained in remote terminal operation for providing appropriate services.

The research activities of PRB were supported with special information retrieval for case selection, tabulations, and creation of subfiles of the Variable File System. Also a major effort has been accomplished with the production of quantities of tabulations for large-scale studies involving considerable amounts of data, utilization of dedicated equipment, extensive systems analysis and complex data processing. A major project was the production of Monograph II in addition to major assignments received from the Research Task Forces organized last year. Supplementary production of tables for the Basic Document was also produced during the year.

The Forms Accountability Program for review of study forms was continued with the objective of maximizing of all recoverable data for inclusion in the PRB master files and data banks. A quality review system of file folders has continued with review of coding, organizing and binding of forms in the file folders which facilitate their use while preventing loss or misfiling of individual study forms. This has been extended by the initiation of file clerk review of folders for organization of forms in a standard order and identification and refiling of misfiled forms. An inventory control card system is completed summarizing the status of each file folder and included in the file.

II. PROBLEMS

The Section, which contains a large number of non-professional staff has continued shifts of assignment of personnel including retraining where required to meet program needs. Continued efforts for retraining and placement of personnel will help in problems that can be expected to arise with a reduced workload in data collection.

III. PROPOSED FUTURE OBJECTIVES

Continuation in the development of sophisticated information retrieval-systems to supply research needs more rapidly is planned with the integrated use of dedicated computer equipment and maximation of remote terminal operation. The conversion and retraining of staff will continue so that present personnel can be reassigned to functions as required. New systems development are planned to be continued which will further effect reduced turnaround time on study requests as well as economy of operation. For example, improved file structure systems tailored to groups of studies with increased flexibility of use are planned.

H. SECTION ON PROJECT SERVICES

Report for the period July 1, 1970 through June 30, 1971

1. SUMMARY OF ACCOMPLISHMENTS

The Section on Project Services, Medical Literature Unit, is responsible for the acquisition of selected medical and statistical textbooks, abstracts, scientific directories, atlases, annual publications of books, and periodicals, and the organizing and indexing of published literature on all medical-scientific subjects, which in any way contribute to the various studies undertaken by the Perinatal Research Branch. The present book collection in the PRB Reading Room numbers 1082. Subscriptions to leading journals and abstracting periodicals pertinent to the program now total 102. In the interest of economy, a number of journals not being utilized to the optimum were discontinued during the past year. Up-to-date listings of journals and new book acquisitions are now in the process of compilation and will soon be circulated to Branch personnel. In addition, our locater file, containing a listing of books by author and title, now numbers 1109, and controls the books housed in the various sections of the Branch, readily retrievable upon request.

Approximately 1200 visits were made to our Reading Room this past year. Its facilities were utilized by PRB personnel, consultants, task forces, and others.

The activities of the Unit are wide and varied and encompass the following functions:

Provides a Reading Room and maintains a lending library.

Processed books and/or journals borrowed from PRB Reading Room collection; processed book, journal and photocopy requests, through NIH-NLM Library, procuring for Branch personnel, items not available in PRB Reading Room; xeroxed from journals and/or books, articles of interest pertinent to the program, and circulated these to the staff; provided a quick service for acquisition of literature, urgently needed and not available in-house, from NIH Library. In most instances material was procured the same day.

Xeroxing on the premises of approximately 25,100 pages of material, requested by PRB Staff.

Dissemination of material and information pertinent to the Program and in answer to a variety of inquiries and requests from the scientific and lay community. This was accomplished by correspondence, by telephone and by mail.

Periodic mailing of NINDS publications and reprints relevant to the Study and of possible interest to PRB Staff, Project Directors, Consultants, Perinatal Research Committee and Task Forces.

Annual distribution of prepared lists regarding new acquisitions of books, journals and abstracts, etc. to those utilizing our facilities.

Literature searches for specific articles, subjects or authors as requested by investigator.

Compilation and maintenance of mailing list for annual distribution of Bibliography of Collaborative Perinatal Project Publications.

Coordination and compilation of Annual Report in proper format for submission to NINDS Director.

Book ordering for PRB Reading Room.

Compilation of Bibliography of Collaborative Perinatal Project Publications. This is updated annually at the end of each fiscal year and includes publications based on pooled core data, local core data, and non-project studies of special interest to authors at PRB and those from collaborating institutions who are paid in part or in toto from Project funds. During the past year a Quarterly Bibliography of all COLR publications, with summaries, was initiated to enhance the visibility of our research effort on a more concurrent basis than is possible with the Annual Bibliography.

Collection of publications by authors from the collaborating institutions prior to 1965 which fall into the following categories: publications using Project population, publications of personnel supported in part or in toto by Project funds, and ancillary studies.

Indexing reprint collection. These are reprints in the perinatal area of critical interest to our scientists. They are retrievable on demand by author, subject and number. Subject headings parallel those used in Index Medicus.

Semi-annual preparation of bibliography of Branch personnel publications for inclusion in Scientific Directory and Annual Bibliography, NIH.

Clearinghouse for scheduling of meetings and conferences in the PRB Reading Room. During the past year 84 meetings were hosted and on occasion information was gathered and material xeroxed and collated for use at a specific meeting.

Reorganized our Collaborative Project reprint file for instant accessibility and retrieval of material.

Arranged tours of National Library of Medicine and Clinical Center for those foreign visitors to the Branch who were interested and also collected literature of interest for them; arranged for the loan of equipment through NIH Audio Visual Unit for use by PRB Staff members, etc.

II. PROBLEMS

Backlog is the big issue and continues in the following areas:

Subject indexing of reprints for Reprint Retrieval File
Cutting, annotating and filing of reprints
Collection of COLR reprints published prior to 1965 from collaborating institutions in our continuing effort to update the file.

III. PROPOSED FUTURE OBJECTIVES

Will continue to concentrate on giving service and meeting the needs of the Branch in literature retrieval, special reference searches, compilation of bibliographies, etc., as requested.

I. SECTION ON SPEECH, LANGUAGE AND HEARING
Report for the Period July 1, 1970 through June 30, 1971

I. SUMMARY OF ACTIVITIES AND DEVELOPMENTS

A. Routine

1. Processing of 3-year Speech, Language and Hearing (SLH) forms. Status: essentially completed (151 forms received last year).

2. Collection of 8-year SLH data. Status: continuing at six collaborating instititions.

3. Processing of 8-year SLH forms. Status: continuing (13,711 forms edited to 2-28-71).

4. Supervision of inter-institutional quality control. Status: continuing on trimester basis.

5. Project site visits by Section Head as Project Officer. Status: continuing.

6. Collection of data for Language Organization Scales. Status: continuing at quality control visits.

B. New

1. Development of forms and method for machine tabulation and analysis of quality control results. Status: essentially completed.

2. Organization of SLH Task Force for data analysis. Status: completed and in operation.

   a. Section Head functions as coordinator, Speech Pathologist as secretary for SLH Task Force.

3. Development of data analysis plan. Status: first steps taken.

4. Analysis of concordance in case identification by exams at 8-years, 7-years and 3-years. Status: completed.

5. Tabulation of first 5000 8-year exams by test, institution, race, sex, birthweight, I.Q. and education of gravida. Status: completed.

6. Tabulation of cases with dysfluency, preliminary to devising study proposal. Status: completed.

7. Preliminary look at cases with significant petechiae and abnormal speech, language or hearing findings. Status: in operation.

C. Discontinued

1. Eight-year SLH exams at 6 institutions (New York Medical College, Columbia-Presbyterian Medical Center, Medical College of Virginia, Philadelphia Children's Hospital, Brown University, Charity Hospital of New Orleans). Reason: recommendation by the Perinatal Research Committee.

2. Updata of 8-year SLH manual and forms. Reason: completed.

D. Non-Project

1. Consultant and training activities by Section Head serving the National Center for Health Statistics regarding hearing tests in the National Health Survey.

2. Active participation by the Speech Pathologist as Chairman and member of the NINDS EEO Advisory Committee, and member of the EEO Task Force whose task is to organize and implement an Affirmative Action Plan for NINDS.

3. Participation by the Speech Pathologist as panel member at the annual convention of the American Speech and Hearing Association in New York, November 18, 1970.

4. Appointment of Section Head as NINDS member of the Committee on Hearing, Bioacoustics, and Biomechanics (CHABA) of the National Research Council-National Academy of Sciences and attendance at its annual meeting at Cape Canaveral, March-April, 1971.

5. Project Head serves as resource person in Speech, Language and Hearing areas for NINDS.

II. PROBLEMS

A. Personnel needs.

1. Language Specialist (Speech Pathology or Psychology)

2. Secretary

3. Statistical assistant (one statistical assistant was transferred to another branch to achieve promotion; this section now is comprised of one statistical assistant, one Speech Pathologist, one Audiologist-Section Head).

III. OBJECTIVES

A. Development of an effective plan for data analysis, leading to the disclosure of relationships between:

1. Perinatal conditions and communicative disorders:

2. Test results at 3-years and at 8-years;

3. Inter-instutional disparities and causative factors;

4. Communicative disorders and learning disabilities.

CONTRACT NARRATIVE
Perinatal Research Branch — Section on Pediatric Neurology
July 1, 1970 through June 30, 1971

WAYNE STATE UNIVERSITY (PH 43-68-669)

Title: Maternal Amino Acid Level as Related to Fetal Birthweight of the Infant

Contractor's Project Director: Kamram S. Moghissi, M.D.

Current Annual Level: $18,000

Objectives:

To investigate relationships in pregnancy between dietary intake of protein, blood amino acid, protein and globulin fraction and weight gain of gravida and dimensions of newborns and Bayley test.

Course of Contract:

The last contract year of the study has ended as of January 25, 1971. The workscope of the project was more than accomplished. There remains only to complete developmental testing of babies as they reach 8 months of age; and analysis of all individual amino acids on those cases tested at 8 months as they are completed.

A new finding of particular interest is an association between branch chain amino acids and the 8-month Bayley test scores.

Major Findings:

The following publications have resulted from the study:

1. Churchill, J.A., Moghissi, K.S., Evans, T.N. and Frohman, C.: Relationships of Maternal Amino Acid Blood Levels to Fetal Development. Obstetrics and Gynecology. 33: 492-495, 1969

2. Moghissi, K.S., Churchill, J.A., and Frohman, C.: Relationships of Maternal Amino Acid Blood Levels to Fetal Development. In Perinatal Factors Affecting Human Development. Proceedings of the Special Session held during the Eighth Meeting of the PAHO Advisory Committee on Medical Research, Washington, D.C., June 10, 1969, Washington, D.C., Pan American Health Organization, Sci. Publ. No. 185, 1969, pp. 16-19.

Significance:

The study may furnish a means by which to identify gravida's providing sub-optimum amounts of nutrients to the developing infant. Nutrient supply may improve the outcome of these pregnancies.

Proposed Course of Contract:

Study is in last contract year.

CONTRACT NARRATIVE
Perinatal Research Branch - Section on Infectious Diseases
July 1, 1970 -- June 30, 1971

JOHNS HOPKINS UNIVERSITY SCHOOL OF MEDICINE (PH-43-68-710)

Title: Long-range effects on the fetus of certain maternal infections during pregnancy

Contractor's Project Director: Janet B. Hardy, M. D.

Current Annual Level: $22,207

Objectives: The project is designed to study the long-range effects of certain maternal infections during pregnancy on fetal outcome with observation of the identified children for a period of at least seven years. Serologic specimens and pertinent clinical information relating to perinatal infections and pediatric clinical findings are being obtained longitudinally from approximately 2100 children. Handprints for dermatoglyphics are available from approximately 800 children with particular emphasis on children with congenital infections. Serial serum specimens are available from approximately 300 children with definite or suspect congenital infections and complete data and specimens are available from the Johns Hopkins Comprehensive Care and the Frederick County Study of cytomegalovirus, rubella and toxoplasmosis. Clinical data and longitudinal follow-up is available for approximately 200 non-Collaborative Study children with congenital rubella.

Major Findings: A list of the publications assisted at least in part from this contract includes a number of papers. Of particular importance is the data published on cord immunoglobulin levels from 2750 cases. Additional important data has been generated concerning age specific distribution of certain infections in the inter-city population as compared to the Frederick County population. This study included one phase of analysis of buccal smears for sex chromatin. The rubella studies have been extremely productive.

Significance to NINDS Program and Biomedical Research: The present study of the long-range effects of certain maternal infections on the fetus provides the longitudinal observations necessary to define the complete spectrum of fetal damage caused by these agents. Combined clinical and laboratory observations permit the analysis of the long-term follow-up data.

Proposed Course of Contract: The total contract period is designed to complete the collection of clinical and laboratory data pertaining to cord immunoglobulins; age specific distribution of certain infections (cytomegalovirus, rubella and toxoplasmosis); effect of perinatal toxoplasmosis; dermatoglyphics and Barr Body studies; and longitudinal studies of children with congenital rubella.

CONTRACT NARRATIVE
Perinatal Research Branch - Section on Infectious Diseases
July 1, 1970 — June 30, 1971

UNIVERSITY OF CALIFORNIA (NIH 69-4)

Title: Cytomegalovirus infections in pregnancy

Contractor's Project Director: Dr. Margaret Jones

Current Annual Level: $33,300

Objectives: Study of maternal and congenital infections with cytomegalovirus and herpesvirus hominis utilizing serologic techniques as well as virus isolation from urine and tissue specimens.

Major Findings: During the second year of the contract approximately 1287 patients have been enrolled. To date 3140 urine specimens and 4308 blood specimens have been collected. In addition, 7 abortion tissue specimens have been obtained. A total of 685 throat swab specimens have been collected along with 410 cervical swabs and 159 cervical smears. The sera are being tested for antibody to cytomegalovirus and herpesvirus hominis.

Serology specimens taken through December 1970 have been tested for antibody to cytomegalovirus. The rate of seroconversions for CMV approaches 1% in the first 1500 women. These results are being reconfirmed; checked for Herpes I and II; tested for cord IgM; analyzed in relation to virus shedding, time of pregnancy and clinical findings in the child.

Significance to NINDS Program and Biomedical Research: Congenital cytomegalovirus infections can cause severe fetal damage and death. The present study is designed to determine the time during pregnancy in which maternal infections may result in congenital disease. The data will also define for the first time the frequency and persistence of maternal and fetal infections.

Proposed Course of Contract: The contract will provide the continued study of pregnant women. We anticipate being able to study 400 patients during the third contract year, bringing the total number of patients studied to 2000. Specimens of urine, blood and tissue will be forwarded, as in the past, for laboratory study of the time of infection during pregnancy and the trend of congenital infection.

Serial No. NDS (CF)-57 PR/ID 402
1. Perinatal Research Branch
2. Section on Infectious Diseases
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Serological and Virus Isolation Studies of Infectious Diseases in the Collaborative Study on Cerebral Palsy, Mental Retardation, and Other Neurological and Sensory Disorders of Infancy and Childhood.

Previous Serial Number: Same

Principal Investigators: Dr. John L. Sever, PRB, NINDS
Dr. David A. Fuccillo, PRB, NINDS
Mrs. Anita Ley, PRB, NINDS
Mrs. Renee Traub, PRB, NINDS
Mrs. Mary Ruth Gilkeson, PRB, NINDS

Other Investigators: Dr. Gabriel Castellano, MBA
Dr. Janet Hardy, Baltimore, Maryland
Dr. Sheldon Korones, Memphis, Tenn.

Cooperating Units: Collaborative Institutions in the Perinatal Research Study
Laboratory of Infectious Diseases, NIAID
NICHD
Cooperating Institutions in California and Hawaii (with the Section on Infectious Diseases)
Microbiological Associates

Man Years:

Total:	3.5
Professional:	5.5
Other:	8

Project Description:

Objectives: The purpose of the infectious disease investigations in the Perinatal Research Study is to determine insofar as possible the role of infections in the production of abnormal pregnancy outcomes. To accomplish this, serial specimens are taken throughout pregnancy, at delivery, and at six weeks postpartum. These sera are being tested with antigens to determine the antibody responses of the patients during pregnancy and postpartum, and then to relate this serological information to the clinical data for the pregnancy and the child. In addition, serum specimens from the children at one year of age were obtained from the last 10,000 study pregnancies and

these are now being studied. In special cases when congenital infection was suspected on the basis of clinical or laboratory findings, throat swabs and blood specimens were obtained from the children.

Methods Employed: To accomplish this program, blood specimens were obtained from pregnant women at set intervals throughout pregnancy and postpartum. Initially, during 1949, the collection of blood specimens was made once every trimester. Late in 1960, the requirements for the collection were increased and strengthened so that blood specimens were taken at the time of registration, at set intervals of approximately every two months throughout pregnancy, at delivery, and at six weeks postpartum. At the same time, a uniform method for the collection and processing was adapted as a required study procedure. The use of special vacutainers, sterile technique, special sterile vials, and new shipping containers were all required. Intensive laboratory training sessions were held at NIH by the Section on Infectious Diseases for all technicians in the collaborating institutions. These sessions were repeated each year and a training film was prepared and sent to collaborating groups on request. Careful, complete control was mainatined on all collection and processing of serum. At the Serum Center of the Section, personnel checked every vial of serum as received for quality and quantity. Regular reports of this information were sent every three months to each study institution. Telephone calls were made immediately to Project Directors whenever there was a decrease in the quality and quantity of sera received. Completeness of sets of specimens from each patient was also reviewed by Serum Center personnel. To improve the collection of complete sets of sera, a special reporting form was devised for the collaborating institutions and monitored by the Section.

By the spring of 1961 all institutions attained the minimum requirement of 90% satisfactory specimens for quality and quantity. Satisfactory quality was defined as straw colored sera with no hemolysis. Satisfactory quantity was a minimum of 4 vials, each with 3 ml of serum and proper labels. At each meeting of the Project Directors a report was given concerning the quality and quantity of specimens submitted by each institution. This detailed intensive review was conducted throughout the entire time of sampling of sera from the mothers and continues at present with specimens being received from children at one year of age, special study specimens, and sera from cooperating groups.

Completeness of sets of serial specimens was determined from the sera submitted to the Serum Center of the Section and the special reporting form sent to the Section. Data for the first 28,386 patients showed that there was at least one specimen submitted for 94.2% of the patients. The majority of the omitted specimens occurred during the first months of 1959. A computer analysis of specimen set completeness was prepared.

With the development of a firm base for obtaining the required specimens and tissues, the program embarked on a large commitment for the development of necessary antigens, new techniques, and the training of a competent laboratory staff for the study of the specimens. Antigen development was

conducted by the personnel of the Section and experienced investigators under contract. Professional laboratory personnel were selected for the Section's Units on Statistics and Design, Serology, Virology, Experimental Animal Research, Immunological Studies, Epidemiology, and Experimental Pathology and Neurology. Three years ago the Unit on Cytogenetics was added. This program is jointly sponsored by the National Institute of Child Health and Human Development and the National Institute of Neurological Diseases and Stroke.

To document the occurrence of an infection, two approaches are available: 1) Isolation of the microorganism from the patient, or 2) Detection of an antibody rise in serum specimens. Both approaches are being used in the present studies. Appropriate isolation procedures for virus, bacteria, and protozoa are being used with throat swab specimens from the children. This approach was not used for detecting infections in the mothers since it would have required obtaining throat swab and anal specimens at the time of each infection. When this has been tried in the past, it has been unsuccessful because the women are usually unable to come to a laboratory for the collection of specimens when they have minor illnesses. Furthermore, a great many of the infections under consideration frequently do not result in significant illness of the women. These subclinical infections go unnoticed and unrecognized. For these reasons, the serological approach was selected. By collecting serial serum specimens, antibody levels for various viruses and protozoa can be determined. The development of antibody to a microorganism in a patient who was previously antibody negative provides indirect evidence for infection. The presence of specific antibody indicates prior exposure to that antigen or microorganism.

The serological test most frequently employed in these studies is the complement fixation method. This basic method has been used for many years as the Wasserman test for the diagnosis of syphilis. With the use of viral antigens the test is very versatile, performed rapidly, and provides broad coverage of a great many of the more than 125 viruses which are known to be of importance to man. Antigens were prepared for most of these viruses. Tests of specificity were conducted with animal sera. For man, considerable data is available from our studies and those of many other laboratories to indicate that both group and specific reactions occur with these antigens. The adenovirus CF (Type 2) antigen, for example, is group reactive and provides evidence for adenovirus infections in general. To date, there are 31 adenoviruses recognized for man. Rubella CF antigen on the other hand is very specific and detects infection with rubella only. The sensitivity, specificity, and persistence of the test is also known. With this type of information, it is pissible to design serological studies for the viruses and protozoa. Bacterial antigens are usually not available for specific serological tests. Only direct evidence for these infections can be used. The history of infection as reported by the patient has proven to be quite unreliable in most cases and is used only in general information.

The majority of initial serological studies are conducted with the use of the complement fixation method. All tests are reproduced completely and a minimum of 90% agreement within twofold variation is required. All sera

showing significant change in antibody, together with any sera which did not reproduce, are tested a third time. For more specific testing or confirmation of these results the hemagglutination inhibition and neutralization methods can be used. These latter serological procedures are very specific and are also employed for follow-up testing whenever the initial studies with the complement fixation method suggest the need for further investigation.

Major Findings: A total of 140 complement fixing antigens have been developed. Approximately three-fourths of the antigens have been thoroughly evaluated and are now being applied in routine testing of study sera. The development and maintenance of large quantities (1,000 ml) of satisfactory antigens for 50 viruses is an integral part of the investigation being carried on by the study. The other antigens are receiving intensive developmental work and 20 of these antigens are under test for specificity. Specific control antisera have been prepared for 90 microorganisms. In addition, to provide improved safety, extensive work has been conducted on the inactivation of the live virus antigens.

The serological studies are being conducted in accordance with three major study designs: First is the epidemiological studies to determine the frequency of virus experience among study populations. Specimens from representative patients at study hospitals are being tested for evidence of antibody. By studying these specimens, it is possible to establish the frequency of antibody and change in antibody titer to each virus. The data for each hospital is then analyzed in relation to other information from the Collaborative Perinatal Study, and in relation to other information from epidemiological data concerning the seasonal occurrence of abnormal pregnancies and children.

The second and most active category of study involves the selection of particular microorganisms for intensive testing. Studies of this type have involved, for example, the testing of sera from a large number of patients for antibody to toxoplasmosis or rubella. Intensive studies are now being conducted utilizing antigens for Australia antigen influenza A, mumps, cytomegalovirus, herpes simplex, and rubella. Patients identified as having serological evidence for an infection are then grouped and clinical data for these groups and the remaining patients and their children are compared and analyzed.

Third, studies are designed to obtain maximum data concerning the virus experience of patients with abnormal pregnancy outcomes, and "matched controls." The results of this type of study are then analyzed in terms of differences in frequency of antibody and antibody change among the abnormals and matched controls. The matching of the patients include factors which are known to influence virus experience, such as time of the year during which the specimens were obtained, race, age, number of living children in the family, and geographic location of the patients. These studies are conducted when a sufficient number of abnormals of a particular type have been identified so that statistical analysis might establish valid information. The initial studies were directed at abnormalities which are relatively frequent, such as abortions, stillbirths, and neonatal deaths. The less frequent abnormalities

or those which cannot be recognized in infancy or early childhood are being studied as greater numbers of these patients are identified in the Collaborative Study population. These studies have included spastic diplegia, CNS malformation, cranio-facial abnormalities, and mongoloid infants.

Collaborating Studies: The primary deficiency of data in the Study has long been recognized as the late registration of Study patients. Since only 20% of the patients register during the first trimester of pregnancy, it is impossible to document adequately the infectious diseases experience of patients during the first trimester. To provide data on the first trimester of pregnancy, one additional Collaborative Study was joined with the program of the Section on Infectious Diseases.

Study of Viral Infections in Pregnancy
Dr. Margaret Jones, UCLA and Kaiser Hospital in Los Angeles, Calif.

In addition to this study, the collaboration with the Perinatal Study in the Kaiser Hospital in Oakland with Dr. Yerushalmy has been an integral part of the program since its initiation in 1959.

Significance to the Program of the Institute: The use of micro-serological techniques for a large group of new viruses provides an opportunity to investigate the course of human disease caused by viruses which are either difficult to isolate or are resistant to evaluation because the clinical effects are delayed until a long time after infection has subsided. This is particularly true in the case of birth defects. The application of this tool of analysis is providing valuable information on the epidemiological aspects of virus infections.

Proposed Course of the Project: The serological program will continue to be expanded in terms of antigenic materials and the performance of tests.

As additional abnormal pregnancy outcomes are reported, these will be added to existing studies on abortions, stillbirths, neonatal deaths, congenital malformations, and mongols. New studies will include congenital malformations of various types and low I.Q. at four years of age.

A specific two-year commitment has been made to complete tests on 4,000 abnormals and 4,000 controls using 10 antigens. The PRS will identify the specifically abnormal children and the laboratory will match controls and perform the necessary tests. The cord sera from 30,000 children are being tested for IgM levels. This work will be completed next year (if materials are provided by the PRC). The 1,000 children with high IgM are being tested and studied in detail.

Publications:

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Henson, T. E., Brody, J. A., Sever, J. L., Dyken, M. L., and Cannon, J.: Measles antibody titers in multiple sclerosis patients, siblings and controls. JAMA 211: 1985-1988, 1970.

Korones, S. B., Todaro, J., Roane, J. A., and Sever, J. L.: Maternal virus infection after the first trimester of pregnancy and status of offspring to 4 years of age in a predominantly Negro population. J. Pediat. 77: 245-251, 1970.

Asbed, R. A., Masland, M. W., Weinberger, M. M., and Sever, J. L.: Early case finding of children with communication problems. Part I. Report of a community screening program. Volta Review. 72: 23-49, 1970.

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Weinberger, M. M., Masland, M. W., Asbed, R. A., and Sever, J. L.: Congenital rubella presenting as retarded language development. Amer. J. Dis. Child. 120: 125-128, 1970.

Sever, J. L., Kurtzke, J. F., Alter, M., Schumacher, G. A., Gilkeson, M. R., and Ellenberg, J. H.: Virus antibodies and multiple sclerosis. Arch. Neurol., In press.

Newman, S. J., McCallin, P. F., and Sever, J. L.: Attempts to isolate H-1 virus from spontaneous human abortions. A negative report. Teratology. 3: 279-281, 1970.

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Sever, J. L.: Virus infections and malformation. Fed. Proc. 30: 114-117, 1971.

Sever, J. L.: Infectious agents and fetal disease. In Waisman, H. A., and Kerr, G. (Eds.): Fetal Growth and Development. New York, N.Y., McGraw-Hill Book Company, 1970, pp. 221-233.

Sever, J.L.: Viroses e o feto, Revista de Atualizacao en Ginecologia et Obstetricia. Vol. IV, No. 10, 1970, pp. 36-54.

Serial No. NDS (CF)-61 PR/ID 835
1. Perinatal Research Branch
2. Section on Infectious Diseases
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Clinical Investigations in Human Volunteers and Other Populations of Virus Effects and Production of Prototype Human Antisera, Vaccines, and Other Agents.

Previous Serial Number: Same

Principal Investigators: Dr. John L. Sever, PRB, NINDS
Dr. Earl Matthew, PRB, NINDS
Dr. Donald Henson, PRB, NINDS
Dr. Dale Dietzman, PRB, NINDS

Other Investigators: Dr. David A. Fuccillo, PRB, NINDS

Cooperating Units: Bureau of Prisons, Department of Justice
(Dr. Myrl Alexander, Director)
Petersburg Federal Reformatory
(Dr. Joel Rascoff, Chief Medical Officer)

Man Years:

Total:	1.00
Professional:	.50
Other:	.50

Project Description:

Objectives: To study the efficacy of prophylactic and therapeutic materials for the prevention and control of infectious diseases. To study the safety, antigenicity, communicability and immunogenicity of candidate rubella vaccines. To determine whether intravenous mannitol causes cytomegalovirus.

Methods Employed: Human volunteer studies are conducted in collaboration with the Federal Bureau of Prisons. These studies are reviewed and approved by the Clinical Research Committee and the Medical Board of the National Institutes of Health, and the Vaccine Development Board of the National Institute of Allergy and Infectious Diseases. Intravenous mannitol was given to human volunteers who were studied for CMV virus shedding excretion and antibody. Vaccine studies with low temperature adapted rubella vaccine are scheduled.

Major Findings: Volunteer studies were performed to evaluate the effect of mannitol on excretion of CMV and antibody response. The mannitol proved to be safe in that it did not increase excretion or antibody.

Significance to the Program of the Institute: Volunteer studies provide the basic data necessary to evaluate potential rubella vaccines. These studies should provide important and necessary information on the effectiveness and safety of such preparations. It will also elucidate the epidemiology of CMV in man.

Proposed Course of the Project: Additional studies will be performed when necessary or appropriate.

Honors and Awards: None

Publications: None

Serial No. NDS (CF)-62 PR/ID 972
1. Perinatal Research Branch
2. Section on Infectious Diseases
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Experimental Animal Tissue Culture, Histopathological and Serological Investigations of the Role of Viruses and Other Microorganisms in the Perinatal Period.

Previous Serial Number: Same

Principal Investigators: Dr. William T. London, PRB, NINDS
Dr. David A. Fuccillo, PRB, NINDS
Dr. John L. Sever, PRB, NINDS
Mr. William Weiss, OB, NINDS

Other Investigators: Mrs. Anita C. Ley, PRB, NINDS
Mrs. Blanche Curfman, PRB, NINDS

Cooperating Units: Dr. Andre J. Nahmias, Department of Pediatrics, Emory University School of Medicine, Atlanta, Ga.

Dr. J. M. Rice, Bioassay Section, EPB, NCI

Dr. Donald B. Cheek, Department of Pediatrics Johns Hopkins University Medical School, Baltimore, Md.

Dr. Louis W. Catalano, Jr., Department of Neurology, College of Physicians and Surgeons, Columbia University, New York, New York

Man Years:

Total:	8
Professional:	2
Other:	6

Project Description:

Objectives: To study the role of viruses and other microorganisms in the perinatal period, the infection of gravid and nongravid animals of several different species by parenteral routes with various viruses and other microorganisms to determine the effects of these agents on the animals and their fetal tissues.

Attempt to recover inoculated agents from the various animals and fetal tissues and the correlation of these reisolations with time (in gestation) of inoculation, and dosage given.

Correlate these findings with gross and histopathological findings. Correlate all of this information with serological findings.

Initiate a nutritional study of non-human primates using pregnant rhesus monkeys to test the hypothesis that there is no causal relation between maternal nutrition during pregnancy and certain sensory, pathological, immunological and biochemical characteristics of the infant. It is considered that a monkey trial in which the concept of randomness is permitted is a necessary preliminary to a human trial.

Methods Employed: An investigation of the role of viruses and other microorganisms in the perinatal period by the continual use of experimental animals; tissue culture techniques; histopathological studies; and serological testing.

Pregnant mice, rabbits and monkeys are being inoculated by various routes with viruses and other microorganisms. These animals are being observed and checked for evidence of disease and/or effects on fetal tissues.

Virus isolation investigations utilizing tissue culture to recover viruses from tissues and fluorescent antibody technique to study the location of virus infection produced in experimental animals.

Histopathological and gross anatomical studies are conducted on specimens obtained from the experimental animal studies.

Extensive serological studies are conducted with the many viral antigens developed for the Collaborative Study and new antigens with materials being studied previously mentioned.

In the non-human primate nutritional study, pregnant rhesus monkeys will be maintained throughout pregnancy on one of four diets restricted in either calories or protein. One diet will be deficient in both. The pregnant animal will be delivered at 158 days of gestation by cesarean section and the infant's tissues will be processed for biochemical analysis by the Hopkins Unit. The nutritionally deprived female monkeys will be continued on their respective diets and studies for immunological responses to various antigens such as tuberculin, rubella and mumps virus will be performed.

Major Findings: The inoculation of rabbits with low passage wild rubella virus strains and vaccine strains gave variations in antibody response and virus shedding. These variations can be useful as markers of strain differences. The low passage wild virus strains produce high antibody levels and variable amounts of virus shedding. The vaccine strains resulted in little or no antibody and no virus shedding.

Genital infection was readily established in 10 female Cebus monkeys when inoculated intravaginally with type 2 Herpesvirus hominis. Infection was proven by virus isolations and serological studies and the demonstration of herpetic vesicles and/or ulcers on the vulva and vagina with moderate cervicitis. Reinoculation with HVH type 2 was performed in all 10 animals with resulting vaginal reinfection being established in 3 monkeys despite the presence of serum neutralizing antibodies. The lesions produced in the Cebus monkey appear to parallel closely the disease observed in human genital infections.

Polyinosinic - polycytidylic ribonucleic acid (Poly I:C) complexed with poly-D-lysine was studied as a prophylactic and therapeutic material in mice inoculated with herpes simplex virus. With a treatment regimen over a period of several days mice treated with Poly I:C - poly-D-lysine had significantly less mortality than animals treated with Poly I:C alone or saline only. This enhanced protection was still detectable if treatment was begun as late as 4 days following inoculation of HSV, at which time an occasional mouse was showing signs of early CNS illness.

A pilot nutrition study using 38 rhesus monkeys has been started. To date 8 animals have become pregnant and at least one animal is on each of the four diets. It appears that the animals will eat the diets as prepared and maintain pregnancy.

Significance to the Program of the Institute: A program using experimental animals, tissue culture techniques, and histopathological investigations complements the strict serological approach being used on human sera obtained from the Collaborative Study and thus balances the investigations of the role of viruses and other microorganisms in the perinatal period. It presents the direct means of investigation of these agents which may contribute to perinatal pathology.

Proposed Course of the Project: Further studies using tissue from patients with subacute sclerosing panencephalitis, Creutzfeldt-Jakob disease, amyotrophic lateral sclerosis and progressive multifocal leucoencephalitis, inoculated intracerebrally into the fetus of pregnant rhesus monkeys are now in progress.

The Cebus herpes monkey model provides an experimental approach for the study of congenital infection and possible prevention or control of congenital disease. We propose a pilot study, first, to determine if congenital infection can be produced and, second, to explore methods of prevention or treatment if this pilot is successful.

If the above studies were successful in producing congenital herpes type 2 infection in the Cebus, we would propose to proceed with a study of chemotherapy using cytosine arabinoside, 5-IDU, Poly I:C, and Poly Lysine for clearing the maternal vaginal infection prior to delivery of the baby.

Second, we would investigate immunization with herpes type 2 antigen for its efficacy in eliminating vaginal infections and, third, we would study cesarean section as a means of by-passing the infected vaginal area and preventing congenital infection.

At the completion of the pilot primate nutrition study, the data will be analyzed and any correction in methods and diets will be made. The main phase will then be started using 136 rhesus monkeys.

Honors and Awards: None

Publications:

Nahmias, A. J., London, W. T., Catalano, L. W., Fuccillo, D. A., and Sever, J. L.: Genital Herpesvirus hominis type 2 infection: An experimental model in Cebus monkeys. Science 171: 297-298, 1971.

London, W. T., Catalano, L. W., Nahmias, A. J., Fuccillo, D. A., and Sever, J. L.: Genital Herpesvirus hominis type 2 infection of monkeys. Obstet. & Gynec. 37: 1971. (In press).

London, W. T., Fuccillo, D. A., Anderson, B., and Sever, J. L.: Concentration of rubella virus antigen in chondrocytes of congenitally infected rabbits. Nature 226: 172-173, 1970.

London, W. T., Fuccillo, D. A., Ley, A., and Sever, J. L.: Antibody response of various strains of rubella virus when inoculated into rabbits. Proc. Soc. Exper. Biol. Med. (In press).

Catalano, L. W., London, W. T., Rice, J. M. and Sever, J. L.: Treatment of herpesvirus encephalitis with Poly I:C - Poly-D-Lysine complexes. Obstet. & Gynec. (In press).

Serial No. NDS (CF)-65 PR/ID 1270
1. Perinatal Research Branch
2. Section on Infectious Diseases
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Toxoplasmosis: Serological and Clinical Studies.

Previous Serial Number: Same

Principal Investigator: Dr. John L. Sever, PRB, NINDS

Other Investigators: Dr. Joseph S. Drage, PRB, NINDS

Cooperating Units: Section on Pediatric Neurology, PRB, NINDS

Man Years:

Total:	.2
Professional:	.2
Other:	.2

Project Description:

Objectives: This study relates rises in antibody titer to abnormal pregnancy outcomes.

Methods Employed: Computer analysis of multiple variables relating to injection with toxoplasma and pregnancy outcome.

Major Findings: Within the group of 47 patients with titer elevations of greater than 4096, or significant increases in antibody titer, five were found to have definite toxoplasmosis and ten were suspected of having toxoplasmosis. The ten included six with motor retardation, two pregnancies resulting in stillbirths, and two in neonatal deaths. The sera from ten of the remaining 32 apparently normal children were tested for antibody to toxoplasmosis and one was found to have a high titer.

Proposed Course of the Project: Publication of findings.

Honors and Awards: None

Publications: None

Serial No. NDS (CF)-67 PR/ID 1503
1. Perinatal Research Branch
2. Section on Infectious Diseases
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Epidemiologic Studies of Perinatal Infections

Previous Serial Number: Same

Principal Investigators: Dr. John L. Sever, PRB, NINDS
Mrs. Dorothy M. Edmonds, R.N., PRB, NINDS

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	1.50
Professional:	1.50
Other:	0

Project Description:

Objectives: To utilize information from the Collaborative Perinatal Research Study and other cooperative studies to identify pregnancies complicated by maternal infections or infections in childhood; to further delineate these cases by serologic testing of the stored sera; to utilize Collaborative Study and related data to determine outcome of these pregnancies in relation to the outcomes of matched controls and the general study population in order to gain information on the frequency of maternal infections during pregnancy and their effects on the developing fetus. Studies include: Microcephaly, Hydrocephaly, Abortions, Cataracts, and Bacterial and Protozoal Infections.

Methods Employed: Primary material utilized for these studies comes from the Perinatal Research Study and other cooperative studies. For this reason the serologic data developed by the Section on Infectious Diseases is correlated with the clinical information available either from print-outs or direct hand review of the charts stored in the Perinatal Research Branch.

Major Findings: The frequency of some virus antibodies has been found to be increased in several of the abnormal patient groups. These findings are being followed with additional testing of similar groups of patients.

Significance to the Program of the Institute: The development of the serologic data requires the further analysis in relation to the epidemiology of infections in the Perinatal Research Study as well as the epidemiology of perinatal infections with other collaborating groups. This data then provides

the basis for correlating serologic and clinical information. Special studies are initiated in populations where high frequencies of infection or abnormal pregnancy outcomes have been noted.

Proposed Course of the Project: Special emphasis will be placed on the possible association of neoplastic diseases and other tumors with herpes simplex infections. We will analyze the clinical data for the 5500 reported infections in the Perinatal Research Study. Detailed epidemiological studies will be conducted on stillbirths, congenital malformations of various types, and children with low IQ's at 4 years of age.

Honors and Awards: None

Publications:

Sever, J.L.: Viruses and embryos. In Fraser, F.C. and McKusick, V.A. (Eds.): Congenital Malformations, Proceedings of the Third International Conference, The Hague, The Netherlands, September 7-13, 1969, Amsterdam, Excerpta Medica Foundation, International Congress Series No. 204, 1970, pp. 180-186.

Sever, J.L.: Viral Teratogens: A Status Report. Hospital Practice. 5: 75-83, 1970.

Sever, J.L.: Viruses and The Fetus. Int. J. Gynaec. Obstet. 8: 763-769, 1970.

Serial No. NDS (CF)-67 PR/ID 1506
1. Perinatal Research Branch
2. Section on Infectious Diseases
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Maternal Infection and Pregnancy Outcome

Previous Serial Number: Same

Principal Investigators: Dr. David A. Fuccillo, PRB, NINDS
Dr. John L. Sever, PRB, NINDS

Other Investigators: Dr. William T. London, PRB, NINDS
Mrs. Renee Traub, PRB, NINDS
Mrs. Mary Ruth Gilkeson, PRB, NINDS
Mrs. Flora Meder, PRB, NINDS

Cooperating Units: University of California (Dr. Margaret H. Jones)
Hawaii Permanente Medical Group (Dr. Paul F. McCallin)
University of Puerto Rico, School of Tropical Medicine (Dr. Dolores Mendez-Cashion)
Pennsylvania Hospital (Dr. Corson and Dr. Bolognese)

Man Years:

Total:	4.5
Professional:	1.0
Other:	3.5

Project Description:

Objectives: To utilize various virological techniques in an intensive study of viruses to determine their role in the production of birth defects and related abnormalities. To develop virological techniques necessary for the investigation of the natural course of the disease as caused by the infectious agents.

Methods Employed: New virus isolation techniques and serum neutralization tests are used for large scale testing in the study of pregnant women and their children. The development of new techniques, such as the HAI test for Herpes hominis Types I and II and a new test for cytomegaloviruses will now permit the determination of the frequency of virus experience among study populations along with the presence of antibody and change in antibody titer to the virus. These tests are being used to establish the reliability of other tests and to determine their sensitivity and specificity. A serologic study utilizing these tests on sera collected on the Collaborative Study

population was conducted to determine the frequency of antibody changes during pregnancy and the effect of these infections on the developing embryo. We have completed the testing of 2500 placentas for rubella virus for the purpose of identifying children with congenital rubella by the presence of rubella in the placental material. These reports are being prepared for publication. The use of a fluorescent, specific anti-IgM test is proving to be a valuable method for identifying congenital infections.

Vaccine for rubella is being given to pregnant women (at the Pennsylvania Hospital) who are subsequently being aborted. This will help determine the effect of vaccine on the fetus.

Major Findings: Three new tests were developed for specific virus serology. These were hemadsorption tests for Herpes I, Herpes II and cytomegalovirus. The tests now provide highly specific, reliable, rapid, micromethods for virus serology with these agents. This is of particular value because previous methods were particularly laborious and expensive or not specific. Also, since these are hemagglutination tests, the anti-complementary effects found in many Perinatal Research Study sera can be avoided and the sera are now usable.

Virus isolation studies of women for cytomegalovirus show transient as well as persistent infections. Studies of the children of these pregnancies are now in progress.

The fetal abortion studies, after rubella vaccinations, have been completed for about 75 cases and results will be reported at a later date.

Rubella vaccine studies are continuing with the temperature adapted vaccine material.

Significance to the Program of the Institute: The results from these studies help determine what effect virus infection has on abnormal pregnancy outcomes and also provide valuable information on the epidemiological aspects of virus infections.

Proposed Course of the Project: Studies are now in progress on cytomegalovirus infections during pregnancy and in several population groups in Maryland. Additional studies are being conducted on Herpes Types I and II infections in women. Drug evaluation of Cytosine Arabinoside for CMV and 5-IDU for Herpes hominis is being conducted in monkeys.

Honors and Awards: None

Publications:

Fuccillo, D. A., Moder, F. L., Traub, R. G., Hensen, S. and Sever, J. L.: Micro Indirect Hemagglutination Test for Cytomegalovirus. Appl. Microbiol. 21: 104-107, 1971.

Catalano, L. W., Fuccillo, D. A., Traub, R. G., and Sever, J. L.:
Isolation of Rubella Virus from Placentas and Throat Cultures of Infants.
Amer. J. Obstet. Gynec. In press.

Serial No. NDS (CF)-69 PR/ID 1729
1. Perinatal Research Branch
2. Section on Infectious Diseases
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Study of the Possible Transmission of Toxoplasmosis in Humans and Mammals

Previous Serial Number: Same

Principal Investigators: Dr. John L. Sever, PRB, NINDS
Dr. Stephen J. Newman, PRB, NINDS
Dr. David A. Fuccillo, PRB, NINDS
Dr. Dolores Mendez-Cashion, Puerto Rico
Dr. William T. London, PRB, NINDS

Other Investigators: None

Cooperating Units: Department of Pediatrics
Centro-Medico
San Juan, Puerto Rico

Study has been completed.

Honors and Awards: None

Publications:

Newman, S. J., Fuccillo, D. A., Sever, J. L., London, W. T. and Mendez-Cashion, D.: Toxoplasmosis in Puerto Rico. II. A serological and epidemiological study in Puerto Rican children. Boletin de la Asociacion Medica de Puerto Rico, in press.

Serial No. NDS (CF)-69 PR/ID 1731
1. Perinatal Research Branch
2. Section on Infectious Diseases
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Experimental *in vitro* and *in vivo* techniques for isolation of infectious agents from chronic diseases. Use of zonal centrifugation and isopycnic ultracentrifugation for purification of the suppressed form of measles virus from SSPE brains and PML papovavirus from progressive multifocal leukoencephalopathy brain specimens. Study of the role of interferon in chronic infection. Comparative study of the role of delayed hypersensitivity, humoral immunity and interferon in neurotropic measles infections in rats. Study of the fetal immunological competence in rhesus monkeys. Development of immunologic methodology in evaluating intra-uterine infections and CNS infections.

Previous Serial Number: Same

Principal Investigators: Dr. Luiz Horta-Barbosa, PRB, NINDS
Dr. Dale Dietzman, PRB, NINDS
Dr. David A. Fuccillo, PRB, NINDS
Dr. William T. London, PRB, NINDS
Dr. John L. Sever, PRB, NINDS

Other Investigators: Mrs. Rebecca Hamilton, PRB, NINDS
Mrs. Barbara Wittig, PRB, NINDS
Miss Helen Kreba, PRB, NINDS
Mrs. Anita Ley, PRB, NINDS

Cooperating Units: University of Tennessee (Dr. J. T. Jabbour and Dr. Charles Cape)
University of Vermont (Dr. George Schumacher)
Indiana University Medical Center (Dr. Wolfgang Zeman)
University of California L.A. (Dr. Gary Gitnick)
Wilmington General Hospital (Dr. George Boines)

Man Years:

Total:	5
Professional:	2
Other:	3

Project Description:

Objectives: To establish whether persistent or tolerant viral infections are associated with chronic diseases such as polymiositis,

ulcerative enterocolitis, amyotrophic lateral sclerosis, Creutzfeldt-Jakob disease, progressive multifocal leukoencephalopathy, and multiple sclerosis.

To purify and concentrate PML and SSPE viruses and examine these agents electron microscopically, biochemically, and antigenically in parallel with the conventional forms of these viruses.

To determine interferon levels in patients with ulcerative enterocolitis, hepatitis, and SSPE, as well as to establish whether their peripheral white cells are capable of producing interferon to normal levels when compared with cells from normal subjects.

To evaluate the role of cellular immunity, humoral immunity, and interferon in protecting experimental animals against neurotropic measles virus.

To elucidate the maturation of the immune system in embryonic life and correlate this process with intra-uterine infections.

To separate IgM and IgG from cord sera from children with congenital diseases and determine the serological specificity of the 19S immunoglobulin using fluorescent antibody assays in an attempt to develop a diagnostic test for fetal infections.

Methods Employed: Brain specimens from well-documented cases of MS, PML, ALS, SSPE, etc, were homogenized to a 10% suspension and inoculated intracranially in groups of 5 rhesus monkey fetuses during the first third of gestation when the animals are expected to be immunologically immature and hence more susceptible to infectious agents. These fetuses were carried to term and, immediately after birth, one animal was killed and examined histopathologically for CNS lesions. The remaining animals were carefully observed for clinical symptoms, abnormal behavior, and antibody pattern. A 2-year follow-up has been scheduled.

Brain tissue provenient from patients with encephalopathies, intestinal mucosa from patients with ulcerative enterocolitis, muscle from patients with polymiositis, and lymph nodes from individuals with SSPE were examined by electron microscopy, fluorescent microscopy and, whenever possible, cultured in vitro. These tissue cultures were submitted to virologic and serologic assays, inoculated into experimental animals, and co-cultivated with human diploid and heteroploid cell lines in efforts to isolate and identify intracellular agents.

Brain autopsies from patients with PML and known to contain intranuclear virus were frozen and thawed three times, the nuclei extracted and disrupted by physical means. Nuclear extracts were isopycnically banded in CsCl and fractions examined under the electron microscope. Fractions containing the papovavirus were used to immunize guinea pigs in order to

produce monospecific antisera intended to identify the virus. Similar approach, but using zonal centrifugation to purify the virus, was planned for SSPE and is awaiting approval of the necessary contract.

Interferon levels in serum of hepatitis and enterocolitis patients were obtained by HA inhibition test using Sindbis virus as the challenge virus. SSPE patients' white cells were grown in vitro, infected with different viruses (including measles), and interferon on supernatants measured by the HA inhibition method. Adequate controls were utilized.

Lewis' rats were immunized with measles vaccine combined with Freund's adjuvant and after antibodies developed and delayed hypersensitivity became manifest as measured by skin tests with PPD, these animals served as serum and lymphocyte donors to groups of histocompatible, non-immunized animals. Interferon was produced by in vitro grown macrophages and 40,000 units were inoculated into other groups of rats. Passively immunized animals, i.e., those rats receiving pre-determined amounts of antibody, competent lymphocytes and interferon were challenged with 10 LD_{50} neurotropic measles virus and protection conferred measured by comparing death rates with those of control groups.

Mumps and chykungunia viruses were inoculated into groups of rhesus monkey fetuses at first, second, and third periods of gestation. At appropriate intervals the fetuses were removed and blood collected for immunological evaluation. Antibody curve was determined by CF test; lymphocyte transformation was measured by uptake of tritiated thymidine in the presence of mumps antigen; interferon production was established by HA inhibition assays. Concomitantly, the pathogenesis of mumps virus was studied in some fetuses to determine the fetal susceptibility in terms of stage of gestation and to correlate these observations with the immunological data.

Immunoglobulins from cord sera provenient from cases of congenital infections were precipitated by the ammonium sulfate method and concentrated 10 times in saline. These preparations were ultracentrifuged in linear gradients of sucrose and 7S gamma globulins separated from 19S molecules. The latter fractions were serologically evaluated against different antigens (Toxoplasma, rubella, CMV) to determine antibody specificity and thus the identity of the causative agent.

Sera and spinal fluid from patients with SSPE were compared with specimens from patients with other neurological diseases using the immunodiffusion technique in gel plates. Highly concentrated measles virus antigens were utilized in the test.

Major Findings: Virological studies with SSPE lymph node biopsies resulted in the isolation of measles virus and in the demonstration of an unidentified bullet-shaped virion in the cytoplasm of several stroma cells. The measles virus was isolated in mixed cultures with HeLa cells. This

finding suggests that SSPE is a systemic infection with possible specific defectiveness of the cellular immunity.

A simple diagnostic test for SSPE was developed by the Ouchterlony technique using the patient's spinal fluid and concentrated measles antigen. The assay proved highly specific and hence of considerable diagnostic value.

A herpes-like virus was found associated with cases of ulcerative enterocolitis. The virus particles were visualized by electron microscopy and tissue cultures of mucosa and sub-mucosa specimens showed scattered intracellular virus when assayed by immunofluorescence.

Circulating interferon was not found in patients with chronic or acute hepatitis nor in patients with enterocolitis. Serum and spinal fluid from patients with SSPE also failed to show detectable levels of interferon. However, the peripheral white cells from these patients responded to in vitro infection by producing normal amounts of interferon, indicating that the IF system is not impaired in these conditions.

Piconavirus-like crystals were seen by electron microscopy in several muscle biopsies from patients with polymiositis. Mixed cultures of muscle cells and simian cells were prepared in attempts to isolate viruses. A papovavirus was purified from a PML brain autopsy and subsequently used to inoculate laboratory animals. The purification was achieved by banding the virus in CsCl (1.6 grs/ml). Spectrophotometry and electron microscopy monitoring resulted in a rather pure virus preparation of approximately 10^7 intact particles/5 grams of tissue.

Significance to the Program of the Institute: The development of mixed cell cultures to unmask latent infections provides an excellent methodology for the study of chronic diseases of possible viral etiologies.

The successful development of a simple diagnostic test for SSPE warrants further immunological studies with cerebrospinal fluid from patients with other neurological diseases in an effort to define antibody patterns of diagnostic value.

The presence of a latent, intracellular measles virus in the lymph node of patients with SSPE suggests a tolerant infection with deficiency of the delayed hypersensitivity. This finding should lead to detailed examination of the immune competence of SSPE patients as well as that of individuals with other CNS illnesses.

The understanding of slow virus infections of the CNS will depend upon purification of the suppressed form of these agents followed by careful biochemical and biophysical analysis. To accomplish this goal, the use of zonal centrifugation for cellular fractionation should be emphasized.

Proposed Course of the Project: Special emphasis will be placed upon a brain culture research program. Investigation of the mechanism of pathogenesis and possible immune deficiencies in patients with neurological diseases will be conducted. Our selection of patients with multiple sclerosis, Parkinson's Disease, progressive multifocal leukoencephalopathy, and amyotrophic lateral sclerosis is supported by the existing data which suggest possible viral etiologies for each of these diseases. Tissue specimens and blood from patients will be provided through collaborative-contract arrangements with investigators throughout the country.

Utilizing the mixed culture technique we hope to determine if it is possible to release suppressed virus from these chronic neurologic diseases and to gain a further understanding of the pathogenesis of latent infections of the CNS. Antibody levels and competence of lymphocytes from patients will be examined using the standard techniques.

The SSPE strain of measles virus will be studied in laboratory animals and efforts will be directed at the development of an animal model system for this disease.

Honors and Awards: None

Publications:

Horta-Barbosa, L., Fuccillo, D. A., Hamilton, R., Traub, R., Ley, A., and Sever, J. L.: Some characteristics of SSPE measles virus. Proc. Soc. Exper. Biol. & Med. 134: 17-21, 1970.

Vernon, M. L., Horta-Barbosa, L., Fuccillo, D. A., Sever, J. L., Baringer, J. R., and Birnbaum, G.: Virus-like particles and nucleoprotein-type filaments in brain tissue from two patients with Creutzfeldt-Jakob Disease. Lancet 1: 964-967, 1970.

Horta-Barbosa, L., Krebs, H., Ley, A., Chen, T. C., Gilkeson, M. R., and Sever, J. L.: Progressive increase in cerebrospinal fluid measles antibody levels in subacute sclerosing panencephalitis. Pediatrics in press.

Horta-Barbosa, L., Fuccillo, D. A., and Sever, J. L.: Viral and protozoan infections of the newborn. In Abramson, H. (Ed.): Resuscitation of the Newborn Infant. St. Louis, Mo., C. V. Mosby Co., in press.

Serial No. NDS (CF)-69 PR/ID 1732
1. Perinatal Research Branch
2. Section on Infectious Diseases
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Investigation of the Role of Mycoplasma spp. and Other Microorganisms in the Perinatal Period.

Previous Serial Number: Same

Principal Investigators: Dr. David L. Madden, PRB, NINDS
Dr. William T. London, PRB, NINDS
Dr. John L. Sever, PRB, NINDS
Dr. Earl B. Matthew, PRB, NINDS
Dr. Dale Dietzman, PRB, NINDS

Other Investigators: Dr. Melvin Museles, NNMH
Mr. Kenneth Moats, PRB, NINDS

Cooperating Units: National Naval Medical Hospital, Bethesda, Md.

Man Years:

Total:	1.60
Professional:	.60
Other:	1

Project Description:

Objectives: To study the role of Mycoplasma spp. in perinatal diseases of man. To develop animal models to determine the pathogenesis of these diseases. To develop new test systems for the rapid diagnosis of perinatal diseases.

Methods Employed: Vaginal and oral swabs are obtained from pregnant women and monkeys at the time of labor or C-section. Swabs are obtained from infants and mothers. These specimens are cultured in standard media. Isolated cultures will be identified by the metabolic-inhibition (M.I.) test. Serum samples obtained prior to delivery, at time of delivery and post-delivery are studied for presence of antibodies by the M.I. test, indirect immunofluorescence and agglutination tests. Pregnant monkeys found to be free of Mycoplasma infections will be inoculated with various strains of Mycoplasma to determine the effect of these strains upon mother and infant. The use of newer techniques for determining antibodies in body fluids will be used in an effort to develop quicker and more accurate tests for detection of viral antigens and antibodies. We will utilize new virus strains and tissues in an attempt to grow higher titered virus antigens. We will use new techniques such as counter-immuno-electrophoresis and radioimmunoprecipitation tests.

Major Findings: Serum from 100 mother-baby pairs, whose Mycoplasma flora and antibody titers for Mycoplasma had been determined, were studied for abnormal amounts of IgG and IgM antibody in an effort to determine if in utero infection occurred. None was found. Post-natal follow-up on children known to have been infected with Mycoplasma at birth has indicated that they were not adversely affected as judged by birth weight, weight gain or increased susceptibility to disease.

Inoculation of M. hominis into the vagina of pregnant monkeys did not cause fetal infections. The pregnant monkeys soon eliminated this agent from their bodies. In non-pregnant monkeys, a severe vaginitis occurred and the organisms have persisted in the vaginal tract for a long period of time. It was also observed that some of the serological characteristics of this organism were altered. Concurrent with this study, a natural spread of M. orale II in the monkey colony was observed.

An SSPE virus antigen was grown to high titers in spinner cultures and then concentrated 100 times in a Spinco centrifuge. A method to concentrate spinal fluid was developed by utilizing evaporation of fluid through cellulose dialysis tubing. This concentrated antigen and spinal fluid in a gel diffusion or counter-immunoelectrophoresis test was of comparable sensitivity to the CF or HI test on unconcentrated spinal fluid and was much less complicated to perform.

Significance to the Program of the Institute: A program devoted to studying the effect of Mycoplasma in perinatal infections complements the virological studies currently being done. This study and the support given to other investigators may more accurately define the role of Mycoplasma in disease. The development of additional techniques for the serological diagnosis of perinatal diseases which are as sensitive or more sensitive than current tests being used, and which by-pass the problems of anti-complementary sera, will increase the usefulness of the stored perinatal serums.

Proposed Course of the Project: Further studies are being done on the association of Mycoplasma in normal deliveries. Study of the pathogenesis of Mycoplasma in monkeys will be continued to see if an animal model can be developed for septic and non-septic abortions and other perinatal infections. Attempts to define the association of Mycoplasma in neurological diseases will be made. Continued effort will be made to apply these techniques to other antigen-antibody combinations in order to develop additional serological methods to detect perinatal and neurological diseases.

Honors and Awards: None

Publications:

Madden, D. L., Horton, R. E. and McCullough, N. B.: Spontaneous infection in Ex-germfree guinea pigs due to Clostridium perfringens. Lab Animal Care 20: 454-455, 1970.

Madden, D. L., Hilderbrandt, R. J., Monif, G. R. G., London, W. T., Sever, J. L. and McCullough, N. B.: The isolation and identification of Mycoplasma from Macaca mulatta. Lab Animal Care 20: 467-470, 1970.

Madden, D. L., Hilderbrandt, R. J., Monif, G. R. G., London, W. T., McCullough, N. B. and Sever, J. L.: The isolation and identification of Mycoplasma from Cercopithecus aethiops. Lab Animal Care 20: 471-473, 1970.

Matthew, E. B., Dietzman, D. E., Madden, D. L., Sever, J. L., Matti, A. and Dodson, W. E.: The possible absence of Australia antigen in G/G translocation type of Down's syndrome. Lancet. (In press)

Serial No. NDS (CF)-69 PR/ID 1733
1. Perinatal Research Branch
2. Section on Infectious Diseases
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Viral Diseases of the Nervous System

Previous Serial Number: Same

Principal Investigator: Dr. David A. Fuccillo

Other Investigators: Dr. J. L. Sever, PRB, NINDS
Dr. S. Baron, LVD, NIAID
Dr. W. T. London, PRB, NINDS

Cooperating Units: Laboratory of Viral Disease, NIAID
Georgetown University School of Medicine,
Washington, D.C., Dr. J. A. Bellanti
Harvard Medical School, Boston, Massachusetts,
Dr. L. Johnson

Man Years:

Total:	2.0
Professional:	1.0
Other:	1.0

Project Description:

Objectives: The main objective of this project is to establish the clinical and biological significance of the two different strains of herpes simplex virus (Type I, "oral" and Type 2, "genital") in the causation of human disease including carcinoma of the cervix.

Methods Employed: The principal methods employed are: (1) the quantal cross-microneutralization test, by which type specific herpesvirus antibody is identified; (2) mass serological surveys of materials from selected patients with specific disease entities; (3) virus isolation, titration, and characterization procedures; (4) interferon titration via plaque reduction assays.

Major Finding: Patients with carcinoma in situ of the uterine cervix were demonstrated to have increased amounts of antibody to type 2 herpesvirus. Antibody was also elevated in these patients two years before they developed cancer (collaboration with Dr. Johnson).

Significance to Biomedical Research and the Program of the Institute: These investigations attempt to elucidate the pathogenesis of viral infections of the adult and fetus using immunological and virological techniques. Herpes

simplex virus has been one agent which has received particilar attention in these studies, since it has significant neurotropic capabilities in terms of newborn and adult encephalitides. There is considerable speculation that this virus may have latent, "slow" virus potential in relationship to chronic diseases of humans, including carcinoma and central nervous system infection. Investigation of the clinical and biological properties of the two strains of herpes simplex virus have permitted more definitive establishment of such capabilities. Furthermore, the therapeutic potential of artificial interferon inducers as anti-viral agents was investigated with herpesvirus in animals and one human, particularly in relationship to central nervous system infections.

Proposed Course of Project: Expanded study of antibody and isolation of virus from women with carcinoma in situ and carcinoma of cervix in the Collaborative Study.

Honors and Awards: None

Publications:

Catalano, L. W., Jr., Fuccillo, D. A. and Sever, J. L.: Piggy-Back Microtransfer Technique. Appl. Microbiol. 18: 1094-1095, 1969.

Catalano, L. W. Jr. and Sever, J. L.: Role of Viruses as Causes of Congenital Defects. Ann. Rev. Microbiol., In Press.

Catalano, L. W. Jr., Fuccillo, D. A., Traub, R., and Sever, J. L.: Isolation of Rubella Virus from Placentas and Throat Cultures of Infants in a Prospective Study Following the 1964-65 Epidemic, J. Pediat., In Press.

Fuccillo, D. A., Moder, F., Traub, R., Hensen, S. and Sever, J. L.: Micro Indirect Hemagglutination Test for Cytomegalovirus. Appl. Microbiol. 21: 104-107, 1971.

Serial No. NDS (CF)-70 PR/ID 1848
1. Perinatal Research Branch
2. Section on Infectious Diseases
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Delayed Hypersensitivity in Chronic Viral Diseases

Previous Serial Number: Same

Principal Investigator: Dr. Earl B. Matthew

Other Investigators: Mrs. Mary Krasny
Dr. Donald Henson
Dr. David A. Fuccillo

Cooperating Units: None

Man Years:

Total:	1
Professional:	1/2
Other:	1/2

Project Description:

Objectives: To determine the role of delayed hypersensitivity (cellular immunity) in chronic viral infections.

Methods Employed: The macrophage migration inhibition test (MI), lymphocyte transformation and lymphotoxin assay were used in the study.

Major Findings: The macrophage migration inhibition test, lymphocytic transformation and lymphotoxin assay were all attempted in studying the mouse cytomegalovirus infection. None were found to give reliable results and no correlation could be made. This was due in most part to the type of animal used, the mouse not developing good delayed hypersensitivity unless an abnormal stimulus is utilized such as Freund's adjuvant. Unfortunately, this distorts the natural disease picture. The mouse CMV system is worthwhile for more refined study when other techniques become available.

Development of the lymphocyte transformation test as a marker of cellular immunity in human virus infections has been a major effort in the past year using various viral antigens. The studies of these in subacute sclerosing panencephalitis and multiple sclerosis are in the last stages of

completion. The study of these in patients with acute lymphocytic leukemia is complete.

Significance to the Program of the Institute: Prior to the development of the above tests, no good tests for measuring cellular immunity in humans to viral antigens existed. Antibody tests to viral antigens have been developed and used in this and other laboratories for many years. This represents a test for only part of the body's immune response. The development of lymphocyte transformation into a clinically usable test for virus infection now allows both forms of the body's protective immune mechanisms, humoral and cellular, to be tested simultaneously.

Proposed Course of the Project: To complete the above mentioned specific projects prior to July 1971 when the principal investigator is terminating his stay in NINDS.

Honors and Awards: None

Publications: None.

Serial No. NDS (CF)-70 PR/ID 1849
1. Perinatal Research Branch
2. Section on Infectious Diseases
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Chronic Infection with Cytomegaloviruses in Man and Animals

Previous Serial Number: Same

Principal Investigator: Dr. Donald Henson, PRB, NINDS

Other Investigators: Mr. Leonard Moore, PRB, NINDS
Dr. John L. Sever, PRB, NINDS
Dr. David A. Fuccillo, PRB, NINDS
Dr. Earl B. Matthew, PRB, NINDS

Cooperating Units: NCI, Dr. Edward Henderson, Dr. Ronald Yankee, Dr. Stuart Siegel, and Dr. Arthur Levine
Armed Forces Institute of Pathology, Dr. A. J. Strano

Man Years:

Total:	2
Professional:	1
Other:	1

Project Description:

Objectives: Study chronic cytomegalovirus infection, antibody levels, virus excration, lymphocyte response, disease in man and mice. Relate specific pathologic processes to antibody levels and virus excretion patterns.

Methods Employed: Children with congenital CMV and patients with leukemia with CMV infections are being followed for virus excretion, antibody response and lymphocyte response. Techniques for CMV isolation have been perfected. Antibody response is being followed with CF, HAI and neutralization methods. Lymphocyte transformation is determined with tritiated thymidine.

Major Findings: Chronic infection of the tissues of the mouse have been demonstrated. Results have shown that the response of the host to infection depends on the anatomy of the organs infected.

Eighty-seven children with acute lymphocytic or acute myelocytic leukemia have been longitudinally studied for 3 to 18 months. Results indicate a correlation between clinical symptoms and rises in antibody titers to CMV. There is no association between antibody titers and frequency of

virus isolation from urine or throat or length of virus excretion in these children. Basic problem now is to develop tests that discriminate between CMV dissemination and focal chronic infection.

Significance to the Program of the Institute: Cytomegalovirus causes congenital disease and death as well as significant infection in patients with malignancies. An understanding of this chronic infection and the immune responses of the host should be of great value in the prevention and treatment of these diseases.

Proposed Course of the Project: This is a new study. We will continue along the lines outlined above.

Honors and Awards: None

Publications:

Henson, D. and Sever, J. L.: Effects of Viruses on the Fetus. In Marcus, S. L. and Marcus, C. C. (Ed.): Advances in Obstetrics and Gynecology. Baltimore, Md., Williams and Wilkins Co., Vol. 2, 1971. In press.

Serial No. NDS (CF)-71 PR/ID 1903
1. Perinatal Research Branch
2. Section on Infectious Diseases
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Investigation of the Etiology and Effect of Serum and Infectious Hepatitis in the Perinatal Period.

Previous Serial Number: None

Principal Investigators: Dr. David L. Madden, PRB, NINDS
Dr. John L. Sever, PRB, NINDS
Dr. Dale E. Dietzman, PRB, NINDS
Dr. Earl B. Matthew, PRB, NINDS

Other Investigators: Dr. Benedict Nagler

Cooperating Units: Lynchburg Training School and Hospital

Man Years:

Total:	1.75
Professional:	1.75
Other:	0

Project Description:

Objectives: To determine the etiology of Australia antigen associated (serum) and infectious hepatitis. To determine the relationship of hepatitis/ congenital jaundice and postnatal jaundice. To develop animal models and new diagnostic tests for these diseases.

Methods Employed: A large epidemic of infectious hepatitis (600 cases) occurred in the Lynchburg Training School and Hospital during the summer of 1970. Over 5000 blood and 400 fecal samples were collected during a 4-month period. These samples represent serial specimens obtained prior to the development of disease, at time of acute disease and post-infection. A 6-month follow-up has now been carried out. Australia antigen determinations were performed on 1200 patients. A controlled study of the effect of gamma globulin on this strain of infectious hepatitis was initiated. Serum and fecal samples will be cultured in a variety of tissue culture systems and inoculated into experimental animals. Material from patients with hepatitis hospitalized at Suburban Hospital has been collected and is being utilized in comparison studies.

Major Findings: The results of the epidemiological study undertaken at the Lynchburg Training School and Hospital indicate that the disease was not associated with Australia antigen. It was found that the disease was transmitted from ward to ward by infected patient workers or through contact with the patients. It appears that this outbreak is the largest non-common source of infectious presently known. In this outbreak, the use of gamma globulin was not effective unless given 14-21 days before the first clinical case. This was partly related to the occurrence of sub-clinical cases which was followed by mass contamination of the ward. The occurrence of circulating Au antigen in chronic carriers did not alter the course of the infectious hepatitis. Preliminary results suggest that the incidence of Au antigen in trisomy 21 karyotype patients was much higher than in patients with Down's syndrome due to other karyotypes or in patients with normal karyotypes.

Significance to the Program of the Institute: The finding of an etiological agent or an infectious hepatitis associated antigen would be an important step in the prevention of this disease which affects several thousand individuals each year. The material collected from the Lynchburg outbreak is probably the largest accumulation of pre-jaundice, acute, and convalescent materials ever collected from a single outbreak. It will be extremely valuable for future reference.

Proposed Course of the Project: Attempts to define the role and cause of serum and infectious hepatitis will be continued. The tissue culture study has just been initiated. Attempts to detect specific antigen and antibody in these feces and serums will be made.

Honors and Awards: None

Publications: None

Serial No. NDS (CF)-63 PR/OC 1144
1. Perinatal Research Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: An Instrument For The Conduct Of A Retrospective Study Of Seizures, Cerebral Palsy, Mental Retardation and Other Neurological and Sensory Disorders of Infancy and Childhood.

Previous Serial Number: Same

Principal Investigators: Z.A. Shakhashiri, M.D., PRB, NINDS
Leonard V. Phelps, Clearwater, Florida
Glen S. Bartlett, M.D., Case Western Reserve Univ., Cleveland, Ohio

Other Investigators: Lenore Bajda, M.D., PRB, NINDS
John R. Day, M.D., Chevy Chase, Maryland
Blanche L. Vincent, S.N.O., Greensboro, North Carolina
Zula C. Meekham, B.S.N., PRB, NINDS
Rose R. Tortorella, PRB, NINDS

Cooperating Units: Georgetown University Hospital, Retarded Children's Clinic, Selected Maternity Hospitals and Physicians in Metropolitan Washington.

Man Years:

Total:	.35
Professional:	.30
Other:	.05

Project Description:

Objectives: Design an instrument for the conduct of a retrospective study of seizures, cerebral palsy, mental retardation and other neurological and sensory disorders of infancy and childhood in order to test certain basic and important hypotheses concerning the occurrence of neurological damage.

Methods employed: Recognized damaged outcomes of pregnancy, such as seizures, diplegias, hemiplegias and choreoathetoids are to be studied and related to defined perinatal or postnatal events. These outcomes were selected because they were construed to be related to or manifestations of or involved in the biological or psycho-sociological mechanism underlying the following hypotheses: (1) anoxia, (2) toxic influences on the brain, (3) metabolic influences, (4) trauma to the head, (5) infection of the brain, (6) dehydration of the child, (7) genetic or familial patterns, and (8) socioeconomic status.

Proposed Course of the Project: The abstraction of the maternity and nursery records and interviewing of the mothers have been completed. The physicians of sib controls have been contacted and information attesting to the health status of these sibs has been obtained. All forms have been edited and coded, and IBM cards have been keypunched. Partial analysis of the data has been carried out. Comparison of findings in this project with findings in the Collaborative Study will be carried out at a later time.

Preparation of the manuscript has been delayed until the end of FY '72.

Honors and Awards: None

Publications: None

Serial No. NDS (CF)-63 PR/OC 1146
1. Perinatal Research Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Public Health Implications Study of Perinatal Mortality in the Collaborative Study and in the Collaborative Study Cities.

Previous Title: Revision and Expansion of Previous Project Entitled A Commentary On The Appropriateness Of The Use Of Certain Tabular Data, For Formulating Generalizations Concerning Populations In The Same Cities As Those In Which The Collaborative Study On Cerebral Palsy, Mental Retardation And Other Neurological And Sensory Disorders Of Infancy And Childhood Is Being Conducted.

Previous Serial Number: Same

Principal Investigators: Z.A. Shakhashiri, M.D., PRB, NINDS
Leonard V. Phelps, Clearwater, Florida
Glen S. Bartlett, M.D., Case Western Reserve Univ., Cleveland, Ohio

Other Investigators: None

Cooperating Units: The Census Bureau and the National Center for Health Statistics cooperated in the furnishing of necessary statistical information for the United States and cities. The respective state or city health departments are providing natality and mortality data for the Project cities.

Man Years:

Total:	0.50
Professional:	0.30
Other:	0.20

Project Description:

Objectives: To evaluate fetal and infant mortality of the Collaborative Study population and of the cities from which that population is drawn, with the aim of comparing the two populations, city by city, and institution by institution, on mortality characteristics.

Methods employed: In addition to the data previously available from the National Center for Health Statistics for perinatal events, detailed data on natality and perinatal mortality are being sought for the study cities from

either the state or city health departments, whichever has jurisdiction for these records. The data include figures for livebirths, stillbirths, and deaths under 24 hours, 1 day to 7 days, 8 days to 28 days and 1 month to 12 months, evaluated by birthweight, length of gestation, race and sex, and plurality for the years 1959 through 1966. Corresponding data will be compiled by institution for the PRB study population. The state or city health departments have been asked to furnish either completed tabulations or raw data to be tabulated (arrangements finalized) by the PRB Section on Data Management and Retrieval.

Considerable effort has been and is being expended, in connection with the Section on Data Management and Retrieval, PRB, and the Office of Biometry, NINDS, to create a usable data file of the external data being obtained in connection with this study. The aim is to provide a file with more general utility than the limited scope of this study. When such a file is created, the information necessary to make use of the file will be made available to interested persons in PRB.

Major Findings: Evaluation of birthweight and length of gestation data for all core live births (first and subsequent pregnancies) reveals differences between races and between sexes that are generally persistent among all the institutions. Birthweights are lighter among non-whites than among whites, and among females than among males. With white males the heaviest, the order of decline is next white female, then, at about the same weight, non-white males, then non-white females. There is a particular excess of non-whites at low birth weights (2500 grams or less). Length of gestation is shorter among non-white than among whites by about 1 week, with an excess of both short-gestation and long-gestation deliveries among non-whites. Length of gestation is slightly shorter among males than among females in both races, but less consistently so among whites.

Perinatal mortality has declined in the study population since its first year, with a transient elevation in 1962. Group I mortality (fetal deaths 1001 grams and over plus deaths under 7 days of age per 1000 total births) declined from 28.5 in 1959 to 17.6 in 1966 (mean 21.7) among whites and from 33.2 to 21.8 (mean 28.4) among non-whites. Group II mortality (fetal deaths 501 grams and over plus deaths under 28 days per 100 total births) declined from 34.9 in 1959 to 19.1 in 1966 (mean 26.0) among whites and from 39.8 to 25.5 (mean 33.3) among non-whites. These trends tended to persist from institution to institution, though to varying degrees.

Previous deadline could not be met because of delays in receipt of data from the cities and in preparing these data from their various sources into a single program for the computer.

It is anticipated that the file will be completed and the present phase of the study reported by the end of June 1972.

Honors and Awards: None

Publications: None

Serial No. NDS (CF)-66 PR/OC 1378
1. Perinatal Research Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Maternal Factors Affecting Birth Weight.

Previous Title: The Prediction of Birth Weight-Multivariate Analysis.

Previous Serial Number: Same

Principal Investigator: Heinz W. Berendes, M.D., PRB, NINDS

Other Investigators: W. Weiss, Office of Biometry, NINDS
J. Deutschberger, Office of Biometry, NINDS*
E. Jackson, Office of Biometry, NINDS

Cooperating Units: Perinatal Research Branch, NINDS

Man Years:

Total:	0
Professional:	0
Other:	0

This study has been completed.

Honors and Awards: None

Publications: Weiss, W., and Jackson, E.C.: Maternal factors affecting birth weight. In Perinatal Factors Affecting Human Development. Proceedings of the Special Session held during the Eight Meeting of the PAHO Advisory Committee on Medical Research, Washington, D.C., June 10, 1969. Washington, D.C., Pan American Health Organization, Sci. Publ. No. 185, 1969, pp. 54-59.

* Deceased

Serial No. NDS (CF)-68 PR/OC 1618
1. Perinatal Research Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: The Effect of Labor on the Outcome of the Child.

Previous Serial Number: Same

Principal Investigators: Z.A. Shakhashiri, M.D., PRB, NINDS
W. Lawrence Holley, M.D., Children's Hosp., Akron, Ohio
A.A. Lilien, M.D., Somerville, New Jersey

Other Investigators: None

Cooperating Units: Perinatal Research Branch, NINDS

Man Years:

Total:	.08
Professional:	.06
Other:	.02

Project Description:

In order to single out the effect of labor, if any, on fetal growth and development, a study is made of outcomes of normal pregnancies terminated spontaneously, compared to outcomes of similar pregnancies terminated by elective induction of labor and to outcomes of similar pregnancies terminated by elective cesarean section.

Methods Employed: Project infants of normal pregnancies terminated as described above will be compared for selected outcome variables such as mortality, Apgar, bilirubin, mental and motor scores at eight months and I.Q. at four years. All three groups will be controlled for race, birth weight and gestation length.

Major Findings: A search is under way to ascertain whether cases are available in adequate numbers to make the design feasible. Additional non-Project cases might be needed.

The scope of this Project has been referred to and is being undertaken by the Ad Hoc Task Force on Labor and Delivery. See Serial No. NDS (CF)-71 PR/OB 1904.

Honors and Awards: None

Publications: None

Serial No. NDS (CF)-66 PR/OB 1331
1. Perinatal Research Branch
2. Section on Obstetrics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Obstetric Factors in Twin Pregnancies.

Previous Serial Number: Same

Principal Investigator: Rudolf F. Vollman, M. D., PRB, NINDS

Other Investigators: Jose G. Marmol, M.D., PRB, NINDS
Irene B. Ross, PRB, NINDS

Cooperating Units: All institutions participating in the Collaborative Study

Man Years

Total:	0.2
Professional:	0.1
Others:	0.1

Project Description:

Objectives: The early diagnosis of a twin pregnancy remains still an important problem on which depend the prenatal care and the management of the labor and delivery. The study has two objectives:

1. To study the outcome of twin pregnancies in relation to the time the diagnosis was first established.

2. To make a comparison of the obstetric problems presented by the first versus the second twin and their effect upon the fetal outcome.

Methods: With the help of a computer printout, an established case and card file, and an additional review of a current file on abortions and fetal deaths, the twins delivered in the Collaborative Project through December 31, 1965, have been identified. All case records were reviewed and additional information or clarification was solicited from the collaborating hospitals as needed. The mothers' medical, family and reproductive history, together with information on the course of the study pregnancy, intercurrent diseases, drugs, obstetric complications, labor and delivery, and outcome of pregnancy were abstracted. These data have been used to prepare a set of tabulations for the study of the variables specified above. This study has been completed and the variables tabulated. The preparation of the manuscript has been delayed

due to a shortage of manpower.

Honors and Awards: None

Publications: None

Serial No. NDS (CF)-66 PR/OB 1333
1. Perinatal Research Branch
2. Section on Obstetrics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Distribution of Abortions by Chronologic and Gynecologic Age of the Gravida.

Previous Serial Number: Same

Principal Investigator: Rudolf F. Vollman, M.D., PRB, NINDS

Other Investigators: Jose G. Marmol, M.D., PRB, NINDS
Irene B. Ross, PRB, NINDS

Cooperating Units: All institutions participating in the Collaborative Study

Man Years

Total:	0.3
Professional:	0.1
Others:	0.2

Project Description:

Objectives: Information is accumulating which demonstrates that endocrine and morphologic conditions for optimal reproductive performance are reached only several years after menarche. The conventional association of pregnancy outcome by chronologic maternal age will be compared with the mother's gynecologic age, based on the age at menarche.

Methods: For study pregnancies terminating in abortion, a number of important maternal variables have been abstracted from the study record: race, chronologic and gynecologic age, marital status, gravidity and parity, length of the menstrual cycle, medical and obstetric complications of the study pregnancy, duration and outcome of the study pregnancy. These variables serve as controls in the analysis of chronologic versus gynecologic maternal age.

Based on the preliminary findings, this study has been extended to include all fetal deaths in the Perinatal Research program. Work is progressing very slowly due to a shortage of manpower.

Honors and Awards: None

Publications: None

Serial No. NDS (CF)-68 PR/OB 1623
1. Perinatal Research Branch
2. Section on Obstetrics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Prenatal Drugs.

Previous Serial Number: Same

Principal Investigator: Alan L. Scriggins, M.D., University of Vermont Hospitals

Other Investigators: Rudolf F. Vollman, M.D., PRB, NINDS
Seymour Katsh, Ph.D., University of Colorado Medical Center
S. Barbara Katz, Office of Biometry, NINDS

Cooperating Units: All institutions participating in the Collaborative Study

The study has been completed.

Honors and Awards: None

Publications: None

Serial No. NDS (CF)-68 PR/OB 1625
1. Perinatal Research Branch
2. Section on Obstetrics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Menstruation and Ovulation in the Monkey.

Previous Serial Number: Same

Principal Investigator: Rudolf F. Vollman, M.D., PRB, NINDS

Other Investigators: Irene B. Ross, PRB, NINDS

Cooperating Units: Department of Embryology, Carnegie Institution of Washington

The study has been discontinued.

Serial No. NDS (CF)-70 PR/OB 1850
1. Perinatal Research Branch
2. Section on Obstetrics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Toxemia in Pregnancy and Its Relationship to the Outcome of Pregnancy.

Previous Serial Number: Same

Principal Investigator: Rudolf F. Vollman, M.D., PRB, NINDS

Other Investigators: Leon C. Chesley, M.D., Downstate Medical Center, Brooklyn, New York
Russell R. de Alvarez, M.D., Temple University School of Medicine, Philadelphia, Pa.
R. Gordon Douglas, M.D., Providence Lying-In Hospital, Providence, R.I.
Emanuel A. Friedman, M.D., Harvard University Medical School, Boston, Mass.
Adolph H. Sellmann, M.D., Charity Hospital, New Orleans, La.
Gilbert J. Vosburgh, M.D., Columbia University, College of Physicians and Surgeons, New York, N.Y.

Cooperating Units: All institutions participating in the Collaborative Project

Man Years

Total:	1.6
Professional:	0.8
Others:	0.8

Project Description:

The purpose of this study on toxemia of pregnancy is threefold:

1. To establish quantitative gradients for defined areas of the individual signs of toxemia versus outcome of pregnancy.

2. To measure the relative weights of the clinical signs of toxemia -- hypertension, proteinuria, edema -- singly and combined, in their contribution to perinatal mortality and birthweight.

3. To define a cohort of cases based on 1 and 2 as "pregnancies complicated by toxemia" in the Perinatal Research Study for further research in association with the long-term development of the children.

Methods: It was demonstrated in a preliminary study of the clinical reports of toxemia in the Study that the rates of toxemia vary widely between the participating institutions and the 2 different Study forms used, even when the populations of the gravidae were controlled for race, age, and parity. It was concluded that this large variability in the incidence of toxemia must be related to the use of different diagnostic criteria by the individual collaborating hospitals. It was therefore decided to use the original (raw) data of the clinical symptoms of toxemia and to analyze them individually and in combination to establish a uniform cohort of cases with toxemia for the study. The study will be carried out in cooperation with a special ad hoc Task Force on Toxemia and will absorb all of the professional and none professional time available.

Some preliminary findings were presented at the International Symposium on Gestosis in Basel, Switzerland, April 25-26, 1969.

Honors and Awards: None

Publications: Vollman, R.F.: Rates of toxemia by age and parity. In Rippmann, E.T. (Ed.): Die Spaetgestose (EPH-Gestose). Basel, Switzerland, Schwabe & Co. Verlag, 1970, pp. 338-342.

Serial No. NDS (CF)-70 PR/OB 1851
1. Perinatal Research Branch
2. Section on Obstetrics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Low Eclampsia Rate in the Collaborative Project.

Previous Serial Number: Same

Principal Investigator: Jose G. Marmol, M.D., PRB, NINDS

Other Investigators: None

Cooperating Units: None

The study has been completed.

Honors and Awards: None

Publications: Marmol, J.G.: Cases of eclampsia in the Collaborative Project. Schweiz. Z. Gynaek. Geburtsh. 1: 169-180, 1970

Serial No. NDS (CF)-71 PR/OB 1904
1. Perinatal Research Branch
2. Section on Obstetrics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: The Study of Labor and Delivery

Previous Serial Number: None

Principal Investigator: Rudolf F. Vollman, M.D., PRB, NINDS

Other Investigators: Emanuel A. Friedman, M.D., Harvard University Medical School, Boston, Mass.
Luke Gillespie, M.D., Boston Lying-in Hospital, Boston, Mass.
Marvin Green, M.D., New York Medical College, New York, N.Y.
Esther Jackson, Office of Biometry, NINDS
Schuyler G. Kohl, M.D., State University of New York, Downstate Medical Center, Brooklyn, N.Y.
Kenneth R. Niswander, M.D., University of California at Davis, Calif.
David Rubinstein, Office of Biometry, NINDS
Vincent Tricomi, M.D., Brooklyn-Cumberland Medical Center, Brooklyn, N.Y.

Cooperating Units: All institutions participating in the Collaborative Project

Man Years

Total:	0.3
Professional:	0.2
Others:	0.1

Project Description:

This study has two objectives:

A. To determine the influence of specific labor and delivery factors on the fetus in terms of immediate outcome and later neurologic and psychologic development.

B. To develop a reference standard of actuarial, obstetric, and labor and delivery features that will result in optimal outcome.

Labor is usually reported in quantitative units of hours and minutes and is conventionally divided into short, normal, and long labor by arbitrarily selected time intervals. These intervals, in turn, have been associated with certain complications of labor. FRIEDMAN has demonstrated in a long series of publications that these associations do not necessarily reflect the underlying physiology or pathology of uterine activity. He devised a methodology to quantify uterine activity and its deviations in relation to the phases of labor, which are based upon the observed progress of labor in time. Possible damage to the fetus is not necessarily dependent upon the total duration of labor, but on the dyscoordination or changes in the duration of any one or combinations of the phases of labor.

The identification of gravidae with specified types of labor will produce several standard cohorts that may be used by other Study sections and task forces (Pediatric Neurology, Pathology, Physical Growth and Development etc.) as variables for their respective special studies.

Methodology:

A reference cohort of gravidae with "normal labors" is defined, for which the specified outcome will be tabulated. This reference cohort will be compared with cohorts of specified types of dysfunctional labor and their respective outcomes.

Those gravidae with incomplete data on labor and delivery in the Study record will be identified and their general characteristics (race, age, parity, sex of fetus) and outcomes will be tabulated separately.

Honors and Awards: None

Publications: None

Serial No. NDS (CF)-71 PR/OB 1905
1. Perinatal Research Branch
2. Section on Obstetrics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Reference Bibliography on Human Teratology.

Previous Serial Number: None

Principal Investigator: Rudolf F. Vollman, M.D., PRB, NINDS

Other Investigators: None

Cooperating Units: Reference and Interlibrary Loan Sections of the NIH Library
Reference Section of the National Library of Medicine

Man Years

Total:	0.3
Professional:	0.2
Others:	0.1

Project Description:

The scientific publications on human teratology during the past 170 years have been reviewed and approximately 4000 items have been selected, analyzed and coded by content. The criteria for selection were:

1. originality of observation reported
2. morphological classification of malformations
3. morphogenesis of malformations in association with embryological stages
4. hypothesis on etiology of malformations in time and teratogenic factors
5. experimental teratology
6. review articles.

Findings: The study of teratology was initially stimulated by the curiosity to explore the range of the lusus naturae from which very early concepts on the embryological potential were deducted. It thus contributed to the development of systematic research in normal human embryology. From experimental embryology, critical times, effective factors, their quantity and quality of interaction with the formative process were identified and have been extrapolated to explain possible mechanisms in human teratology.

The manuscript is ready for publication.

Honors and Awards: None

Publications: None

Serial No. NDS (CF)-63 PR/PN 1163
1. Perinatal Research Branch, NINDS
2. Section on Pediatric Neurology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: An Investigation into the Relationship Between Congenital Heart and Great Vessel Anomalies and Selected Factors as Recorded in the Collaborative Perinatal Research Project.

Previous Serial Number: Same

Principal Investigator: Lenore Bajda, M.D., PRB, NINDS

Other Investigators: Heinz W. Berendes, M.D., PRB, NINDS
John L. Sever, M.D., PRB, NINDS

Cooperating Units: Office of the Director, NHI

Man Years:

Total:	0
Professional:	0
Other:	0

Project Description: The primary objective of this study is to assess relationships between certain maternal variables and congenital heart-great vessel anomalies.

Additional objectives include investigating relationships between early signs of abnormality and the existence of definitive congenital heart lesions and determining the existence of congenital heart-vessel anomaly in conjunction with mental retardation as recorded in the eight-month psychological examination, the one-year summary records, four-year psychological examination, and seven-year neurological and psychological examinations.

To date, maternal parameters analyzed included age of gravida, parity, prior pregnancy outcome, prior and current health status, ABO blood group, current smoking pattern, and viral antibody status.

Study data was obtained from Collaborative Study records received by PRB from the onset of the Study (January 1959) through December 1964. These records provided 112 live and stillbirth cardiac cases for study out of a population pool of approximately 38,000. Analysis of an expanded Study cohort through 1965, with a population pool of approximately 55,000 providing additional cases, is underway in anticipation that the additional cases will support earlier findings and perhaps provide further clues for identifying etiological relationships.

There was a definite preponderance of mothers over 30 in the C-V Study group. Controling for race, and removing cases with chromosomal aberrations, there were more white mothers in the 30 and over age group than expected at the .05 level. This trend is also noted among Negroes. There was a greater than expected number of gravida with systemic disease complications and prior pregnancy loss among the mothers of the cardiacs. A breakdown of these factors for greater specificity is pending.

Because the number of patients with each specific cardiac abnormality was small, specific associations between serological findings and clinical observations were not possible, although several interesting trends were noted. Analysis on the larger cohort is nearing completion.

A preliminary report on the 1964 cohort study was presented at the 1966 Annual Meeting of the Teratology Society.

The urgent priority of processing the entire Collaborative Project study data has temporarily delayed further work on this study.

Honors and Awards: None

Publications: None

Serial No. NDS (CF)-63 PR/PN 1164
1. Perinatal Research Branch, NINDS
2. Section on Pediatric Neurology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Early Signs as Predictors of Death and Neurological Abnormality Among Premature Infants Weighing 1000-2000 Grams

Previous Serial Number: Same and incorporating Serial No. NDS (CF)-69 PR/PN 1740

Principal Investigator: Joseph S. Drage, M.D., PRB, NINDS

Other Investigators: Karin B. Nelson, M.D., PRB, NINDS
Heinz Berendes, M.D., PRB, NINDS

Cooperating Units: None

Man Years:

Total:	.00
Professional:	.00
Other:	.00

Project Description: A group of 1365 single liveborn premature infants with birthweights of 1000-2000 grams has been prospectively studied relating the presence of specific neurological signs during the nursery period to neurological status at one year of age. Of the 1365 infants, 917 were examined at one year, 274 died during the first year, and 173 were lost to follow-up. There were 201 neurologically abnormal infants among the 917 examined at one year. The first four PED-2's (Neonatal Pediatric Examinations) were reviewed for the presence or absence of early signs. A positive early sign was defined as the occurrence, on at least one examination of the specific early sign being studied. Specific signs studied included cry, suck, palmar grasp, traction response, Moro reflex, eye movement, muscle tone, local convulsions, general convulsions, highest serum bilirubin, Coombs' test, procedures of resuscitation, etc. Each of these signs was considered separately, and those showing significant association with death and abnormal outcome were then combined. When three signs were considered in combination, the more that were positive the greater the association of neurological abnormality and death, and of neurological abnormality alone.

A preliminary manuscript has been prepared. Because of Task Force study assignments, this project is in abeyance.

Honors and Awards: None

Publications: None

Serial No. NDS (CF)-66 PR/PN 1335
1. Perinatal Research Branch, NINDS
2. Section on Pediatric Neurology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Mortality and Morbidity Among Infants Weighing 1000-2000 Grams

Previous Serial Number: Same

Principal Investigator: Joseph S. Drage, M.D., PRB, NINDS

Other Investigators: Karin B. Nelson, M.D., PRB, NINDS
B.H. Williams, M.D., PRB, NINDS

Cooperating Units: None

Man Years:

Total:	.00
Professional:	.00
Other:	.00

Project Description: A group of 1364 liveborn infants, with birthweights ranging from 1000-2000 grams, has been studied regarding outcome within the first year of life. This group of 1364 infants represents approximately 2% of the group of 55,000 single live births from which they were drawn.

Within the group of 1364 infants, 917 were examined at one year of age, and of these 201 were considered to have definite neurological abnormality. There were 274 deaths during the first year of life. Thus, 475 of the infants either died during the first year of life or were considered neurologically abnormal by examination at one year. There were 173 infants lost to follow-up and this represents 12% of the original 1364 cases. Over 50% of the deaths occurred during the first 24 hours and over 90% occurred during the first 28 days.

The 201 infants neurologically abnormal at one year were classified in the following way: isolated motor retardation, diplegia, hemiplegia, quadriplegia, monoplegia, athetosis, motor retardation with neurological signs insufficient for other diagnosis, and other(infants with neurological signs, but not fitting a specific diagnostic category). Congenital malformations were also monitored for this group.

Outcome at one year (death, abnormal, normal, and lost) was then tabulated within each 100-gram birthweight interval. In general for 1364 infants, as the birthweight increased, the percent of deaths decreased. For infants

in jeopardy (dead or abnormal at one year), the same relationship held. The percent of lost cases within each 100-gram birthweight interval varied slightly. Data has been obtained and is undergoing analysis

Because of Task Force study assignments, this project is in abeyance.

Honors and Awards: None

Publications: None

Serial No. NDS (CF)-66 PR/PN 1338
1. Perinatal Research Branch, NINDS
2. Section on Pediatric Neurology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: The Association of Mental Subnormality with Head Circumference, Congenital Malformations, and Other Conditions of the Newborn Term Infant

Previous Serial Number: Same

Principal Investigator: Lenore Bajda, M.D., PRB, NINDS

Other Investigators: Karin B. Nelson, M.D., PRB, NINDS

Cooperating Units: Office of Biometry

Man Years:

Total:	0
Professional:	0
Other:	0

Project Description: The objective of this study was to determine the relationship between head size and certain other physical features of the Collaborative Study child noted shortly after birth, at the one-year examination and at 4 year upon completion of the psychological examination. The project was in abeyance pending updating of the data file. Meanwhile Dr. Nelson and Mr. Deutschberger have completed their study on "Head Size at One Year as a Predictor of Four-Year IQ" (PR/PN 1509) using a sample of the Collaborative Study population including a partially selected pediatric group of 9,379 children. They concluded that there is approximately a 50% chance of an IQ of less than 80 at 4 years of age for the one-year male with a head size less than 43 cm. and a one-year female with a head size of less than 42 cm. Plans are under way to examine in detail the low and high head measure sample of the Nelson-Deutschberger study for other factors which might account for the correlation between head size and the four-year IQ values. Depending on the results of this analysis, the original study proposal will then move either forward with a larger sample, or terminate.

The urgent priority of processing the entire Collaborative Project study data has temporarily delayed further work on this study.

Honors and Awards: None

Publications: None

Serial No. NDS (CF)-68 PR/PN 1628
1. Perinatal Research Branch, NINDS
2. Section on Pediatric Neurology
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Effects of Prenatal Protein Deprivation on Behavior and Brain Structure of Mice

Previous Serial Number: Same

Principal Investigators: John A. Churchill, M.D., PRB, NINDS
J. H. Carleton, M.D., Miami, Florida
W. Lawrence Holley, M.D., Children's Hosp., Akron, Ohio

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	0
Professional:	0
Other	0

Temporarily discontinued pending a time when brain specimens can be processed and examined. The brains are now in celloidin blocks. Dr. Jack Carleton, Co-investigator, has left the Branch.

Honors and Awards: None

Publications: None

Serial No. NDS (CF)-68 PR/PN 1633
1. Perinatal Research Branch, NINDS
2. Section on Pediatric Neurology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Neuropsychologic Outcome of Children with Retinal Hemorrhages at Birth

Previous Serial Number: Same

Principal Investigators: Arthur L. Rosenbaum, M.D., U.C.L.A., Los Angeles, Cal.
John A. Churchill, M.D., PRB, NINDS

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	.05
Professional:	.05
Other:	.00

Project Description: Retinal hemorrhages in the newborn occur in approximately 20% vertex births. Much work has been done to elucidate the etiology of these hemorrhages, but little is known about their significance in terms of long term follow-up. This study was designed to study the neuropsychologic outcome of children with retinal hemorrhages, and also the relationship between laterality of the hemorrhage and birth position.

Approximately 200 cases of retinal hemorrhage in the newborn were studied, and matched with "non-hemorrhage" controls. These matched pairs were then compared in relationship to the following outcome variables: a) Birthweight, b) Bayley mental and motor scores given at 8 months of age, and c) The Binet IQ given at 4 years of age.

The retinal hemorrhage cases were also reviewed in an attempt to analyze the possibility of a relationship between birth position and the eye in which the hemorrhage occurred.

This material is still in the process of being prepared for submission for publication.

Honors and Awards: None

Publications: None

Serial No. NDS (CF)-69 PR/PN 1748
1. Perinatal Research Branch, NINDS
2. Section on Pediatric Neurology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Neonatal Polycythemia: I. A Manifestation of Chronic Injury During Distress

Previous Serial Number: Same

Principal Investigator: Miles M. Weinberger, M.D., Nat'l. Jewish Hosp. Denver Colorado

Other Investigators: Arthur Oleinick, M.D., EPID, NCI
John A. Churchill, M.D., PRB, NINDS

Cooperating Units: None

Man Years:

Total:	.05
Professional:	.05
Other:	.00

Project Description: The objectives of the study were to study demographic and maternal factors associated with neonatal polycythemia.

As part of the protocol during the Collaborative Study on Cerebral Palsy, capillary hematocrits were obtained as near to 48 hours as possible (generally between 36 and 60 hours) on 44,683 newborns, or 86% of all 77 and over were identified, and controls, matched for institution and year of birth, race sex, socioeconomic index, and presence or absence of 4-year follow-up examination were selected randomly from all infants with 48-hour hematocrits 50 through 65. Various demographic and maternal factors were identified on both subjects and controls, and differences were statistically evaluated.

The subject cases (those with polycythemia manifested by hematocrits 77 and over) were found to have been the product of longer gestation, but were smaller in weight than the control population. There was an increase in incidence of placental pathology and the placenta of the subject cases was significantly lighter than the weights of the controls. One-minute and five-minute Apgar scores were both lower in the subject cases and there was a greater incidence of dysmaturity diagnosed in the subject cases. When compared with the whole Collaborative Study population, infants with polycythemia were noted to come from lower socioeconomic groups. Data suggest that neonatal polycythemia may be manifestation of chronic intrauterine distress.

This material was presented at a Perinatal Research Branch (NINDS) seminar in April 1970. Considerable progress has been made toward completing this study for submission for publication. However, no additional work will be done on it until after June 1971.

Honors and Awards: None

Publications: None

Serial No. NDS (CF)-69 PR/PN 1749
1. Perinatal Research Branch, NINDS
2. Section on Pediatric Neurology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Neonatal Polycythemia: II. Outcome

Previous Serial Number: Same

Principal Investigators: Miles M. Weinberger, M.D., Nat'l. Jewish Hosp., Denver, Colorado, John A. Churchill, M.D., PRB, NINDS

Other Investigators: Arthur Oleinick, M.D., EPID, NCI

Cooperating Units: None

Man Years:

Total:	.05
Professional:	.05
Other:	.00

Project Description: The objectives of the study were to determine polycythemia in the neonatal period as an adverse effect on neuropsychological outcome.

See project report on Neonatal Polycythemia: I. A Manifestation of Chronic Injury During Distress covering demographic and maternal factors. In addition, the neuropsychological status of these infants was examined at various intervals through four years of age.

Examining only the outcome of those infants without recognized congenital malformation and gestational ages of 36 weeks and greater, it was found that while these infants tended to be small for gestation age and have lower Apgar scores, there was no significant difference in other findings in the newborn period. There was also no difference in psychological scores at eight months. At one year, there was no difference in abnormal neurological findings between subjects and control cases. At four years, however, the subjects did manifest a statistically-significant lower score on the 4-year Stanford-Binet examinations than the controls. This difference was greatest among Negroes, especially among the Negro females. White males did not manifest any difference in 4-year IQ.

Significance is not so much that some differences in 4-year IQ were found among some of the subgroups of the polycythemic infants, but that contrary to the expectations from the literature the differences between polycythemic infants and non-polycythemic controls were so small.

This material is still in the process of being prepared for submission for publication. However, no additional work will be done on it until after June, 1971.

Honors and Awards: None

Publications: None

Serial No. NDS (CF)-69 PR/PN 1750
1. Perinatal Research Branch, NINDS
2. Section on Pediatric Neurology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Congenital Marrow Dysfunction in Down's Syndrome

Previous Project Title: Abnormal Hematopoiesis in Newborns with Down's Syndrome

Previous Serial Number: Same

Principal Investigators: Miles M. Weinberger, M.D., Nat'l Jewish Hosp.
Denver, Colorado
Arthur Oleinick, M.D., EPID, NCI

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	.05
Professional:	.05
Other:	.00

Study has been completed.

Honors and Awards: None

Publications: Weinberger, M.M. and Oleinick, A.: Congenital marrow dysfunction in Down's syndrome. J. Pediat. 77: 273-279, 1970

Serial No. NDS (CF)-70 PR/PN 1853
1. Perinatal Research Branch, NINDS
2. Section on Pediatric Neurology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: The Effects of Protein Malnutrition on Ontogeny of the Brain

Previous Serial Number: Same

Principal Investigator: John A. Churchill, M.D., PRB, NINDS

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	0
Professional:	0
Other:	0

Project Description: A review was made of the literature pertaining to the effect of protein-calorie malnutrition of the mother during pregnancy on the nervous system structure and function of the offspring. From this review, a critique was prepared and presented at the meeting of the American Association for the Advancement of Science, held in Boston, Massachusetts on December 26-31, 1969. Study has been completed.

Honors and Awards: None

Publications: None

Serial No. NDS (CF)-70 PR/PN 1854
1. Perinatal Research Branch, NINDS
2. Section on Pediatric Neurology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: The Etiology of Cerebral Palsy in Prematures.

Previous Serial Number: Same

Principal Investigator: John A. Churchill, M.D., PRB, NINDS

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	.30
Professional:	.25
Other:	.05

Project Description: Theories of the etiology of cerebral palsy were studied in a group of 1364 singleton liveborn infants weighing 2.0 Kg. or less at birth, the group comprising all such small babies in the Collaborative Project totalling 54,370 births. Subjects diagnosed as having cerebral palsy at 1 year of age numbered 45.

No support for the genetic theory was obtained by a study of siblings of the spastics.

The anoxia theory gained no support in that Apgar and other indirect measures of asphyxia differed insignificantly between the spastic and equally premature non-spastic groups.

Hyperbilirubinemia did not appear to cause spastic diplegia since bilirubin levels differed very little between spastics and non-spastics. Of 18 cases with erythroblastosis, none had cerebral palsy.

Intrauterine blighting of the fetus was considered unlikely. Intrauterine disturbances often produce growth retardation. None of the small-for-dates infants had spastic diplegia. Furthermore, 5 spastics were born by "elective" caesarean section, one being remarkably free from abnormal prenatal events.

Evidences of cranial injury or of factors conducive to birth trauma were no more frequent in spastic than in other prematures. The spastic cases born by caesarean section, where trauma should be minimized, weighed against the trauma theory.

Cerebral hemorrhage as the cause of premature spastic diplegia was found to

deserve attention. Hematocrits obtained in the first few postpartum days were significantly lower in spastic than in non-spastic prematures. Hemodilution did not explain the observation. Others have shown that low hematocrits occur in prematures who have cerebral hemorrhage.

Compelling and direct evidence that cerebral hemorrhage causes spastic diplegia of prematurity cannot be derived from this study. However, the results make clear a need to investigate hemorrhagic mechanisms in prematures.

This has been submitted for publication.

Honors and Awards: None

Publications: None

Serial No. NDS (CF)-68 PR/BS 1640
1. Perinatal Research Branch
2. Section on Behavioral Sciences
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: The relationship of demographic, perinatal and other developmental characteristics to intellectual and motor performance of pre-school children.

Previous Serial Number: Same

Principal Investigator: Sarah H. Broman, Ph.D., PRB, NINDS

Other Investigators: Jaswant Khanna, Ph.D., University of Tennessee
Joseph Weber, formerly Office of Biometry, NINDS

Cooperating Units: University of Tennessee
Office of Biometry, NINDS

Man Years:

Total:	.25
Professional:	.20
Other:	.05

Project Description:

30 characteristics of some 13,000 study children have been related to their pass-fail performance on individual items in the psychology battery administered at four years of age. Preliminary analyses relating four demographic variables to performance on the Stanford-Binet show that: (I) differences among white, Negro and Puerto Rican children in IQ, socioeconomic index, educational level of mother and proportion of males to females are highly significant. Whites exceed the other two groups in mean IQ and socioeconomic index. Differences among the groups in mean number of years of mother's education are more evenly spaced with whites > Negroes > Puerto Ricans. Sex ratios in these groups follow a different order with Puerto Ricans > whites > Negroes; (II) Highly significant differences in percent pass by ethnic group occur on all 28 of the Stanford Binet items examined. These items cover the test levels from 2 years 6 months through 6 years. Among whites percent pass is significantly higher than among the total group on 27 items. Among Negroes percent pass is significantly lower than the total group on 26 items. Among Puerto-Ricans, percent pass is significantly lower than the total group on 16 items, does not differ from the total group on nine items and is higher on three items: (III) Within both the white and Negro groups, children who pass have a significantly higher socioeconomic index than those who fail on all of the 28 Binet items. Among Puerto-Ricans however these differences occur on only 10 items; (IV) Similarly, among both whites and Negroes, educational level of mother is significantly

higher for passers than for those who fail on all 28 items. However for Puerto Ricans this occurs on only five items; (V) Among whites, sex differences in performance occur on 13 of the 28 Binet items with proportionally more males than females failing these items. Among Negroes sex differences in favor of females occur on 15 items and in favor of males on one item. Among Puerto Ricans sex differences are significant on only five items, again with more males than females failing these items.

The content of the items on which all of the above differences occur is of course highly relevant and is now being categorized. The majority of test items administered at this age are verbal. However the following trends in ethnic group differences have been noted. Using McNemar's classification of items, verbal item differences are largest between white and Puerto Ricans; memory item differences are largest between whites and Negroes, non-verbal item differences are relatively small among all groups. Using five factors identified by Valett, whites are consistently higher than both Negroes and Puerto Ricans on groups of items representing the three factors of general comprehension, vocabulary and verbal fluency, and judgement and reasoning. For the factor of visual motor ability, whites consistently exceed Negroes but only slightly exceed Puerto Ricans. For the last factor considered, memory and concentration, whites are higher than Negroes and slightly higher than Puerto Ricans. When Negro-Puerto Rican differences are examined, the two groups differ very little on the factors of general comprehension and memory and concentration. Puerto Ricans are higher on the visual motor items and Negroes are higher on vocabulary and verbal fluency and to a lesser extent on judgement and reasoning items.

Subsequent analyses will explore the relationship of perinatal, neurological and other psychological variables to performance at four years of age.

Honors and Awards: None

Publications: None

Serial No. NDS (CF)-69 PR/BS 1752
1. Perinatal Research Branch
2. Section on Behavioral Sciences
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Duration of Membrane Rupture and Psychological Outcome

Previous Serial Number: Same

Principal Investigator: Dr. Lee Willerman, PRB,NINDS

Other Investigator: Dr. John A. Churchill, PRB,NINDS

Cooperating Unit: Section on Pediatric Neurology, PRB,NINDS

This project has been discontinued.

Serial No. NDS (CF)-69 PR/BS 1753
1. Perinatal Research Branch
2. Section on Behavioral Sciences
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Growth and Intellectual Development of Children from Consanguineous Matings

Previous Serial Number: Same

Principal Investigator: Dr. Lee Willerman, PRB, NINDS

Other Investigators: Dr. Ntinos C. Myrianthopoulos, PRB, NINDS
Dr. Alfred F. Naylor, PRB, NINDS

Cooperating Unit: Section on Epidemiology and Genetics, PRB, NINDS

Man Years:

Total:	.10
Professional:	.05
Other:	.05

Project Description:

Objectives: Consanguineous matings have been reported to be associated with a higher incidence of fetal and neonatal mortality as well as mental retardation in the offspring. The present study will examine outcomes in the offspring of 148 sets of parents in the Collaborative Study who are second cousins or closer. Cases coded as consanguineous are now being reviewed for validity of such codes in the Section on Epidemiology and Genetics. Following this review, analysis of the outcome variables is expected to proceed quickly.

Honors and Awards: None

Publications: None

Serial No. NDS (CF)-69 PR/BS 1754
1. Perinatal Research Branch
2. Section on Behavioral Sciences
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Growth and Intellectual Development of Children From Interracial Matings

Previous Serial Number: Same

Principal Investigator: Dr. Lee Willerman, PRB, NINDS

Other Investigators: Dr. Alfred R. Naylor, PRB, NINDS
Dr. Ntinos C. Myrianthopoulos, PRB, NINDS
Dr. John A. Churchill, PRB, NINDS

Cooperating Units: Section on Epidemiology and Genetics, PRB, NINDS
Section on Pediatric Neurology, PRB, NINDS

Man Years:

Total:	.40
Professional:	.35
Other:	.05

Project Description:

Objectives: Offspring from Negro-white matings (n=171) were individually matched to children from white-white and Negro-Negro matings on hospital of birth, socioeconomic index, and marital status. Though not significantly different from the controls in either length or weight at birth, by four months of age the interracials were significantly smaller than the controls. At one year the interracials were still smaller, but the magnitude of the differences had diminished considerably. On psychological test performance at eight months, no differences were observed. At four years the IQs of the interracial children were significantly lower than the white controls, but not significantly lower than the Negro controls. Birthweights and lengths of the interracial children were intermediate to the larger white and smaller Negro children regardless of the race of the interracial mother. These results suggest a genetic basis to the findings that Negro children weigh less and are shorter than white children at birth.

Honors and Awards: None

Publications:

Willerman, L., Naylor, A. F., Myrianthopoulos, N. C.: Intellectual Development of Children from Interracial Matings. Science, 1970, Vol. 170, pp. 1329-1331.

Serial No. NDS (CF)-69 PR/BS 1756
1. Perinatal Research Branch
2. Section on Behavioral Sciences
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Preschool Stuttering and Early Maternal Attitudes

Previous Serial Number: Same

Principal Investigator: Raymond H. Holden, Ed.D., Brown University

Other Investigator: Paul J. LaBenz, Sc.D., PRB, NINDS

Cooperating Unit: Child Development Study, Brown University

Man Years:

Total:	.35
Professional:	.30
Other:	.05

Project Description:

This study evaluates the relationship of dysfluency in speech noted in children at age three years, and attitudes of their mothers as reported on the Parental Attitude Research Instrument (PARI) when the children were 8 months old. A sample of 1100 children in the Providence Child Development Study was screened for speech, language and hearing problems at three years of age. Twenty six children were identified as dysfluent. Two random samples consisting of 26 cases showing no dysfluency and 26 cases rated as unknown were selected from their respective groups within the sample.

No significant differences were found between the dysfluent and normal groups with regard to total PARI scores, socioeconomic status or mean IQ (Revised Stanford-Binet Form L-M, 1960) at age 4 years. The group rated unknown was 19 points lower than both of the other groups in mean IQ. Review of their records indicated that they could not be rated for dysfluency because they were uncooperative, untestable and/or mentally retarded. Follow-up on partial samples of the dysfluent group revealed a marked drop in mean verbal IQ at age 7 years, and a high proportion of abnormal articulation ratings at age 8 years. This study will be completed when additional follow-up data is available.

Honors and Awards: None

Publications: None

Serial No. NDS (CF)-69 PR/BS 1757
1. Perinatal Research Branch
2. Section on Behavioral Sciences
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Social Class and Outcome in the Neurologically Abnormal Infant

Previous Serial Number: Same

Principal Investigator: Raymond H. Holden, Ed.D., Brown University Providence, Rhode Island

Other Investigator: Lee Willerman, Ph.D., PRB, NINDS

Cooperating Unit: Brown University, Providence, Rhode Island

This study has been completed.

Honors and Awards: None

Publications:

Holden, R. H. and Willerman, L.: Social class and outcome in the neurologically abnormal child. In Trapp, E. P. (ed.) Readings on the Exceptional Child. 2nd Ed. Appleton-Century-Crofts, 1971. In Press.

Serial No. NDS (CF)-69 PR/BS 1758
1. Perinatal Research Branch
2. Section on Behavioral Sciences
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Neonatal, Ethnic and Social Class Factors in Infant and Pre-school Test Performance

Previous Project Title: Maturity at Birth, Mental and Motor Performance at Eight Months and Intelligence Quotient at Four Years

Previous Serial Number: Same

Principal Investigator: Sarah H. Broman, Ph.D., PRB, NINDS

Other Investigator: Heinz W. Berendes, M.D., PRB, NINDS

Cooperating Unit: Office of the Chief, PRB, NINDS

Man Years:

Total:	.25
Professional:	.20
Other:	.05

Objectives: This study examined the effects of two measures of maturity at birth on mental and motor development at eight months and on a subsequent measure of intelligence at four years in two large samples of children who are being followed longitudinally in the Collaborative Study in the National Institute of Neurological Diseases and Stroke. Birthweight and gestational age were related to Bayley Mental and Motor scores at eight months and to Stanford-Binet IQs at four years within the context of ethnic, social class and sex classifications. The relationship of Bayley scores to Binet IQ was also examined. A multiple linear regression model was used.

The major findings were as follows:

1. Among infants tested at eight months (N=31,678) there is a highly significant association between the group of predictor variables of ethnicity (white and Negro) sex, social class, (four levels of maternal education), birthweight and gestational age and the criterion of Bayley mental score. However only seven percent of the variance was explained in the criterion measure. The most "useful" predictor was birthweight, followed by gestational age.

2. A similar pattern resulted from the analysis of Bayley motor scores. Nine percent of the variance was explained and again birthweight, followed by gestational age, was the best predictor.

3. Among children tested at four years (N=16,638) there is a highly signi-

ficant association between the group of predictor variables of ethnicity, sex, social class, birthweight, gestational age, eight month mental scores and eight month motor scores and the criterion of Stanford-Binet IQ. Twenty-eight percent of the variance was explained in the criterion measure. The most useful predictor was ethnicity followed by social class (maternal education).

The implications of these findings and other derived from analyses of the relationship of the predictors to the criterion measures within the four separate ethnic-sex groups are discussed in the context of the characteristics of the samples studied. This study has been completed.

Honors and Awards: None

Publications: None

Serial No. NDS (CF)-70 PR/BS 1856
1. Perinatal Research Branch
2. Section on Behavioral Sciences
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Biosocial Influences on Human Development

Previous Serial Number: Same

Principal Investigator: Dr. Lee Willerman, PRB, NINDS

Other Investigator: None

Cooperating Unit: None

This paper has been completed and was presented at the 47th Annual Meeting of the American Orthopsychiatric Association, San Francisco, California, March 23-26, 1970. The abstract has been published in the American Journal of Orthopsychiatry, 40: 324, 1971.

Honors and Awards: None

Publications: None

Serial No. NDS (CF)-70 PR/BS 1857
1. Perinatal Research Branch
2. Section on Behavioral Sciences
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: The Genetics of Intellectual and Motor Performance

Previous Serial Number: Same

Principal Investigator: Sarah H. Broman, Ph.D., PRB, NINDS

Other Investigators: N. C. Myrianthopoulos, Ph.D., PRB, NINDS
Lee Willerman, Ph.D., PRB, NINDS
V. L. Anderson, Ph.D., University of Minnesota
Paul Nichols, Ph.D., University of Minnesota

Cooperating Units: Section on Epidemiology and Genetics
The Dight Institute for Human Genetics, University of Minnesota

Man Years:

Total:	.25
Professional:	.20
Other:	.05

Project Description:

Objectives: The objective of the study is to assess the contribution of genetics to the variance of behavioral measures, particularly intellectual and motor performance, by the calculation of heritability in twins. The results of the psychological tests given at ages eight months, four years and seven years have been analyzed for all twins of known zygosity and for all sibs from first and second study pregnancies. It is also planned to analyze these data for an appropriate sample of unrelated child pairs. As a physical parallel, the heritabilities of height, weight and head circumference at several age levels have been computed.

Some selected findings are as follows: (1) heritability of IQ at age four does not appear to be lower than in older populations; (2) the between family variance componant of the four-year IQ test was nearly twice as large in the white population as in the Negro population suggesting that heritability is lower in Negroes than in whites; (3) an analysis of variance of weight and height measured at four years revealed that, unlike IQ, the variance componants were not different in whites and Negroes; (4) at seven years, a high correlation (.86) was found between 12 subtests social class loading and the Negro-white differences on the test, while no relationship was found between Negro-white differences and heritabilities of the subtests

after controlling for the subtests' social class loading; (5) the discrepancy in IQ between twins and singletons decreased in both races from four to seven years.

Analyses of these data are continuing.

Honors and Awards: None

Publications: None

Serial No. NDS (CF)-63 PR/EG 1174
1. Perinatal Research Branch
2. Section on Epidemiology and Genetics
3. Bethesda, Maryland

PHS
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Birthweight in Relation to Selected Socioeconomic Variables

Previous Serial Number: Same

Principal Investigator: Dr. N.C. Myrianthopoulos, PRB, NINDS

Other Investigators: Dr. Joshua Lederberg, Stanford University

Cooperating Units: None

Man Years:

Total:	.10
Professional:	.05
Other:	.05

Project Description:

Objectives: This is a continuation of a study to relate birthweight to socioeconomic and medical variables. The results of the first part of the study have already been published (Ann. Hum. Genet. 31:71-83, 1967).

Proposed course: In the second part the plan is to compare children in the 100th percentile of birthweight with children in the 50th percentile of birthweight and children of diabetic mothers, in terms of several socioeconomic, biological and medical variables. A magnetic tape with all the necessary data has been prepared. Analysis is done at Stanford University under Dr. Lederberg's direction. No further progress has been made in this study.

Honors and Awards: None

Publications: None

Serial No. NDS (CF)-63 PR/EG 1175
1. Perinatal Research Branch
2. Section on Epidemiology and Genetics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Determination of the Zygosity of Twins Born to Mothers in the Collaborative Study

Previous Serial Number: Same

Principal Investigator: Dr. N.C. Myrianthopoulos, PRB, NINDS

Other Investigators: None

Cooperating Units: All Institutions participating in the Collaborative Study

Man Years:

Total:	.15
Professional:	.15
Other:	.00

Project Description:

Objectives: This is a continuing project to determine the zygosity and epidemiologic characteristics of twins born to Study mothers.

Methods employed: Twin zygosity is determined by comparison of sex, placentation, blood groups, and finger and palm prints. This information is forwarded by all Institutions to the Section on Epidemiology and Genetics where it is classified and analyzed by special methods.

Major findings: In addition to the findings reported in last year's report, an analysis has been made of respiratory distress syndrome in the twins. Respiratory distress syndrome occurred in 77 of 1130 liveborn twins (or 1 in 15) and appears to be from 5 to 9 times more frequent than in singletons. This increase cannot be entirely accounted for by the higher prematurity rate of the twins over singletons. In 46 twin pairs with at least one affected, there was a significantly higher concordance rate among MZ than DZ pairs, suggesting that genetic factors are of some etiologic importance in this disease.

An analysis of congenital malformations in the twins is also being made.

Proposed course: To study all twins in terms of a variety of genetic and socioeconomic variables.

Honors and Awards: None

Publications: Myrianthopoulos, N.C.: A survey of twins in the population of a prospective collaborative study. Acta Genet. Med. Gemellol. 19: 15-23, 1970.

Myrianthopoulos, N.C.: An epidemiologic survey of twins in a large, prospectively studied population. Amer. J. Hum. Genet. 22: 611-629, 1970.

Myrianthopoulos, N.C.: Respiratory distress syndrome in twins. Acta Genet. Med. Gemellol., in press.

Serial No. NDS (CF)-63 PR/EG 1177
1. Perinatal Research Branch
2. Section on Epidemiology and Genetics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Genetic and Socioeconomic Factors in Early and Late Fetal Death

Previous Serial Number: Same

Principal Investigator: Dr. N.C. Myrianthopoulos, PRB, NINDS

Other Investigators: Esther Jackson, OB, NINDS

Cooperating Units: None

Man Years:

Total:	.10
Professional:	.10
Other:	.00

Project Description:

Objectives: To determine what effect do genetic and socioeconomic factors have on pregnancy wastage and if a distinction can be made etiologically between early and late fetal deaths.

Methodology and major findings: Discriminant analysis of 1,877 early and late fetal deaths with respect to 40 medical, genetic and socioeconomic variables, shows that toxemia, anemia and complications of pregnancy are good discriminants in both whites and Negroes; prior pregnancies and prior pregnancy wastage in whites only; and socioeconomic status in Negroes only. Knowledge of sex of study child improves the power of the discriminant function.

When the sample is restricted to fetal deaths of mothers who registered in the first trimester the results are not appreciably changed. This project is now completed.

Honors and Awards: None

Publications: None

Serial No. NDS (CF)-63 PR/EG 1184
1. Perinatal Research Branch
2. Section on Epidemiology and Genetics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Population Dynamics of Tay-Sachs Disease and other Sphingolipidoses

Previous Serial Number: Same

Principal Investigator: Dr. N.C. Myrianthopoulos, PRB, NINDS

Other Investigators: Dr. Stanley Aronson, Brown University

Cooperating Units: None

Man Years:

Total:	.15
Professional:	.15
Other:	.00

Project Description:

Objectives: This is a continuation of a study to determine whether differential fertility favoring the Jewish heterozygote can account for the 100 fold higher frequency of Tay-Sachs disease and the gene responsible for it among the Jewish compared with non-Jewish population in the U.S. Evidence for selective advantage of the heterozygote was found in the first part of the study (Am. J. Hum. Genet. 18:313-327, 1966).

Proposed course: The plan is to investigate whether or not such advantage can be demonstrated in other sphingolipidoses, to study the demography of these disorders and to determine whether resistance to tuberculosis or some other infectious disease confers a selective advantage to the heterozygote.

Methodology: About 4,000 cases of Jewish immigrant patients who had TB have been collected from the records of the American Chronic Disease Center, Denver, Colorado. The distribution of places of origin of these patients has been compared with that of ancestors of Tay-Sachs disease and a negative rank correlation has been found suggesting that the frequency of TB had been low in areas of high concentrations of Tay-Sachs disease, and vice versa. A more sophisticated analysis is now being performed.

Honors and Awards: None

Publications: None

Serial No. NDS (CF)-65 PR/EG 1274
1. Perinatal Research Branch
2. Section on Epidemiology and Genetics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Genetic Bases of Neonatal Reflexes

Previous Serial Number: Same

Principal Investigator: Dr. A.F. Naylor, PRB, NINDS

Other Investigators: Dr. N.C. Myrianthopoulos, PRB, NINDS

Cooperating Units: None

Man Years:

Total:	.05
Professional:	.05
Other:	.00

Project Description:

Objectives: To investigate the validity of regarding the suck, rooting and other neonatal reflexes as genetic entities.

Major findings: An initial set of cases retrieved for absence of one or more of these reflexes was reviewed and seemed to have high frequencies of various kinds of trauma whose base line frequencies were unknown.

Proposed course: To place limits on the frequencies of losses of suck, rooting, palmar grasp, plantar grasp and Moro reflexes because of mutation or segregation at gene loci specifically affecting manifestation of these reflexes.

The completion of the (condensed) Variable Data File makes practical the reactivation of this project along proper lines. Base populations can be selected for general health, especially neurological, and frequencies of isolated absence or weakness of single neurological signs can be tested. Active work on this project will be undertaken when most current tasks have been carried out.

Honors and Awards: None

Publications: None

Serial No. NDS (CF)-65 PR/EG 1276
1. Perinatal Research Branch
2. Section on Epidemiology and Genetics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Sequential Aspects of Occurrence of Spontaneous Abortion in Family Histories

Previous Serial Number: Same

Principal Investigator: Dr. A.F. Naylor, PRB, NINDS

Other Investigators: Dr. Dorothy Warburton, College of Physicians and Surgeons of Columbia University

Cooperating Units: None

Man Years:

Total:	.20
Professional:	.20
Other:	.00

Project Description:

Objectives: To relate the risk of spontaneous abortion to maternal age and prior reproductive experience. A special point under investigation is whether apparent age effects are explicable by a tendency for intrinsic habitual aborters to remain in the reproductive population longer in attempts to compensate for unsuccessful pregnancies. Also conditional risks have been estimated.

Proposed course: Although certain portions of the data analysis support a maternal aging explanation for part of the trend in abortion risk, a reconsideration of the original tabular outputs shows that a very substantial additional parity exists. An existing manuscript must be completely redrafted to reflect this change in interpretation.

Honors and Awards: None

Publications: None

Serial No. NDS (CF)-67 PR/EG 1510
1. Perinatal Research Branch
2. Section on Epidemiology and Genetics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: The Study of Maternal Effects in the Production of Congenital Malformations

Previous Serial Number: Same

Principal Investigator: Dr. N.C. Myrianthopoulos, PRB, NINDS

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	.10
Professional:	.10
Other:	.00

Project Description:

Objectives: To determine the extent to which maternal factors are involved in the production of congenital malformations, and to single out those malformations and conditions of the newborn in which the role of maternal factors, genetic and environmental, appears to be deciding.

Methods employed: The GEN 5-8 was used to obtain a history of outcome of prior pregnancies, in families in which half-siblings are present. Study pregnancies were also included.

Major findings: In all, 152 cases were identified and these were screened for occurrence of congenital malformations and other conditions among children by different fathers. Six conditions were found to occur in high frequency among half sibs: Rh trouble, convulsions, congenital heart disease, club foot, mental retardation and polydactyly.

Analysis of the distribution of these abnormalities in first and second sibships of the same family showed that in the case of seizures and mental retardation the experience in the first sibship was significantly different from that in the second, indicating a genetic etiology; in the case of congenital heart defects and club foot the experience seemed to be the same in both sibships, indicating that environmental maternal factors predominate.

Additional cases have now become available and a more complete analysis is being made. The study is in progress.

Honors and Awards: None

Publications: None

Serial No. NDS (CF)-67 PR/EG 1514
1. Perinatal Research Branch
2. Section on Epidemiology and Genetics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Record Linkage of Relatives Registered in the Collaborative Study

Previous Serial Number: Same

Principal Investigator: Dr. A.F. Naylor, PRB, NINDS

Other Investigators: Dr. N.C. Myrianthopoulos, PRB, NINDS

Cooperating Units: None

Man Years:

Total:	.60
Professional:	.15
Other:	.45

Project Description:

Objectives: To identify all relatives of gravidae registered in the Collaborative Study.

Methodology: Abstracting of reports of relatives has been completed and the information key-punched into card images on magnetic tape.

Proposed course: In its current form the file lists, case by case, the NINDS number of reporter and reported relatives and relationship code. The file must be reprocessed into pairs so that the relative making the report is retrievable when the reported relative is an index case; also transitive pairing -- linkage of two relatives who are both reported by one woman -- must be established.

Honors and Awards: None

Publications: None

Serial No. NDS (CF)-67 PR/EG 1515
1. Perinatal Research Branch
2. Section on Epidemiology and Genetics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Rh Hemolytic Disease in Negro and White Infants

Previous Serial Number: Same

Principal Investigator: Dr. A.F. Naylor, PRB, NINDS

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	.01
Professional:	.01
Other:	.00

Project Description:

Objectives: To confirm a report that high Rh antibody levels have smaller morbid effects in Negro than in White babies, although this is not true for ABO antibodies.

Major findings: Preliminary and indirect confirmation has been obtained, from a small data sample under study in this Section, for reports in the literature that high Rh antibody titers are not as highly associated with serious morbidity in Negroes as in whites.

Proposed course: The existence of the Variable Data File will make possible the easy execution of the required data processing.

Honors and Awards: None

Publications: None

Serial No. NDS (CF)-67 PR/EG 1516
1. Perinatal Research Branch
2. Section on Epidemiology and Genetics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Size of Placenta in Relation to Mother-Fetus Antigenic Difference

Previous Serial Number: Same

Principal Investigators: Dr. Dorothy Warburton, College of Physicians and Surgeons of Columbia University
Dr. A.F. Naylor, PRB, NINDS

Other Investigators: Dr. G. Nicholas Rogentine, Immunology Branch, NCI
Dr. Robert Cefalo, Obstetrics, National Naval Medical Center
Mrs. Lois Dienes, PRB, NINDS
Mrs. Jean Nemore, PRB, NINDS

Cooperating Units: Obstetrics Department, National Naval Medical Center

Man Years:

Total:	1.20
Professional:	.80
Other:	.40

Project Description:

Objectives: To investigate the hypothesis published by Billington in 1964, that sensitization of the mother during pregnancy against paternal antigens leads to non-pathological placental hypertrophy and increased birthweight in succeeding pregnancies.

Major findings: Analysis of a large sample of study data has given convincing support to the hypothesis. A paper reporting the evidence has been published.

Proposed course: The arrangements for a laboratory study, described in the 1969-70 Annual Report have been active and 25 maternal sera have been tested against paternal leucocytes. No attempt at analysis will be made until many more cases have accumulated.

Honors and Awards: None

Publications: Warburton, D. and Naylor, A.F.: The effect of parity on placental weight and birth weight: An immunological phenomenon? A report of the Collaborative Study of Cerebral Palsy. Amer. J. Hum. Genet. 23: 41-54, 1971.

Serial No. NDS (CF)-68 PR/EG 1646
1. Perinatal Research Branch
2. Section on Epidemiology and Genetics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Study of the Physical Growth of Children in the Collaborative Study

Previous Serial Number: Same

Principal Investigator: Dr. Glen S. Bartlett, Case Western Reserve University

Other Investigators: None

Cooperating Units: Office of Biometry, NINDS

This project has been transferred to the jurisdiction of the task force on Physical Growth and Development.

Serial No. NDS (CF)-68 PR/EG 1647
1. Perinatal Research Branch
2. Section on Epidemiology and Genetics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Development and Evaluation of an Index of Reproductive Performance for the Purpose of Identifying High Risk Pregnancy Suspects

Previous Serial Number: Same

Principal Investigator: Dr. Glen S. Bartlett, Case Western Reserve University

Other Investigators: None

Cooperating Units: None

This project has been temporarily discontinued and will be held in abeyance.

Serial No. NDS (CF)-68 PR/EG 1648
1. Perinatal Research Branch
2. Section on Epidemiology and Genetics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Further Investigation of the Socioeconomic Index as a Descriptive and Predictive Instrument

Previous Serial Number: Same

Principal Investigators: Dr. Glen S. Bartlett, Case Western University
Dr. N.C. Myrianthopoulos, PRB, NINDS

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	.00
Professional:	.00
Other:	.00

Project Description:

Objectives: The socioeconomic status score or socioeconomic index, developed by the Bureau of the Census for use with the 1960 Census, has been used with good results in this Section to describe the Collaborative Study population, and by others to describe other populations. This study is designed to explore mathematically the three component parts of the index -- education and occupation of the head of household, and family income -- in order to assess their relative contributions to the index; to evaluate the ability of the index to encompass other socioeconomic variables not used in deriving the inde ; and to investigate whether or not the index, in addition to being descriptive, can also be used as a reliable predictive instrument.

Methodology: Linear discriminant function and multiple regression analysis will be used to determine appropriate weights to be applied to the component factors, and correlation coefficients will be obtained between the index and its components, and external variables.

Current status: A working paper proposing three models has been written. Data from first study pregnancy and from the 7-year follow-up examination will be used to test these models. No further progress has been made in this project.

Honors and Awards: None

Publications: None

Serial No. NDS (CF)-71 PR/EG 1906
1. Perinatal Research Branch
2. Section on Epidemiology and Genetics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Blood group effects in mother and offspring

Previous Serial Number: None

Principal Investigators: Dr. N.C. Myrianthopoulos, PRB, NINDS
Dr. Bernice Cohen, Johns Hopkins University

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	.10
Professional:	.10
Other:	.00

Project Description:

Objectives: To determine the influence, if any, of maternal and/or infant ABO and/or Rh types upon maternal manifestations and fetal-infant-child survivorship, morbidity and development.

Proposed course and methodology: A comprehensive investigation will be made of the blood group relationships, including possible deleterious consequences or beneficial effects in mothers and offspring of various maternal ABO and/or Rh types and ABO-Rh combinations both per se, and grouped as potentially "compatible" and "incompatible" combinations of mother and conceptus. Maternal and offspring effects will be examined in terms of immediate and acute effects and in terms of long-range effects in mother and offspring, through developmental milestones at 7 years of age. Comparisons will be made by institution, race, age of mother, previous reproductive history, complications of pregnancy, immunoglobulin levels, and socioeconomic status.

Honors and Awards: None

Publications: None

Serial No. NDS (CF)-71 PR/EG 1907
1. Perinatal Research Branch
2. Section on Epidemiology and Genetics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Epidemiologic and genetic study of congenital malformations

Previous Serial Number: None

Principal Investigator: Dr. N.C. Myrianthopoulos, PRB, NINDS

Other Investigators: Dr. J. Drage, PRB, NINDS
Dr. C.S. Chung, University of Hawaii

Cooperating Units: None

Man Years:

Total:	.20
Professional:	.15
Other:	.05

Project Description:

Objectives: To study the epidemiology of all congenital malformations in Collaborative Study children at birth and one year; to test the hypothesis of quasi-continuous distribution as an inheritance mechanism for most malformations; to assess the genetic load due to congenital malformations; to test the etiologic significance of several medical, genetic and socioeconomic factors in the occurrence of selected malformations; and to explore more fully previous findings suggesting a relationship of diabetes in the mother and the occurrence of congenital heart disease in the offspring.

Proposed course and methodology: The plan is to draw a definitive list of congenital malformations and develop a file of all Collaborative Study children who had a malformation between birth and one year of age. Consanguinity data will be used to assess the genetic load and an extension of Falconer's method will be employed for the quasi-continuous distribution analysis. A prospective analysis will be used to test the etiologic significance of medical, genetic and socioeconomic factors.

Honors and Awards: None

Publications: None

Serial No. NDS (CF)-71 PR/EG 1908
1. Perinatal Research Branch
2. Section on Epidemiology and Genetics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: A chromosomal study of children in the Collaborative Study

Previous Serial Number: None

Principal Investigators: Dr. N.C. Myrianthopoulos, PRB, NINDS
Dr. H. Lubs, University of Colorado

Other Investigators: None

Cooperating Units: Boston Children's Hospital Medical Center, Children's Hospital University of Buffalo, University of Tennessee, Children's Hospital of Philadelphia and University of Oregon Medical School

Man Years:

Total:	.20
Professional:	.15
Other:	.05

Project Description:

Objectives: To study the epidemiology of chromosomal aberrations in approximately 10,000 Study children at age 7 years; and to relate major and minor chromosomal deviants to growth, mental and motor development and neurological status of these children.

Proposed course and methodology: A blood sample will be taken from children at the five collaborating institutions, during their 7-year examination. Two cells will be immediately analyzed; ten cells will be analyzed when chromosomal anomalies are found or when the children have some mental or motor anomaly or a congenital malformation. All technical work will be done in accordance with standardized techniques and new techniques such as fluorescence staining, will be employed. All measurements and analysis will be done at Denver with the aid of an antomatic chromosome analyzer. A second phase of the study will be concerned with the chromosomal-clinical correlations.

Honors and Awards: None

Publications: None

Serial No. NDS (CF)-71 PR/EG 1909
1. Perinatal Research Branch
2. Section on Epidemiology and Genetics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Continuation of study of twins born in the Collaborative Study beyond the age of seven years

Previous Serial Number: None

Principal Investigator: Dr. N.C. Myrianthopoulos, PRB, NINDS

Other Investigators: None

Cooperating Units: All Collaborating Institutions

Man Years:

Total:	.10
Professional:	.05
Other:	.05

Project Description:

Objectives: To follow all living twins and their siblings born in the Collaborative Study to age 15 years in order to study their physical and mental development through puberty.

Proposed course and methodology: All living twins, including unlike-sexed twins, will be typed with a broader spectrum of genetic markers to insure utmost accuracy in zygosity and to obtain a distribution of these markers in twins and their Study siblings for further studies. The twins and their Study siblings will be given an annual physical examination which will include physical and anthropometric measurements, and more extensive examinations with appropriate IQ and behavioral tests at ages 12 and 15 years. Investigations will include growth and development of genetically identical and environmentally related individuals; neurological evaluation, especially in regard to minimal abnormalities; differences in mental development; determination of the occurrence of a third type of twin; genetic factors in induction of puberty; and host resistance to disease.

Honors and Awards: None

Publications: None

Serial No. NDS (CF)-71 PR/EG 1910
1. Perinatal Research Branch
2. Section on Epidemiology and Genetics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Genetics of Obstetric Variables and the Role of Maternal Factors in the Determination of Intelligence and Neurological Performance

Previous Serial Number: None

Principal Investigators: Dr. A.F. Naylor, PRB, NINDS
Dr. N.C. Myrianthopoulos, PRB. NINDS

Other Investigators: Dr. D. Warburton, College of Physicians and Surgeons of Columbia University

Cooperating Units: Department of Human Genetics and Development, Columbia University

Man Years:

Total:	.50
Professional:	.50
Other:	.00

Project Description:

Objectives: To analyze the variation of obstetric and gynecological factors into heritable and non-heritable components.

Proposed course: The genetic linkage file necessary for full realization of the objectives, is near but short of completion. Specialized programs necessary for genetic analyses are being procured from the University of Hawaii but will need modification for use on NIH computers. An expanded version of the Variable Data File incorporating needed additional information has been created. This expanded file will be used immediately (1) to investigate biases in obstetric variation among women reporting relatives in the Study compared to those not reporting relatives (2) to investigate obstetric heritability at a "primary" level to identify conditions which tend to recur in repeat Study pregnancies.

Honors and Awards: None

Publications: None

Serial No. NDS (CF)-65 PR/P 1278
1. Perinatal Research Branch
2. Section on Pathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Biologic Pattern Data Processing

Previous Serial Number: Same

Principal Investigators: Lewis E. Lipkin, M. D.
Russell A. Kirsch

Other Investigators: Peter F. Lemkin
Philip G. Stein
Stanley E. Shackney, M. D.

Cooperating Units: Artificial Intelligence Group
National Bureau of Standards

Office of Associate Scientific Director
Clinical Trials
National Cancer Institute

PDP-10 Group
Division of Computer Research and Technology

Man Years

Total:	8.2
Professional:	5.0
Others:	3.2

Project Description:

This project has been extensively reviewed during the past calendar year. The first review in June 1970 was by the NCI directorate with uniformly favorable comment. The second more formal review in December 1970 by an external ad hoc committee, chaired by Dr. Kenneth Earle, Chief of Neuropathology of AFIP, concluded: "The committee was unanimous in its support of this important project..."

1. Substantive Projects: There have been three major biologic projects begun during the reporting period. These are totally dependent on both the concepts and facilities of the emerging image processing facility in the Section on Pathology.

a. The Quantitative Characterization of Chromatolysis: Serial histologic sections of hypoglossal neurons of rabbits rendered chromatolytic by nerve section one or two weeks prior to sacrifice by perfusion fixation and the corresponding contralateral controls are selected by the observer for automatic scanning. The tapes so generated are displayed on the PDP-10's 340 display, where under the biologist's visual control and by means of a Grafcon stylus and tablet cells and their components are segregated. The computer is providing data on each cell and its components that are measures of Nissl distance dispersion and/or loss, nuclear and nucleolar swelling, nuclear eccentricity, change in cell size, change in cell shape and changes in patterns of distributions of cytoplasmic RNA. At this writing, additional subprograms are being written to enable us to determine quantitative measures of satellitosis in terms of such parameters as the probability of encountering a glial cell as a function of distance from the cytoplasmic boundary of the cell body. Since the magnetic tapes of the scanned images are preserved, these results may be obtained without rescanning. Indeed, as additional algorithms are developed, they may be applied to exactly the same cell images without further microscopy.

b. Identification of Marrow and Peripheral Blood Cells in Radioautographs: This ability is one of the remaining requirements for specifying the automatic grain counting device requested by the National Cancer Institute. A library of scans is now under construction,so that following leads such as those provided by Mendelsohn and Prewitt, this major requirement for successful completion may be met. Of particular interest is the very great utility of image masks generable from multiple wavelength scans.

c. Concurrent DNA Content and Synthesis Rate Determinations in Embryonic Neural Tube and Transplanted Lymphomas: The image processing facility provides the unique ability to determine not only the rate of DNA synthesis in a cell population, but also to determine the quantity of DNA/cell in a Feulgen-stained autoradiograph. This ability will provide data which will provide information which hitherto has only been available from laboriously examined time-lapse studies and labeled mitosis counts. Material presently being examined are transplants of rodent leukemia. In process, however, are embryonic neural tissues with the expectation of obtaining more quantitative information concerning mantle-marginal relationships.

In addition to the foregoing specific projects, extensive experiments involving three-dimensional reconstructions, automatic morphologic analysis, etc. were performed. Some indications of their scope is given under "4. Computer Analysis and Image Processing Results."

2. Apparatus Design, Construction and Procurement: Significant design and construction attention has gone into improving the scanning microscope, while keeping it concurrently usable for productive scanning. Some of the modifications have been discovered to be necessary as a consequence of having used the system. For example, a new rigid table was constructed and procurement was initiated for anti-vibration mounts to cure a building vibration induced problem that was discovered during the course of scanning at high magnification. In preparing to use the scanner, photometric calibration of the photo-multiplier and associated circuitry is necessary. To facilitate this, new gain controls were constructed and low density filters were obtained which can be used to simulate the optical density of biological materials to be scanned, so as to facilitate calibration of the scanner over the full optical density range. A standard procedure was also adopted for recording on the magnetic tape along with an image that has been scanned, the corresponding values of the reference beam photomultiplier, so as to make completely available for subsequent analysis the information about incident illumination necessary to refine the calibration of the photometric part of the scanner. For point by point rather than raster scanning, the scan axis drivers and associated motor controls necessary for driving the scanner mirrors were constructed. Immediately after scanning it is often useful to have a crude visual presentation of the image as scanned. A beam storage tube display was obtained and connected so as to provide both intensity versus x and y type of display as well as the previous contouragram form of display to exhibit the complete scan output for a single scan.

During this period the LINC-8 and the PDP-10 computers were connected together in a flexible manner to enable the interchange not only of scan data but of programs in both directions from the LINC-8 and hence from the microscope to the PDP-10 and conversely. The RF08/RS08 disk system was connected to the LINC-8 and is currently in operation. An A.B. Dick Videojet Line Printer was connected to the LINC-8 and has made it possible to produce full resolution displays of scanned images through the use of about 16 gray levels of intensity encoded with suitable print characters.

A large disk pack drive installed on the PDP-10 has a five-million word capacity and is used exclusively by the image processing project.

A major effort has been going on in the design and construction of an optical bench to provide the capabilities of the present scanner with more flexibility for the addition of new scanner options. The basic optical bench mount which had previously been obtained had several pieces of equipment added to it. A vertical stage to take the place of the present optical microscope stage was constructed and installed, as was a mount for the use of the monochromator and a mount and changing system for mounting microscope objectives on the bench. Investigation was initiated for procurement of a new scanner to be used on the optical bench and a microscope tube length correction system was designed.

Special image measuring devices for use on the optical bench were obtained to be used in precise calibration of the optical system when it is in operation on the optical bench. The design of a system for distance measurement using interferometric techniques on the optical bench was begun.

3. Programs: Many programs have been written during this period for the LINC-8 and the PDP-10 which served to facilitate experiments using the scanning microscope and to remove the burden from the user of having to write detailed programs. Notable among such efforts is the construction of successive versions of the MOSS microscope operating supervising system which enables complex sequences of scanning and processing on the LINC-8 to be initiated by the user with a minimal amount of programming. Prior to obtaining special line printer equipment for producing images, a program was written to use the NBS Stromberg-Carlson Graphic Printer to make digitized printouts of scanned images. This served not only the purpose of visual representation of scans, but enabled us to gain assurance that the scan data was as intended when the microscope was set up.

During this period the PDP-10 has become the main production device for processing scanned data. Largely because of fine cooperation from personnel in DCRT, the PDP-10 has proved powerful and has generally been satisfactorily available. The total use of the PDP-10 has, at various times, consumed over 20 hours of CPU time per month for NBS users alone engaged in program writing and development as well as production computing. At peak usages, NINDS and NCI users on this project have exceeded this total. This total of 40-45 hours of CPU time/month can probably be taken as a reasonable projected upper bound for computer use during the next year.

During this period several specialized and some general purpose tools have been constructed. A version of the morphological analysis procedure for decomposing scanned images that had previously been converted from the Q-32 computer to the PDP-10 was modified to use the full resolution of the data obtained from the microscope scanner. Another somewhat more general version was constructed in the LISP language to provide the full generality over all possible scanned images of decomposition methods. Of the generalized tools, a systematic effort enabled the construction on the PDP-10 system of general purpose image processing facility more powerful than the corresponding one previously built on the Q-32 computer. This facility is written to operate in the LISP language and hence to provide the high level language processing capability of LISP with the corresponding efficiency of lower level code written either in assembly language or in FORTRAN through the use of a FORTRAN-LISP interface written by S. Bryan (DCRT). The list of major functions available in the LISP image processing facility is as follows:

a. A routine for reading whole or partial scans from magnetic tape into memory performing optical thresholding on the data. This routine also allows the reference photomultiplier data optionally to be read instead of the photometric information from the scan data.

b. A routine for variously thresholding image arrays to convert them to binary form.

c. Very high speed non-buffered input/output for swapping images from disk to core. Because a typical image involves 16,000 words of PDP-10 core storage, it is important to be able to swap such images into and out of memory at high speeds to avoid unconscionably large amounts of processing time.

d. A FORTRAN bit processing package to operate within the LISP language.

e. A high speed assembly language program for computing the derivative of a scanned image. The derivative data contains information about boundaries and gradients and hence is used in morphological analysis extensively.

f. An assembly language program to perform Boolean operations on binary arrays considered as masks.

g. A high speed assembly language program to extract contiguous regions (blobs) from binary arrays obtained from images.

h. A high speed assembly language program for counting the number of elements in a binary array.

i. A FORTRAN routine for circumscribing an extracted object in an image with a circumscribing rectangle.

j. A general purpose statistical routine for taking an image which has been previously thresholded into binary form, and then computing centers of gravity, mean densities, and various types of moment computations, as well as obtaining minimum and maximum values of intensity range for an image.

All the above programs are available at the LISP language level and hence can be called with considerable ease by higher-level programs without the need for the user to descend to lower level coding. The above general and special purpose programs operate in a unique environment involving both specialized equipment and users with particular kinds of processing problems. Despite the specialized nature of this environment, a systematic documentation effort is being made to document these programs so as to describe the operations and enable their duplication.

4. Computer Analysis and Image Processing Results: The large amount of PDP-10 computer time used during this period is mostly attributable to the various analyses that have been run on the computer using scan data from various biological sources. We mention below some of these results that have been obtained:

   a. Various fluctuations in scanner illumination and line voltages result in non-uniformities in an otherwise uniform section of a scan. To analyze the degree of non-uniformity, a statistical analysis was made on the values of the reference beam on the microscope scanner. It was found that this illumination is extremely stable, so much so that for a reasonable level of illumination, variations in illumination level were small compared to those attributable to quantization errors and other sources of scanning noise.

   b. In order to test the resolution of the scanner for scanning silver grains on blood cells, the Stromberg-Carlson 4020 Display was used to resynthesize several scanned images of labeled blood cells. In the resynthesized image, the silver grains appeared clearly resolved and hence the data necessary for location and recognition can be considered to be present in the output of the scanner.

   c. An analysis of a small region from one of these cells containing multiple silver grains was made using the morphological analysis algorithm. The result was a proper identification of the location of silver grains and an indication that if this procedure were used over a larger image, the silver grains would properly be identified.

   d. Several experiments were performed using optical serial section data. In the first, a specimen with small polystyrene spheres was sectioned optically and then the image was reconstructed in three dimensions within the computer. Since the spheres were only a few microns in diameter, the reconstructed image, rather than being that of a sphere, was the diffraction pattern that would be expected from such small, largely phase, objects. It was also possible, using the three-dimensional reconstruction inside the computer, to rotate the image through 90 degrees and hence produce the effects of having sectioned perpendicular to the plane of the optical slide. This technique can be directly extended to other natural objects and in particular can be extended to larger objects than these test specimens.

   e. A set of serial sections of two adjacent nerve cells was made which exhibited the appropriate low depth-of-field for the optics that were used and enabled in a resynthesized set of images the easy visual identification of the vertical sectioning process.

f. An extensive analysis was made of several optical sections of monkey vagal nuclei neurons. These analyses consisted of a complete morphological decomposition using the morphological analysis algorithm along with the determination of optical density statistics for the decomposed objects. Using these statistics then, the original objects were resynthesized and the computer's reconstruction was visually compared with the original scan data. The strong correspondence between the original and the resynthesized images suggested that the morphological analysis decomposition procedure preserves the morphological structure of images for purposes of recognition of cells and their components.

g. A program was written by Bryan (DCRT) to enable manual specification of the decomposition procedure analogously to the automatic method used in the morphological analyzer. This program is currently being worked on to make its operation more precise and more convenient. Both versions are being employed particularly in the quantitative analysis of chromatolysis.

Honors and Awards: None

Publications:

Lipkin, L.E.: Resolution, scale change and information distortion. In Lipkin, B.S., and Rosenfeld, A. (Eds.): Picture Processing and Psychopictorics. New York, N.Y., Academic Press, 1970, pp. 203-215.

Lemkin, P.F.: A patch to focal-w to use the LINC-8 display. DECUS Program Library. FOCAL8-58: 1-3, 1969.

Lemkin, P.F.: OCTMON: Octal monitor for the PDP-8. DECUS Program Library. 8-298: 1-7, 1969.

Stein, P.G.: Image-analyzing microscopes. Anal. Chem. 42: 103A-105A, 1970.

Shapiro, H., Bryan, S., Lipkin, L.E., Stein, P.G., and Lemkin, P.F.: Computer aided microspectrophotometry of biological specimens. This paper has been accepted for publication in Exp. Cell Res.

Kirsch, R.A.: Computer determination of the constituent structure of biological images. This paper has been accepted for publication in Comput. Biomed. Res.

Serial No. NDS (CF)-68 PR/P 1650
1. Perinatal Research Branch
2. Section on Pathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Twin Placentation in Relation to Zygosity

Previous Serial Number: Same

Principal Investigators: Toshio Fujikura, M. D.
Luz A. Froehlich, M. D.

Other Investigators: Ntinos Myrianthopoulos, M. D.

Cooperating Units: All Collaborating Institutions

Man Years

Total:	0.0
Professional:	0.0
Others:	0.0

Project Description:

Twins totalling 569 pairs were studied in relation to type of twin placentation. Separate diamniotic-dichorionic placentas were rarely associated with monozygosity. This and the fact that monochorionic placentas are all monozygotic make examination of twin placentas extremely useful in forecasting zygosity. Relatively heavy infants as well as twins with large intrapair birth-weight differences were common in the separate diamniotic-dichorionic group, suggesting independent intrauterine growth of co-twins. The converse was true in twins with fused diamniotic-dichorionic placentas, who also had the lowest death rates. Perinatal death rate was the same in whites (14.7%) and Negroes (14.3%), highest in male-male twin pairs, but lowest in male-female pairs in Negroes and female-female pairs in whites. Compared to singleton deaths, the frequency of congenital malformations was not higher in twin deaths, but the types of malformations found in monochorionic deaths were often multiple and lethal. This study has been completed.

Honors and Awards: None

Publications: Fujikura, T., and Froehlich, L.A.: Twin placentation and zygosity. Obstet. Gynec. 37: 34-43, Jan. 1971.

Serial No. NDS (CF)-69 PR/P 1763
1. Perinatal Research Branch
2. Section on Pathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Kidney Malformations in Fetuses of A x C Line 9935 Rats

Previous Serial Number: Same

Principal Investigators: Toshio Fujikura, M. D.

Other Investigators: None

Cooperating Units: None

Man Years

Total:	0.9
Professional:	0.5
Others:	0.4

Project Description:

Fifty A x C 9935 strain inbred rat litters were examined near term; 88/315 fetuses (27.9%) showed renal malformations (renal agenesis and hydronephrosis). Unilateral renal agenesis occurred more often on the right side (11.5%) than the left (3.6%), especially in males. Renal agenesis was always associated with absence or hypoplasia of a uterine horn or vas deferens and epididymis on the corresponding side. However, the ovaries or testes were present and intact. Hydronephrosis was seen more often on the left side (11.4%) than the right (3.5%). There was no significant sex difference in the frequency of renal agenesis and hydronephrosis. In hydronephrosis the ureteropelvic junction was patent, and hydroureter always accompanied moderate hydronephrosis. Atresia near the vesicoureteral junction was considered as a cause of hydronephrosis. There was a close morphogenetic relation between renal agenesis and hydronephrosis. This study has been completed.

Honors and Awards: None

Publications: Fujikura, T.: Kidney malformations in fetuses of A x C line 9935 rats. Teratology 3: 245-249, Aug. 1970.

Serial No. NDS (CF)-69 PR/P 1764
1. Perinatal Research Branch
2. Section on Pathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Placental Study of Abortion Material
(Obtained by an induced abortion)

Previous Serial Number: Same

Principal Investigators: Toshio Fujikura, M. D.

Other Investigators: Hideo Nishimura, M. D.
Kenichiro Ezaki, M. D.

Cooperating Units: Department of Anatomy
Kyoto University
Kyoto, Japan

Man Years

Total:	0.1
Professional:	0.1
Others:	0.0

Project Description:

The placental materials of 114 induced abortions (normal group) and 87 spontaneous abortions (abnormal group) were histologically compared. Degenerative hydropic and necrotic villi were commonly found in the chorion laeve of the normal group even in early gestation, but not in the chorion frondosum. The sampling site in the chorionic sac was important for histological diagnosis. The mean number of chorionic villi in the abnormal group was not different from that of the normal group within each gestational interval. This indicates that abnormal villous growth may not be the primary factor responsible for spontaneous abortion. Up to 14 weeks gestation, active syncytial proliferation was present but no substantial increase of chorionic villi was found. Beyond this inactive stage the villous number increased rapidly and conversely syncytial sprouts decreased in numbers. The mechanism of syncytial proliferation was discussed in relation to other prenatal conditions. Study completed.

Honors and Awards: None

Publications: Fujikura, T., Nishimura, H., Ezaki, K.: Placental study of abortion material (obtained by an induced abortion). Amer. J. Obstet. Gynec., in press

Serial No. NDS (CF)-69 PR/P 1765
1. Perinatal Research Branch
2. Section on Pathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: The Interrelationship Between Selected Congenital Malformations and Major Pathologic Findings

Previous Serial Number: Same

Principal Investigators: Luz A. Froehlich, M. D.
Toshio Fujikura, M. D.

Other Investigators: None

Cooperating Units: All Collaborating Institutions

Man Years

Total:	0.05
Professional:	0.05
Others:	0.00

Project Description:

The incidences of certain variables in the baby, mother and placenta of core deaths were compared with deaths having selected congenital malformations. Velamentous and marginal cords were more common in single umbilical artery deaths compared to core deaths. There was surprisingly little association with diabetes, except among the cases with agenesis of the kidney. Meconium staining was nearly twice as high in multiple heart malformations compared to controls. Hydramnios was common in association with anencephaly, and to a lesser extent with spina bifida and hypoplasia of lungs. The incidence of toxemia was more than twice as high in anencephaly, spina bifida, and agenesis of the kidney compare to controls. In addition, retroplacental hemorrhage was twice as high in anencephaly. Erythroblastosis was twice as high in accessory spleen but was not found in any of the other malformed cases. These correlations will be tested for possible significance. The study is being readied for publication.

Honors and Awards: None

Publications: None

Serial No. NDS (CF)-69 PR/P 1766
1. Perinatal Research Branch
2. Section on Pathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Reproductive Ability of the American Negro with Sickling and its Public Health Implications

Previous Serial Number: Same

Principal Investigators: Luz A. Froehlich, M. D.
Toshio Fujikura, M. D.

Other Investigators: None

Cooperating Units: All Collaborating Institutions

Man Years

Total:	0.4
Professional:	0.4
Others:	0.0

Project Description:

The reproductive performance of 654 sicklers and 1,890 non-sicklers in the Collaborative Study was compared. The cumulative fertility rate of mothers with sicklemia was the same as that of non-sickling mothers. There was no substantial difference in perinatal death rates, birthweight and gestation age between the sickling and non-sickling group. Only the infant and child death rate was higher in the sickling group. Because of the normal reproductive abilities of the sickler, the sickle cell gene may continue to propagate in the U.S. Negroes for generations to come.

The paper has been approved by the Publications Review Board and the NIH Review Board. Certain modifications and refinements are felt necessary prior to submission for publication. These modifications are strongly dependent on the performing of hemoglobin electrophoretic studies on the sicklers, to distinguish the homozygotes from the heterozygotes. Such a plan to allow uniform testing of all Negroes for sickling and to follow this through with hemoglobin electrophoresis on the proven sicklers is being earnestly pursued.

Honors and Awards: None

Publications: None

Serial No. NDS (CF)-69 PR/P 1769
1. Perinatal Research Branch
2. Section on Pathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: The Significance of Chorioangiomas

Previous Serial Number: Same

Principal Investigators: Luz A. Froehlich, M. D.
Toshio Fujikura, M. D.

Other Investigators: Pearl Fisher, Ph.D., OB, NINDS

Cooperating Units: All Collaborating Institutions

Man Years

Total:	0.1
Professional:	0.1
Others:	0.0

Project Description:

Eighty-one cases of chorioangioma in the Collaborative Study were analyzed, of which five were in twin pregnancies. Chorioangiomas were more frequent in whites (0.33%) than in Negroes (0.20%) and in females (49 cases) more than in males (37 cases). Two infants had neonatal thrombocytopenic purpura unassociated with skin hemangioma. Congenital anomalies were high in twins; in single births, the incidences of several malformations were significantly higher than in the general Collaborative Study population. An interesting correlation was noted among chorioangioma, skin hemangioma and single umbilical artery. In Negroes, in particular, twin rates in current and prior pregnancies were high as was the fact that the gravida herself was a twin. Acute toxemia was a significant complication in both whites and Negroes. This study has been completed.

The paper was presented at the Teratology Society Meeting in Annapolis, Maryland on May 20-22, 1970.

Honors and Awards: None

Publications: Froehlich, L.A., Fujikura, T., and Fisher, P.: Chorioangiomas and their clinical implications. Obstet. Gynec. 37: 51-59, Jan. 1971.

Serial No. NDS (CF)-69 PR/P 1772
1. Perinatal Research Branch
2. Section on Pathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Pathologic Effects of Ligation of the Anterior Spinal Artery and/or the Great Radicular Artery in Monkeys

Previous Serial Number: Same

Principal Investigators: Oscar Aparicio, M. D.

Other Investigators: Larry C. Fried, M. D.

Cooperating Units: Surgical Neurology Branch
Intramural Research
National Institute of Neurological Diseases and Stroke

Section on Neuroradiology
Medical Neurology Branch
Intramural Research
National Institute of Neurological Diseases and Stroke

Man Years

Total:	.05
Professional:	.05
Others:	.00

Project Description:

Objectives: The neuropathological evaluation of spinal cords of monkeys subjected to vascular ligations, in order to determine the practical feasibility of sacrificing any of these vessels if necessary during the course of a surgical procedure.

Methods Employed: The neuropathologic evaluation of the spinal cords by means of multiple staining methods, including H&E, Luxol Blue with Cresyl Violet, and others, in order to determine the presence, extent and location of any lesions due to ischemia.

Proposed Course: The histopathological evaluation has been completed and a publication is being prepared.

Honors and Awards: None

Publications: None

Serial No. NDS (CF)-70 PR/P 1858
1. Perinatal Research Branch
2. Section on Pathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Thrombocytopenic Purpura and Placental Hemangioma

Previous Serial Number: Same

Principal Investigators: Luz A. Froehlich, M. D.

Other Investigators: Mary Housler, R. N.
University of Buffalo

Cooperating Units: None

Man Years

Total:	0.1
Professional:	0.1
Others:	0.0

Project Description:

A case is presented in which neonatal thrombocytopenic purpura (TP) was associated with hemangioma of the placenta. This combination has not been reported in the literature, although the combination of TP and skin hemangioma is well known. The baby had no skin hemangioma. Other known causes of TP in the newborn such as maternal TP and sepsis were not present. The mother took a thiazide during the last eight weeks of pregnancy. However, the incidence of TP among those whose mothers took thiazide (0.01%) was not higher than among those whose mothers did not take thiazide (0.01%), indicating that thiazides and TP are not related. Study completed.

Honors and Awards: None

Publications: Froehlich, L.A. and Housler, M.: Neonatal thrombocytopenia and chorangioma. J. Pediat. 78: 516-519, 1971.

Serial No. NDS (CF)-70 PR/P 1859
1. Perinatal Research Branch
2. Section on Pathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Mental and Motor Development in Monozygotic Co-Twins with Dissimilar Birthweights

Previous Serial Number: Same

Principal Investigators: Toshio Fujikura, M. D.
Luz A. Froehlich, M. D.

Other Investigators: None

Cooperating Units: All Collaborating Institutions

Man Years

Total:	0.2
Professional:	0.2
Others:	0.0

Project Description:

Developmental measures in 125 monozygotic twin sets with unequal birthweights between co-twins were studied. There were no significant differences between co-twins in the Bayley mental and motor scores at eight months nor the Stanford-Binet I.Q. at four years. A reportedly higher I.Q. for the heavier monozygotic twins was not confirmed in this study, even among pairs with large birthweight differences (mean differences 26-28%). Although the effects of nutrition on the mental development of the fetus are currently of great concern, these data suggest that the developing human brain seems to have a strong resistance to intrauterine deprivation.

The future of this manuscript is uncertain. Because interpretation of the data is controversial the paper is apparently being shelved until data on the seven year I.Q. have been similarly analyzed.

Honors and Awards: None

Publications: None

Serial No. NDS (CF)-71 PR/P 1911
1. Perinatal Research Branch
2. Section on Pathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: A Follow-up of Children with Single Umbilical Artery

Previous Serial Number: None

Principal Investigators: Luz A. Froehlich, M. D.

Other Investigators: Toshio Fujikura, M. D.
John Churchill, M. D.
Pearl Fisher, Ph.D.

Cooperating Units: All Collaborating Institutions

Man Years

Total:	0.3
Professional:	0.2
Others:	0.1

Project Description:

There are 306 presently living core Study children who were born with single umbilical artery (SUA). These are from a total of 355 core SUA cases (identified by microscopic examination) of whom 49 (13.8%) have died, mostly in utero or during the first week of life. Associated congenital malformations were a significant cause of death. The surviving cases were compared with surviving controls matched for race, sex, institutions, birthweight, gestational age and S-E index. Velamentous and marginal insertions of cord were about six times as frequent in SUA. There were also slightly more neurologically abnormal cases (7.0%) than controls (4.9%). On the whole, however, mean mental and motor and four year I.Q. scores, and parameters of physical growth did not distinguish between cases and controls. Statistical analysis has been temporarily halted because of Dr. Fisher's departure, but all attempts will be made to see the paper to completion despite this inconvenience.

Honors and Awards: None

Publications: None

Serial No. NDS (CF)-71 PR/P 1912
1. Perinatal Research Branch
2. Section on Pathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Birthweight in Relation to Renal Glomerular Development and Gestational Age in Whites and Negroes

Previous Serial Number: None

Principal Investigators: Toshio Fujikura, M. D.
Luz A. Froehlich, M. D.

Other Investigators: None

Cooperating Units: All Collaborating Institutions

Man Years

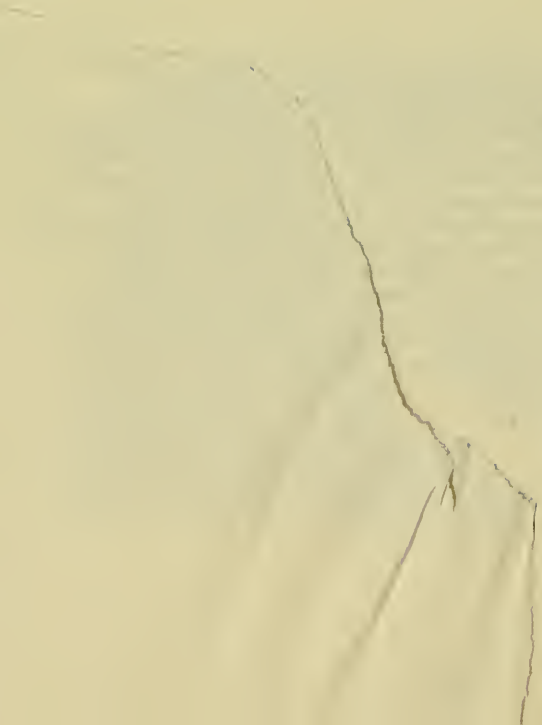
Total:	0.25
Professional:	0.20
Others:	0.05

Project Description:

Birthweight, body length and X-ray evaluation of bone development continue to be widely used as measures of fetal maturity. However, these are factors of somatic growth and are not necessarily directly related to growth and maturity of internal viscera. On the conviction that histologic examination of an organ would give a more accurate picture of the maturation of internal viscera, a histologic evaluation of the kidneys of 514 neonatal deaths and fresh stillbirths was undertaken. In the early phases of pregnancy, somatic growth appeared to proceed at a significantly faster pace in the Negro, as evidenced by higher birthweights in this race compared to whites. However, the developmental rate of viscera such as the kidney seemed to proceed at similar rates in the two races. Racial differences were observed in the percent of the presence of nephrogenesis (PPN) in the "small for dates" as well as "large for dates" infants. In both races about half of the "small for dates" infants still exhibited a nephrogenic zone compared to more than 95% of the truly prematures. At the same time two to four times as many "large for dates" infants showed nephrogenesis as those which were mature both for birthweight and gestational age. In maternal diabetes more than 80% of infants between 35-37 weeks gestation showed nephrogenesis where only 66.6% were expected. The paper is being readied for publication.

Honors and Awards: None

Publications: None



Serial No. NDS (CF)-71 PR/P 1913
1. Perinatal Research Branch
2. Section on Pathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: The Clinical Significance of Generalized Petechiae at Birth

Previous Serial Number: None

Principal Investigators: Luz A. Froehlich, M. D.
Jean G. Oliver, M.A.

Other Investigators: Toshio Fujikura, M. D.
Jean S. Nemore, R.N., B.A.

Cooperating Units: Section on Infectious Diseases, PRB, NINDS
All Collaborating Institutions

Man Years

Total:	0.2
Professional:	0.2
Others:	0

Project Description:

In a hand review of charts of some 30 of about 120 living infants diagnosed as having had significant skin petechiae at birth, six were found to have significant hearing loss, an incidence far greater than expected. Speech and behavior problems were also common. Mental retardation was found in a mature male along with marked visual impairment.

On the hypothesis that significant skin petechiae could be accompanied by hemorrhages in vital structures such as the brain, inner ear, retina, etc., the review of charts of all 120 children will be continued and findings compared with those of controls matched for institution, race, sex, birthweight and socio-economic index.

Honors and Awards: None

Publications: None

Serial No. NDS (CF)-70 PR/SLH 1520
1. Perinatal Research Branch
2. Section on Speech, Language and Hearing
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Explorative Study for the Use of a Speech and Language Screening Examination for 3-Year Old Children in the Home Situation

Previous Serial Number: NDS-(CF)-63 PR/BS 1167

Principal Investigator: Dr. Miriam F. Fiedler

Other Investigator: Dr. Eric H. Lenneberg, Cornell University

Cooperating Units: Children's Medical Center, Boston, Massachusetts
Section on Behavioral Sciences, PRB, NINDS
Section on Pediatric-Neurology, PRB, NINDS

Man Years:
Total: .5
Professional: .2
Other: .3

Project Description:

The study concerns children with non-normal speech identified at age three years by means of interviews with the mothers of Perinatal Project children in Boston. Findings were confirmed by subsequent speech, language and hearing examinations. The speech data were considered in relation to perinatal findings as an initial study, and, in a second study, with regard to outcome at age seven years in the light of psychological and neurological examinations. Most of the children identified as abnormal or suspect at age three years were found to be similarly regarded by other measures at age seven years.

Part of the study was presented by Dr. Fiedler as a paper delivered at the Tri-City Meeting of the Obstetrical Society in Boston on May 16, 1967, under the title "Delayed Speech Development in Children at 3 Years of Age Related to Peri and Postnatal Findings". The study has been completed.

Honors and Awards: None

Publications:

Fiedler, M. F., and Lenneberg, E. H.: Explorative Study for the Use of a Speech and Language Screening Examination for 3-Year Old Children in the Home Situation. Pediatrics. In press.

Annual Report
Associate Director's Report
July 1, 1970 through June 30, 1971
Extramural Programs
National Institute of Neurological Diseases and Stroke

A detailed summary of the NINDS extramural research grant effort and training accomplishments in Fiscal Year 1971 are provided in the reports of the Research Grant and Training Branches; organizational progress including program reporting activities are included in the report of the Administrative Officer. In broad terms, the highlights of NINDS Extramural Programs for Fiscal Year 1971 have been characterized by:

A. A CONTINUED REDUCTION IN TRAINING ACTIVITIES: With a "hold the line" philosophy in the training activities of the Institute, 10 additional grant supported training programs have had to be discontinued. This was necessitated by the continuing increase in funding requirements of active programs despite an across-the-board "negotiated cut" for the third successive year, this year at the 15% level. The plan to stabilize the number of new RCDA awards each year at 20 is reaching fruition; if there are no further reductions in this program area in the future, the fiscal requirements for this minimal level of activity will be reached in Fiscal Year 1975. Fiscal Year 1971 will end with $8.5 million approved training grant and traineeship applications unfunded and $1.5 million approved fellowship applications unfunded. The President's Budget for Fiscal Year 1972 will require further reductions in NINDS training activities. This will have particularly unfavorable effects on the recruitment and training of personnel for careers in teaching and research in neurology, child neurology, neurological surgery and otolaryngology. In addition, desperately needed new training programs in the basic science areas of neurochemistry, neurophysiology and neurobiology will not be activated.

B. A TEMPORARY STABILIZATION OF THE LEVEL OF RESEARCH GRANT ACTIVITY: An increase of $5 million in research grant funds in Fiscal Year 1971 permitted the Institute to maintain its research grant activities at approximately the same level as in Fiscal Year 1970. The additional funds were utilized to help offset a 7% increase in cost-of-living; to provide for a reduction to 10% of the previous "across-the-board" budget negotiation level of 15%; to meet a peak in the triannual level of previously committed applications; and to initiate feasibility studies for the establishment of acute spinal cord injury research centers. The President's Budget for Fiscal Year 1972 reduces the research grant activity by over a half-million dollars. This reduction will interfere seriously with the Institute's plan to further reduce across-the-board negotiations to the 5% level, to meet the continuing increase in research costs, and to provide additional emphasis on selected high priority research programs.

C. RESEARCH AREAS OF SPECIAL EMPHASIS:

(1) Stroke: The Institute's principal instrument for research grant support of stroke research is its program of stroke clinical research centers, eighteen of which are now active. These are centers of coordinated clinical and basic research focused on problems such as local and regional cerebral blood flow, cerebral hypoxia, cerebral edema, cerebral arterial thrombosis,

hemorrhage and spasm, stroke epidemiology, the pathophysiology of the transient ischemic episode, etc. In the area of cooperative research: The cerebral aneurysm study has been completed with significant results; the hypertension-stroke study is in its final phases; plans have been completed for the initiation of a study to evaluate the efficacy of amino-caporic acid in the treatment of the acute stages of intracerebral hemorrhage. As a result of the activity of the Joint Council Subcommittee on Cerebrovascular Disease (NINDS and NHLI), plans have been developed for a program of coordinated research on the problem of acute stroke care (nervous system monitoring and acute medical-surgical intervention) through the establishment of acute stroke research units; this activity can be initiated if the additional funds required are made available for stroke research.

(2) Trauma To The Central Nervous System: In 1968, the NINDS initiated its program of Head Injury Clinical Research Centers by means of specialized research center awards to plan and develop these activities. In Fiscal Years 1971 and 1972, the pilot centers will compete for future support in the categorical clinical center program. In order to provide for both a progress report on research accomplishments to date in this area and to establish specific scientific merit criteria for review of applications, the Institute has organized a Head Injury Research Workshop. This Workshop will be held in February 1972 and will include scientists from each of the 6 active head injury research centers and selected scientists from other laboratories.

In the area of acute spinal cord trauma, fifteen applications for feasibility studies have been received. These applications to develop the scientific, medical, community and organizational feasibility of establishing up to 4 acute spinal cord injury clinical research centers in the U.S. will undergo review during the summer of 1971 and receive final Council recommendation in September 1971. Funds for the feasibility studies have been set aside. A minimum of $4 million will be required to launch the 4 center activity in late Fiscal Year 1972 or early Fiscal Year 1973.

(3) Communicative Disorders (Hearing; Speech; Language): A Task Force of the NINDS Communicative Disorders Program Project Review Committee has completed its report on opportunities for research in these areas. The report documents the several research problems of high priority and opportunity which justify increased Institute effort; included are the biological and medical effects of noise on man; chronic and episodic vertigo; genetics and deafness; pre-school deafness; hearing loss and chronic secretory otitis media; and traumatic neural impairment and language. The documents supporting these specific program area needs will serve as a basis for program planning and development in FY 1972.

(4) Parkinsonism; L-DOPA; Neurotransmitters: The availability of L-DOPA and its acceptance as the therapy of choice for moderate to severe Parkinsonism has redirected and accelerated the entire area of research on movement disorders, neurotransmitters and the neural and neuromuscular synapse. L-DOPA potentiators are now in phase 2 and phase 3 study; L-DOPA analogues, dopamine-o-mimetic drugs and antichoreic agents are now potentially available; the delicate balance of protagonists and antagonists of synaptic transmission is being described; and the metabolic pathways of neurotransmitter saturation therapy are being unraveled. The four research centers in this area supported by the Institute

are the leaders in these developments; the additional $3 million in research projects provides for an independent but complementary research effort.

D. PROGRAM REPORTING: With the completion of the conversion of our routine data storage and retrieval system to tape and the development of programmed search procedures, for the first time a comprehensive search and reporting system is now in effect. Staff scientist administrators responsible for program area activities now have regular reports available to them in both the research grant and training grant programs; similar reporting will be available in the fellowship programs in Fiscal Year 1972. Computer data also are being used regularly for fiscal monitoring and reporting. In Fiscal Year 1972, pilot programs will be developed and tested for the reporting of scientific status and accomplishments.

Annual Report
Administrative Report
July 1, 1970 through June 30, 1971
Extramural Programs
National Institute of Neurological Diseases and Stroke

Extramural Programs found the problem of diminishing staff particularly difficult during Fiscal Year 1971. During a year when position strength was more than 15% below normal operating levels and the program data maintenance and analysis organization was being expanded to meet increasing demands against it, the normal ability to reassign support personnel to cover absences or heavy load demands was most difficult. The sacrifice of certain central service positions has caused a further drain on the time and effort of existing clerical staff. Mail and messenger service and local duplication have all become do-it-yourself tasks. The sum of this was that certain minimum compromises with total efficiency had to be made.

The Extramural Programs participated with other Institute program areas in two Equal Employment Opportunity conferences during FY 1971. These conferences were held to identify specific problems and attitudes that foster inequitable job situations and to develop an Institute commitment to improve equal opportunity. An affirmative action plan has been developed and is being implemented at this time.

The Data Analysis and Reports Unit has completed its conversion from manual records to computer files. This new data file has begun to provide Extramural Program's management with an information capability that enhances management planning, analysis, and operations and improves projections of future needs.

The full impact of the concurrent project period ruling was felt in grants management during FY 1971. A significant amount of additional grant support was permitted by this method of allowing the use of unexpended grant funds during an extension of the project period.

The administration of the NINDS Extramural Programs budget was made difficult due to the late release of funds with the appropriation being signed on January 11 and the apportionment approved by the Office of Management and Budget on March 1, 1971. This late release of funds resulted in the delay of the award of many new as well as competing renewal Research Grants until the fourth quarter of the fiscal year. As in the past, reserves were withheld from the appropriation in the amount of $2,957,000 for Research Grants and $672,000 for Graduate Training Grants, a total of $3,629,000. Of this amount withheld from grants, $1,307,000 was allocated to NINDS direct operations for pay raises, and the balance of $2,322,000 to other Institutes of N.I.H. and the DHEW for pay raises. However the amounts released this year were increased over the President's Budget by $4,211,000 for Research Grants and $187,000 for Fellowships. There was no change in Training Grants. Of the increase for Research Grants, $3,522,000 was for research projects and centers and $689,000 for General Research Support Grants. Even with these increases, the level of unfunded approvals continued to climb upward as a result of an in-

creased number of approvals, the average cost of grants increasing coupled with a lesser amount of negotiated reduction. The amounts of unfunded approvals were $15,300,000 for Research Grants, $8,500,000 for Training Grants, and $1,450,000 for Fellowships. To assure the most efficient use of available funds, the following steps were taken.

1. Research Grants were negotiated downward in an amount averaging 10%, resulting in savings of approximately $4.0 million which resulted in the award of 31% more new and competing renewal grants than would have been possible without negotiations.

2. Graduate Training Grants were negotiated downward an average of 15% which made possible the award of twice as many competing grants as would have been awarded without negotiations.

3. Additional savings were realized for Research Grants and Graduate Training Grants by utilizing the concurrent project concept. This means that competing renewal grants were reduced by the amount of funds remaining in the previous project period and extended up to a year so that the grantee could use these unexpended funds during the new project period.

Annual Report
July 1, 1970, through June 30, 1971
Research Grants Branch
National Institute of Neurological Diseases and Stroke

Introduction

The brain is by far the most intricate, sensitive and versatile organ in the body. As a result, it has been the subject of extensive study and research for centuries. However, it has yielded only slowly to scientific exploration because of its complexity and because of its relative inaccessibility due to being enclosed in the skull and due to the blood-brain barrier which separates the brain metabolically in many respects from the rest of the body. Nevertheless, research is gradually bringing a greater understanding of how the 10 to 13 billion individual nerve cells in the brain, together with the additional billions comprising the nervous system, work together to make the human body an effective and coordinated living organism.

The economic burden of the neurological and sensory diseases amounts to billions of dollars each year, with immeasurable human suffering. Although the human spirit can often adjust to the effects of physical disability, even the greatest courage may be broken by the devastating consequences of brain injury or disease which may continue or exacerbate during the remainder of a person's life.

More than 200 disorders are known to afflict the brain, sense organs, nervous system and neuromuscular apparatus, the most familiar of which are stroke, head and spinal cord injury, epilepsy, cerebral palsy, aphasia, multiple sclerosis, muscular dystrophy, parkinsonism, brain tumors, and otosclerosis. These diseases lead to paralysis, loss of speech, paraplegia and deafness, and are among the major causes of death and permanent disability in the United States.

The research grant programs of the National Institute of Neurological Diseases and Stroke include research projects, research program projects, cerebrovascular and communicative disorder outpatient clinical research projects, clinical research centers, and grants to establish cerebrovascular and head injury clinical research centers. The objectives of these programs are the identification, stimulation, and support of important research problems related to the diagnosis, treatment, and prevention of disorders such as those mentioned above.

For many years the question of whether hypertension played a major role in the development of stroke has been controversial. About one year ago the Veterans Administration reported a study indicating that antihypertensive therapy had a marked protective affect on stroke morbidity and mortality. This whole question was then reviewed by the Joint Council Subcommittee on Cerebrovascular Disease of the NHLI and NINDS and by the NANDS Council. Both groups recommended that further

steps be taken as expeditiously and aggressively as possible to implement the application of antihypertensive therapy on community bases. Since this problem is now beyond the clinical research stage and is in the area of community application, this recommendation was referred to the Regional Medical Programs Service which was urged to give it a high priority.

Injuries to the spinal cord are occurring with increasing frequency. Once the spinal cord degenerates in the area of injury, paralysis always develops. In March 1971, the NANDS Council recommended approval of plans to support a few Acute Spinal Cord Injury Centers. At the first stage of development, funds would be provided only to plan the requirements and test the feasibility of a few centers for acute spinal cord injury. Each Center's plan would include investigation, development and evaluation of improved methods of emergency treatment, rapid transportation, diagnostic techniques, medical-surgical repair, and the training of required professional, scientific and technical personnel. Ultimately, it is expected that a fully developed Center would contribute important information on the prevention of degeneration of the spinal cord and on the restoration of spinal cord function. Close liaison with other government and non-government agencies with responsibilities in this area is being maintained.

The importance of improved stroke acute care is obvious since 70 to 80 percent of the mortality occurs in the first ten days. Also, the consequences of not recognizing a progression or extension of the infarct may be catastrophic. After extended review of this problem, the Joint Council Subcommittee on Cerebrovascular Disease, NHLI-NINDS, agreed that the present information about stroke acute care is extremely limited and that the individual efforts on the part of investigators, singly and in teams, to push forward with the problem have met with only limited success to date. In view of these considerations, the NANDS Council in March 1971 approved the organization of a Problem Commission on Stroke Acute Care Research. The Commission will consist of 10-12 experts in this and closely related areas and will be closely linked to the present Stroke Clinical Research Centers. It will be the responsibility of the Commission to (1) develop the strategy required to explore the problem (e.g., identify the neurological variables requiring attention and possible human and mechanical monitoring as a basis for intervention); (2) describe the specifications of the required organization, software and hardware; and (3) assume the responsibility for testing and evaluating these specifications in their own Stroke Clinical Research Units.

This was the third year in which every research grant, competing and committed, was subject to a negotiated reduction. All grants were negotiated downward by an amount which would hopefully still allow the work to proceed, although productivity doubtless was reduced even more than in the previous two years because costs have increased. As a result of this arrangement, however, over 50% more funds were available for competing applications. Even so, approximately half of the approved projects could not be supported. The Institute has been advised that grants may be reduced by only a minimal amount (5%) next year. In effect

this will amount to a reduction of about $2.5 million or 25 percent in the funds available for competing applications. Two years ago, the National Eye Institute was organized and all of the activities related to the visual system and disorders of vision were transferred out of NINDS. This took more than 20 percent of the grants, applications and funds. Although there has been little increase in the funds available, it has been possible to maintain about the same number of projects by reducing each grant as described above. That is, last year 1,267 research grants were supported at a total cost of $48.8 million. This year 1,256 grants were supported at a cost of $53.6 million.

Within one year after the formation of the National Eye Institute, the number of applications to NINDS was just as large as it was before the operation of the two. For example, in March 1970, the NANDS Council reviewed about 375 applications. In June 1971 the Council reviewed about 450 applications, a 20 percent increase over the average for the previous year. This continued and dramatic increase in the number of requests is due primarily to the effectiveness of the research training programs of this Institute in which the output of fully trained investigators has only in recent years reached its full potential.

There were two replacements in the professional staff of the Research Grants Branch since last year. One was due to a retirement and the other was due to a resignation. One of the new staff members is Executive Secretary of Program Project Committee A and the other is responsible for research grants in the areas of pharmacology, medicinal chemistry, and toxicology. Both have proved to be mature and competent scientists and are becoming expert administrators. Therefore, the Branch continued to enjoy the services of an active and experienced staff.

The numbers of grants and amounts of funds in the various disorder categories are shown in Appendix A.

Cerebrovascular Disorders

In FY 1971 support for research in cerebrovascular disease was at the level of $5.6 million, representing 63 research grants and 19 clinical research centers.

Studies directed toward cerebral blood flow continue to be of prime importance in research on cerebrovascular disorders. Some investigators direct their efforts to such facets as development and refinement of methodology and techniques for measurement of cerebral blood flow at a regional and focal level, others to problems of regulation, others to correlative studies of cerebral blood flow with other parameters which might be used as predictive studies in the course of a stroke.

In one clinical research center, the relationship of cerebral blood flow (CBF) to pH in cerebral venous blood and in cerebral cortex is being explored. Dogs were lightly anesthetized, paralyzed and ventilated with oxygen. CBF was measured by an electromagnetic flowmeter in a shunt between the torcular and the superior vena cava. Cerebral venous blood pH, pO_2, and pCO_2 were measured continuously in a flow-through within the shunt. The same parameters were measured on the surface of the cerebral cortex by the application of flat surface counterbalanced electrodes on the exposed brain through a parietal craniotomy. The brain surface was protected by a shallow pool of warmed artificial cerebrospinal fluid. Simple seizures were produced by electroconvulsive stimuli or by pentylenetetrazol I.V. and recorded by electroencephalographic monitor. In eight dogs so studied, CBF increased immediately with the onset of each seizure. Both brain and cerebral venous pO_2 increased consistently with seizures in well ventilated animals, but fell when seizures were accompanied by apnea. Brain pH showed little change initially during the acute phase of cerebral hyperemia. Thereafter brain pH showed variable changes. It decreased by 0.10-0.15 units in most observations, but in several experiments a primary increase in pH was found. In the post-ictal phase brain pH was consistently increased above baseline values at a time when CBF was still elevated. In the post-ictal phase of impaired brains, autoregulation increases, induced by hypertension if CBF was accompanied by increases in brain pO_2 and pH, and decreases in pCO_2.

The lack of consistent correlation between CBF and brain pH during and after seizures suggests that factors other than hydrogen ion activity play a role in regulation of cerebral vascular resistance. Such factors, whether neurogenic or metabolic, serve the important function of augmenting CBF to meet the brain's increased oxidative metabolism during seizures. Further studies are continuing to define mechanisms of functional hyperemia in brain.

Another study cites experimental evidence that microparticles present in blood during cardiopulmonary bypass cause a reduction in cerebral blood flow and metabolism during this procedure. The extent and distribution of ischemic cell change in dog brains following prolonged cardiopulmonary bypass are being investigated. Six animals have undergone

thoracotomy under anesthesia and four were maintained on complete bypass for four hours under conditions of normal temperature, arterial pressure, blood gas tensions, and pH. Two control animals were maintained under identical conditions, but without bypass. Thereafter, perfusion with saline followed by perfusion-fixation with a formaldehyde-acetic acid-methanol mixture was continued for at least 20 minutes at perfusion pressures of 100-110 mg Hg. Following an additional period of immersion fixation, 20 micron sections were prepared from paraffin-embedded blocks at 6 mm intervals throughout the cerebral hemispheres, cerebellum and brain stem, and were stained by several critical staining methods.

The four experimental animals studied following bypass showed early ischemic cell changes by the criteria of Brierley and Brown for light microscopy. These changes were non-focal and present in neurons of layers VI and V of the cerebral cortex, in thalamic nuclei, and in Purkinje cells in the cerebellum. Although there was no absolute regional localization, ischemic changes were more apparent within the parietal cortex than in other cortical areas. While occasionally small and medium sized arterioles were occluded by aggregates of red blood cells and amorphous PTAH positive material, there were no occlusive lesions of larger sized arterial vessels. Small punctate parenchymal hemorrhages were present in two specimens, and two instances of intraventricular bleeding, apparently originating in or near the choroid plexus, were found. Control sections were histologically normal. Continuation of these studies is planned, including microangiographic radiography of thick sections of brain following perfusion with colloidal barium. These studies are to be correlated with direct measurements of microembolic material in blood during bypass by sonar detector methods. Also, the effectiveness of micropore filtration in preventing such changes will be tested.

A serial circular angiotomographic device has been constructed and tested extensively on dogs during angiography. It has been possible to demonstrate a diffuse "brain blush" in tomographic sections 1 cm. thick. Extensive physical tests have been conducted to establish the relation of thickness of cut to tomographic angle and the relation of speed of rotation to resolution. The results of animal studies were so encouraging that the unit was moved to the clinical area and patient studies have been initiated. Fifteen studies have been carried out on patients. In two cases the angiotomogram gave diagnostic information not obtained from conventional films. Continued patient studies will be carried out to evaluate the unit for its clinical value.

In the same radiology group, a new monitoring system for use during cerebral angiography is being evaluated. The system incorporates an industrial transducer and alarm system, and has proved to be an added safety factor during angiography. It indicates blood clots in the needles or catheter, sub-intimal position of the needle tip and changes of blood pressure or pulse rate. A unique feature of the device is that the transducer may be left connected during the injection of contrast media. The part of the pressure tracing immediately after the injection of contrast media is of considerable interest.

In another clinical research center, several members of the group are continuing studies on newly developed techniques for measuring hemispheric blood flow and metabolism in man. They have shown a bilateral reduction in hemispheric blood flow and in metabolism in unilateral stroke due to diaschisis. They have found that cerebral acetoacetate and alpha-butyrate metabolism is not increased in the infarcted hemisphere. The group now plans to direct their attention to the phosphate-oxygen ratio which may define whether or not uncoupling is present in the human brain following cerebral infarction.

Another group of investigators is studying new methods for measuring regional cerebral blood flow using the Anger Camera and a tape storage and retrieval system following the intracarotid injection of radioactive xenon and technecium. These methods provide greater resolution for regional measurements than previously available, and the play back system permits measurement of up to 100 different areas of brain with a single injection. Evidence is being provided of definitive and diagnostic patterns of abnormal flow and changes in blood volume and blood brain barrier in intracranial hematoma and brain tumor as well as in patients with occlusive cerebral vascular disease and infarction.

Another group has induced experimental subarachnoid hemorrhage by the injection of fresh blood into the subarachnoid space. This produced a spasm of the cerebral vessels with a marked reduction of cerebral blood flow on the release of serotonin. The relation of this to ruptured aneurysm and spasm in human subjects is under active investigation and methods of treatment are being considered.

In another set of experiments, regional cerebral blood flow is being measured by implanted electrodes before and after occlusion of the middle cerebral artery and the effects of various forms of treatment on regional blood flow are under study. These studies are being combined with measurements of regional cerebral blood flow using the radioactive antipyrine method originally developed by Landau, Sokoloff, et al, as modified by Reivich and Isaacs. In the human laboratory, using the hemispheric blood flow method and the regional xenon techniques, the therapeutic usefulness of glycerol has been shown, which reduces brain swelling and increases blood flow in the area of cerebral edema.

Therapeutic trials are also ongoing on a double blind basis with two different drugs. One is Hexobendine, a potent cerebrovasodilator. Investigations on man and in animals have shown that it increases cerebral blood flow. The drug has been given to 11 patients intravenously, and it has been observed that five improved, four showed no change and two died of unrelated causes. No toxic effects have been noted. These studies are being extended to evaluation of therapy in cases of acute and chronic cerebrovascular disease. Additionally, drug studies with Cyclandelate are now being initiated.

Researchers in another clinical research center have completed a study in animals of the effect of change in blood pressure on blood flow through cerebral cortex made acutely ischemic by occlusion of a

middle cerebral artery. The effect of CO_2 inhalation on blood flow through cortex which was either acutely or chronically ischemic was studied in animals and revealed the loss of autoregulation in ischemic cortex. The auto-radiographic technique for the measurement of regional cerebral blood flow in animals was used in the study of the effects of occlusion of a middle cerebral artery on blood flow and various regions of the brain correlated with changes in surface vessels. It was then possible to get quantitative estimates of flow in portions of the brain remote to those areas being visualized by the investigator. A study was begun of the effects of stimulation of the sympathetic nerves in the neck on blood flow through the cerebral cortex and on the diameter of surface blood vessels in animals. A pilot study has been initiated in humans of the measurement of cerebral blood flow by inhalation of xenon-133 and has so far shown that the technique is useful but needs further evaluation, particularly in patients with cerebrovascular and other neurological disorders. This evaluation study is continuing.

Another group of investigators at this research center is pursuing the development of clip grafts for aneurysms in small vessel surgery and is beginning to apply the techniques in human subjects. Among personnel of various stroke research centers where carotid endarterectomy is performed, there continues to be active debate concerning the various methods for keeping morbidity and mortality at the lowest possible levels. The ability to rapidly and safely evaluate the changing pattern of regional cerebral blood flow prior to, during and following endarterectomy would be of potential value in making clinical correlations concerning surgical technique, pre-operative neurological findings, including the results of arteriography and the post-operative neurological state of the patient.

Another research group are delineating the temporal profile of acute progressing cerebral infarction in the carotid system and of acute progressing infarction in the vertebral basilar system. This is a problem of practical importance since there are some patients who develop severe worsening of clinical evidences of focal cerebral ischemia some hours after a cerebral infarct is thought to have been "clinically completed." The occurrence of this delayed worsening suggests certain pathophysiologic mechanisms which might be subject to prevention by appropriate therapy.

Research to develop a method for measurement of regional cerebral glucose metabolism has been pursued at another research center. To do this, the characteristics of a tracer for the proposed technique was thought to be one which was taken up by the brain at a rate proportional to that of glucose and whose metabolic products remained in the tissue under study. The use of C^{14}-2-deoxyglucose was selected for study and experiments were conducted to establish a mathematical model and to substantiate the findings by actual experiments in dogs. Favorable results have been obtained to date, and further development to refine the technique is being pursued. It is anticipated that this research will make possible (1) a means of studying regional CBF simultaneously in vivo, (2) will also make possible a direct assessment of the role of regional metabolism in the control of cerebral circulation, and (3) finally when validated it will make possible the extension to human studies, by the use of C^{11}-2-

deoxyglucose which can be detected with external detectors.

The investigator has also directed his efforts to an evaluation of rapid diagnostic methods in extracranial cerebrovascular disease. Four rapid diagnostic tests have been examined (direct thermometry, ophthalmodynamometry, bruit and pulse assessment) to discriminate for the presence or absence of significant extracranial cerebrovascular disease. Utilized separately, thermometry, ophthalmodynamometry and the examination for a bruit will find over 75% to 85% of cases. Pulse assessment alone reveals only 40% of significant lesions. The combination of neck examination for the presence of bruits or pulse deficit alone will discover 93% of the vascular lesions. In this investigator's present series, 100% of the cases were found by including either direct thermometry or ophthalmodynamometry in combination with the aforementioned two tests.

Ophthalmodynamometry accurately located the site of the major lesion in more than 90% of the cases. The direct thermometry technique scored below 40% in lesion localization. It is suggested that the thermometry technique, because of its ease of performance, be utilized in combination with neck examination as the preliminary step in assessing patients with suspected extracranial cerebrovascular disease. Ophthalmodynamometry should then be used for more accurate localization of the lesion prior to angiography.

An understanding of the normal control mechanisms of the cerebral circulation is essential to any investigation of the derangement present in various abnormal conditions. This is especially important in the study of cerebrovascular disease. Knowledge of the important regulating factors in both normal and pathologic states may lead to concepts that will be of use in preventing or correcting abnormalities of the cerebral circulatory system. The relationship between regional cerebral metabolism and regional cerebral blood flow is one important aspect of the control system which has not yet been able to be directly studied. The development of a method for measuring regional cerebral glucose metabolism in vivo should be of significance in this regard.

Considerable interest has developed in studies of cerebral vasospasm. One research group reports progress in determining the actual effect on regional blood flow of angiographic experimental cerebral vasospasm. Vasospasm was produced both by vessel puncture (as recommended by this group) and by subarachnoid injection of blood (as recommended by others). It was found that, despite apparent significant cerebral vasospasm (vessel caliber reduced to 1/2 of control), there was still no statistically significant reduction in cerebral blood flow. Only when the vasospasm became intense, that is the cerebral arteries appeared to be 1/3 or less of the original caliber, was there reduction in cerebral blood flow. When this critical point was reached, the cerebral blood flow dropped dramatically. In a series of 25 monkeys in which subarachnoid hemorrhage was produced by puncture of internal carotid artery, virtually all showed a precipitous drop in cerebral blood flow determinations made within 1/2 hour of puncture. Cerebral blood flow determinations rapidly stabilized and, quite unexplainedly, increased slightly above control from the first to the fifth hour after hemorrhage. Following this, cerebral blood flow would usually stabilize despite the presence of mild

to moderate spasm on the arteriograms. If severe vasospasm developed, angiography would demonstrate marked arterial constriction. CBF values at this time were often half of the control value. This second phase of spasm (probably part of the biphasic response described by Brawley) would persist throughout duration of the survival of the animals. Upon the injection of blood into the chiasmatic cistern, immediate angiographic spasm was noted, associated with a significant drop in cerebral blood flow. The spasm would never become secondarily severe, though some degree of spasm was seen throughout the experiment. Marked reduction in CBF and this characteristic second stage of spasm are not seen with subarachnoid blood injections.

It is the opinion of this investigator that both rupture of an intracranial vessel and subarachnoid injection of blood are capable of producing an immediate transient angiographic spasm which does reduce CBF. Apparently, the puncture technique is more likely to produce the characteristic second phase of spasm which is probably the clinically significant phenomenon.

Concomitant experiments carried out by this researcher related to the determination of stability of regional CBF determinations by the radioisotope method in experimental animals for long durations. A series of long duration regional blood flow experiments (over 36 hours) were carried out in ten animals. It was found that: (1) RBF determinations within the first hour following the preparation fluctuate and are invalid, and (2) the coefficient of variation among the determinations increases with the duration of the experiment, within the first 5-6 hours it being only 3-4%, but after 20 hours it may be as high as 14%. The practicality of this technique for inducing cerebral vasospasm may be open to question since the technique of this investigator produced severe reduction in blood flow in only about 1/3 of the preparations, but not at all in preparations produced by techniques described by others.

Another phase of this investigator's studies consisted of the application of physical forces to overcome the effects of cerebral vasospasm. This researcher has been able to reliably maintain regional cerebral blood flow above control levels in normal autoregulating brains by intermittently occluding the descending aorta with an inflatable balloon passed up through the femoral artery. He has determined the appropriate intervals of inflation and deflation which allow for adequate renal perfusion, yet can increase CBF significantly. The assumption is that in the presence of cerebral vasospasm, increased perfusion pressure for long intervals may adequately nourish the brain until the spasm spontaneously subsides. The technique of intermittent aortic occlusion was performed in 35 dogs and 25 monkeys without pathologic alterations. Intermittent aortic occlusion in the presence of spasm has been attempted. It has been shown that it is capable of perfusing past severely constructed vessels used in the experiment described. In this experiment there was intense spasm of the middle cerebral artery, which barely visualized angoigraphically, associated with a marked reduction in CBF records. On institution of intermittent aortic occlusion, CBF values returned to near normal levels. The results appear encouraging even though there are many problems yet to be solved.

These include cerebral edema which can result in certain instances when the brain is so hyper-perfused. Additionally, certain animals develop alterations in blood pressure after long intervals with intermittent aortic occlusion. The importance of these studies is heavily based on the presumption that a model of cerebral vasospasm, which behaves in a manner like the human vasospasm phenomenon, is necessary before any research in this area can be continued. The significance of this model as it may apply to problems of cerebral blood flow in cerebrovascular disease is not certain. Further studies are needed.

Trauma (Head Injury)

Since trauma is the number one killer of man while he is in the prime of life, it is only natural that a major aspect of trauma, head injury, should receive special attention from the NINDS. During 1971, approximately 70 research grants representing $5,228,000 supported investigators working in medical and scientific areas directly or indirectly related to central nervous system trauma, particularly head injury. More recently, this Institute has also begun to expand its support of research on acute trauma to the spinal cord.

In general, the care and study of the seriously injured head trauma patient has had as its primary concern the prevention of brain swelling and therefore a search for the mechanisms that trigger the onset of brain edema. Recent work in one of the head injury centers demonstrated a marked diminution in arteriolar calibre (720 micra) in the injured area following controlled impact trauma to the intact monkey skull. One day after trauma the microangiograms showed a return toward normal in the calibre of the larger vessels and an increase in tortuosity of these vessels with a continuing diminution of capillary filling. Histologically, the most significant feature was the presence of cerebral edema which became apparent one day after injury, the edema being present in both the cortex and the white matter. The brain swelling was well established between the first and third days when the caliber of the arterioles was larger than that noted in the immediate post traumatic period. This was also the period when the capillary filling was abnormal.

Workers in another head injury center also have been investigating cerebral vasomotor paralysis following artificial concussion in animals and during the decompression which usually occurs in patients following brain tumor extirpation. In the latter case, patients who had had prolonged retraction of the brain during removal of a deep seated tumor or clipping of an aneurysm, had an intracranial pressure which was extremely sensitive to changes in respiration. These patients were maintained on respiratory assistance immediately following surgery and, quite often, removal of the respirator was followed within two minutes by an increase in intracranial pressure of 30-50 mm Hg. The pressure promptly fell when respiratory assistance was reinstituted. Several continued to decompensate and ultimately the responsiveness of intracranial pressure to respiratory control disappeared. These investigators attributed the continued rise in intracranial pressure to brain swelling and the lack of response to changes in arterial pO2 and pCO2 to paralysis of the vasomotor tone of the cerebral vessels.

This concept of cerebral vasomotor paralysis was developed in monkey experiments in which pressure waves were first identified and studied. A series of moderately high intracranial pressure waves that had no effect on the animal's EEG was often followed by a "terminal pressure wave," at which time the intracranial pressure rose to the level of the blood pressure, the EEG became isoelectric, and the animal passed rapidly into shock unless fluid was rapidly withdrawn from the pressure inducing balloon.

In another series of experiments the relationship of cerebral blood flow (CBF) and intracranial pressure (IP) was investigated. During the control period, when the arterial pressure was elevated with norepinephrine, the CBF remained essentially constant. After a series of balloon induced intracranial pressure waves, norepinephrine was injected again. The same increase in blood pressure now produced a greater rise in CBF, leading these investigators to postulate that the vascular autoregulatory mechanism had been damaged. As with the patients discussed above, the response of the cerebral arterioles to hypercapnia and hyperventilation also disappeared. In the terminal stage, when intracranial and arterial pressures were equal, cortical electrical activity had disappeared, spontaneous breathing had ceased, and rapid evacuation of the balloon produced a drop in IP to normal. This sudden expansion of the brain following balloon deflation was attributed to rapid filling of flaccid cerebral arterioles (vasomotor paralysis) and it was suggested that the increase in brain volume was due to the rapid expansion of cerebral vessels, cerebral edema, or a combination of the two processes.

Attempts to control and reverse the trend of progressive brain edema in the head injured patient have involved the energies of scientific personnel in a number of head injury centers. The efficacy of using diuretics to successfully treat brain edema following head trauma has been a subject of major concern because the results have had a wide range of variability. Precisely controlled studies by one head injury center have demonstrated that the brain swelling which follows experimental brain trauma in animals is not reversed by steroid (dexamethasone) therapy. In terms of human disease, this would certainly mean that, in those conditions involving cerebral edema in which there is associated significant necrosis and hemorrhage, the edema would not be expected to respond to steroid therapy. The possible effect of steroids on inflammation and thus on edema has not, however, been entirely ruled out.

Long-term studies on seizure incidence in children (birth to 14 years) following injury has shown that 10 percent of the head injured children had at least one seizure during the first 24 hours after injury. The incidence of cases with seizures dropped to 3 percent by the end of the first year and approximately 10 percent of the children without seizures (at the time of injury) developed EEG abnormalities, including spike foci within 2-4 years after injury. In addition, the head-injured child had an increased incidence of negative behavioral traits, compared with their pre-injury incidence. Among the major symptoms were hyperkinesis, aggression, irritability and poor control of affects.

The effects of brain damage on performance and physiological responses have been studied by another group of scientific investigators who found that brain-injured patients have slower reaction times, diminished alpha blocking, and disturbed orienting responses to auditory stimuli. In addition, cold pressor stimulation to the brain-injured patient results in depressed plethysmographic rebounds, suggesting differently tuned vascular responsivity.

Another area of major concern, and therefore major research effort, is regeneration of nerve tissue following central nervous system trauma. In general most of the research models have employed the spinal cord for study but one group has concerned itself with investigating regeneration in the pyramidal tract. In man, damage of the pyramidal tract produces a total hemiplegia, but these patients generally recover during the following eight months with excellent control of the involved face, arm and leg without developing the "pyramidal syndrome," except for the sign of Babinski. Parallel studies in monkeys revealed that the recovery of motor function was not as complete as that recorded in man. These differing results suggest that in man, as opposed to the monkey, there are at least three motor systems; the first of which is the one mediated by the cortico-spinal tract at the pyramidal level; the second being a corticospinal pontine system; and the third, a system whose cortical origins and paths are unknown.

An investigation concerning the origin and activity of cells invading the damaged animal nervous tissue (spinal cord) has revealed that they are mononuclear leukocytes. Large numbers of these cells enter the white matter of the cord at all levels following root transection. Some of these cells underwent cell division after entering the nervous tissue, as evidence by the labeled mitotic figures. In addition, increased DNA synthesis was recorded in cells adjacent to the nerve cell bodies, both in the cord and in the dorsal root ganglia.

Even more exciting are results of studies carried out in another laboratory on regeneration following spinal cord damage. Axons at the proximal end of the animal spinal cord following transection were found to produce growth cones and characteristic new bouton connections on axons and dendrites. This is the first positive data that mammalian central nervous system neurones have the capacity to regenerate. Additional work on regeneration in damaged animal spinal cord by another group of investigators has demonstrated that one week following cord damage the area of insult becomes filled with Gitter cells. At two weeks, axons begin to appear beneath the pia, following the surface of capillaries, and at seven weeks the area of destruction is filled with a heavy growth of myelinated axons. Electronmicroscopy has shown that the cells giving origin to the myelin are both Schwann cells and glial cells. The premise is that the axons regenerating and encased by a peripheral type of myelin grow in from the dorsal spinal nerve roots and blood vessels of the stumps, while those possessing a central type of myelin grow out from the spinal cord stumps beyond the zone of damage.

That cultured embryonic spinal ganglion cells can form functional synapses has been an area of endeavor in which considerable effort has been devoted by one group of investigators. They have shown that "typical synapses" are formed, even though they lack other innervation territories. The newly formed dendrites have functional meaning as recipients of input rather than fortuitous efforts as abortive growth. Intracellular records of these ganglion cell cultures have been obtained, providing evidence of synaptic interaction. Nerve growth factor (NGF) was employed in the tissue culture medium and future studies are planned to answer the question of how crucial this material is and if so, is it necessary for the formation of functional synapses.

OTHER NEUROLOGICAL DISORDERS

Sclerosing Disorders - General

Studies relating to sclerosing disorders in general continue to focus primarily on detailed investigations of lipids and myelin. The lipid studies pertain to central nervous system lipids, lipid metabolism in normal and pathological states, the relationship between brain lipids and electrolytes, and a biochemical genetic study of bacterial lipids. Phospholipids are the subject of study with regard to their physical state, biological activity in general and nerve-muscle function in particular, together with their metabolism in membrane synthesis. The biochemistry of glycolipids, fatty acids, long chain bases, inositol and phosphoinositides, along with the mechanism of ion transport in nerve membrane and studies on subcellular fractions of developing brain, continues in the service of a better understanding of general sclerosing disorders. Myelin studies continue to emphasize structure, function, metabolism, pathology and comparative studies, together with its formation and relation to the neuron, its abnormal composition in plasma membranes and the interactions of phosphoinositides in myelin itself.

Multiple Sclerosis

Multiple sclerosis is a disorder of the nervous system characterized by the presence of tiny sclerotic areas or plaques of degenerating or destroyed myelin in the white matter of the brain and spinal cord. Its symptoms are referable to disruption of nerve impulses in the affected fiber tracts. Its etiology remains to be determined and treatment is only symptomatic.

A current investigation is aimed at the etiology of multiple sclerosis and the mechanism involved in its development. The work is directed at the identification of infectious agents, measles virus in particular, that may be responsible for the signs and symptoms of demyelinating diseases appearing years after the initial illness. It is postulated that the measles virus in some form may persist and provide active immunity throughout life for most people but on rare occasions may produce demyelination and symptoms commonly associated with central nervous system disease, behaving generally in the manner of the "slow" viruses. Evidence is now available which indicates that the measles virus may be responsible for the pathologic changes known to be present in various forms of measles encephalitis, a world-wide disease with a geographic distribution similar to that of multiple sclerosis, since its incidence tends to be low in tropical areas and high in the more temperate zones.

The discovery of inclusion bodies in the cytoplasm and nuclei of cells and the formation of giant cells in areas of demyelination strongly suggest that the measles virus is related to encephalitic manifestations. The hallmark of the virus has been found in individuals suffering from degenerative brain disease years after childhood measles. Further, several patients with a classic form of multiple sclerosis have shown inclusion bodies and giant cells similar to those seen in acute and chronic measles encephalitis. Continuing attempts will be made to recover active measles virus by co-cultivation with Hela and human embryonic kidney cells.

Amyotrophic Lateral Sclerosis

Amyotrophic lateral sclerosis is a neurological disorder of unknown etiology with an incidence of 8,000 to 10,000 patients in the United States. It is characterized initially by muscle weakness and wasting which is brought about by degeneration of motor cells in the brain and spinal cord. Treatment is symptomatic only.

A new study is in progress to develop an experimental model for amyotrophic lateral sclerosis by immunomanipulation of mice infected with mouse encephalomyelitis virus which causes mouse poliomyelitis.

This study arose from suggestive evidence that certain progressive neurological diseases of later life may be caused by a reactivated virus which seemed to have been inactivated effectively many years earlier. Particularly intriguing are some cases of amyotrophic lateral sclerosis which have appeared many years subsequent to recovery from antecedent poliomyelitis. A not unreasonable conjecture with regard to mechanism might be that the poliovirus has been reactivated. Similar cases can be made for paralysis agitans, multiple sclerosis and possibly Jakob-Creutzfeldt disease and parkinsonism.

The main objective of the program will be to develop a model system for the reactivation of a central nervous system virus which can be applied to human amyotrophic lateral sclerosis. The approach will be to infect animals with mouse poliovirus so they contract the disease but ultimately recover. Following recovery, the immunologic competence of the animals will be compromised by various means with the expectation that latent viruses will manifest themselves under these circumstances.

Experimental Allergic Encephalitis

Experimental allergic encephalitis is an artificial, abnormally induced, auto-immune disease in which the immune defenses of the organism are manipulated and directed against its own tissues. It has been used as a model system for the study of immunological factors implicated in central nervous system disease. Demyelinating diseases bearing resemblance to multiple sclerosis may be induced by this means.

Serial measurement of cerebrospinal fluid beta-glucuronidase has been found to be a sensitive method for detecting experimental allergic encephalitis and for monitoring the course of the disease in the rabbit. Elevated levels have been found only with clinical or histological evidence of the disease. Control studies indicate that elevated cerebrospinal fluid enzyme does not result from induced serum increases of beta-glucuronidase levels. The suspicion is that the increased cerebrospinal fluid enzyme levels in active experimental allergic encephalitis reflect release of enzyme from lymphoid and other inflammatory cells contributing to the encephalitic lesion. There are implications here for human neurological disease states. Continuing studies indicate that cats can develop experimental allergic encephalitis providing an elaborate sensitization procedure is used, but so far, the disease has been induced in a high proportion of animals only after placing them on a thiamine deficient diet.

It was found that experimental allergic encephalitis could be inhibited in rats by cyclophosphamide when given in daily doses beginning as early as the first day of paralysis or as late as the fifth day of advanced paralytic signs. It caused reversal of clinical signs and disappearance of lesions in two thirds of the animals. Fully recovered animals showed a prompt relapse when cyclophosphamide was discontinued. Continued remission could be obtained with continued treatment.

Continuing investigation will determine the relative roles of different cell types, subcellular factors and circulating antibodies in the development of this auto-immune disease. Mechanisms underlying the inhibitory and therapeutic effects of cyclophosphamide will be further investigated as will the apparent ability of thiamine deficiency to enhance the susceptibility of cats to the disease.

Experiments are being devised to discover the phylogenetic capacity to develop auto immune disease and autoantibody along with the role of the thymus and other central lymphoid tissue in the interest of preventing and treating experimental auto-immune disorders.

Parkinsonism

Parkinson's disease is a progressive neurological disorder of unknown cause affecting certain brain areas involved in the control and regulation of movement. Its onset may be incidious and its progression may be almost imperceptible, but is ultimately characterized by tremor, rigidity and bent posture. Standard treatment in the past consisted of physical therapy, a variety of drugs, and surgery. A highly effective form of replacement therapy making use of large oral doses of L-Dopa or thalamic surgery are now the treatments of choice.

A third center for research in parkinsonism has recently come into existence and will be concerned with a correlated approach involving biochemical, ultrastructural and neurophysiological methods in the same systems to elucidate the role of catecholamines in the basal ganglia. The individual projects will be concerned with anatomical studies of biogenic amines in the primate brain, neuromelanin, neurophysiology of the substantia nigracaudate nucleus interactions, blood-brain barrier to L-Dopa, amine metabolism, and motor potentials in parkinsonism.

The anatomical studies of aminergic systems in primate brain that may be related to the effects of L-Dopa administration and nigro-striatal pathology will deal with catecholamine bodies in the substantia nigra and regions rich in catecholamine terminals. They will also include mapping of nigro-striatal dopaminergic pathways in the primate. Experiments are planned which will examine in detail the modulatary effect of the substantia nigra on the cortico-striato-thalamic activity of the brain using both anatomical and physiological techniques. Studies are also designed to elucidate the mechanism of entry of Dopa or its metabolites into the central nervous system across the blood-brain barrier and possibly across the choroid plexus. There are suggestions that a central mechanism, not located in the motor cortex, develops during learning of skilled movements which monitors and controls subsequent

voluntary execution of specific motor acts. Selective inhibition of afferent stimuli is essential to this mechansim. Interference with this system can lead to slow behavior not unlike that seen in Parkinson's disease. It is proposed to examine these hypotheses in animals and in Parkinson's disease patients before and after L-Dopa therapy.

Work is in progress now to determine the effects of Dopa decarboxylase inhibitors alone and in combination with monoamine oxidase inhibitors on the amount of systemically administered L-Dopa that gets into the brain across the blood-brain barrier and to explore dimethyl sulfoxide as a transport carrier of Dopa molecules.

Finally, there is a program for the long-term follow-up of patients with parkinsonism receiving L-Dopa and for investigations of L-Dopa in selected disorders other than Parkinson's disease. These studies are to be supplemented with peripheral metabolic inhibitors and centrally acting monoamine oxidase inhibitors when synthesized. Evaluation of dopamine-like substances, substances which change the sensitivity of the central nervous system to L-Dopa, and the role of serotonin as related to the involuntary movements induced by L-Dopa will be included in these studies.

Neuromuscular Disorders

The most prominent neuromuscular diseases are myasthenia gravis (MG) and the muscular dystrophies (MD) which have prevalence rates of 4 and 6 per 100,000 respectively. While MG has been recognized as a clinical entity since the seventeenth century, the dystrophies constitute a variety of clinical conditions. New dystrophic variants in both animals and humans are described yearly and their classification constitutes a formidable neurological problem. Myasthenia gravis has been related to impaired transmission through the myoneural junction while the dystrophies are variously correlated with deficiencies in muscle, nerve, intermediary metabolism, or the myoneural junction.

MG is characterized by progressive impairment of neuromuscular transmission with continuous exercise. Research in recent years has demonstrated that a diminished quantity of acetylcholine is liberated from nerve terminals, preventing adequate depolarization of the muscle membrane. Current observations suggest that a decreased amount of the neurohumor is synthesized at the nerve terminals, as judged by the lower activity of choline acetyltransterase in muscle biopsies from MG patients. It is not known whether the diminution of enzyme activity results from the presence of an endogenous inhibitor or from more primary structural or metabolic factors.

Treatment, for the most part, consists of the administration of anticholinesterase drugs which act to increase the effective concentration of acetylcholine at the endplate. However, in a significant number of patients, marked improvement may be obtained by the chronic administration of ACTH or prednisone. The basis for the efficacy of these latter drugs is not clear. Finally, the surgical removal of a thymoma in a patient with MG often leads to marked improvement. Curiously, the incidence of thymomas in MG is approximately 15% or about five thousand times more frequent than in control patients.

Recent studies continue to confirm the possibility that MG is an autoimmune disease. The serum of any patients contain antibodies against their own muscle, thymus, thyroid or other tissue. No antibodies have been identified as yet as the myoneural junction, and the presence of antimuscle antibodies does not necessarily correlate with the course of the patients illness. However, it has been reported that the serum of MG patients inhibits the synthesis of acetylcholine by frog or rat brain.

Research efforts in the area of muscular dystrophy follow well-established approaches. Substances which may alter the dystrophic condition are being tested in humans, in animal models, and in tissue culture. Recently, it has been reported that safflower oil reverses the hereditary muscular distrophy of chickens. The testing of components of the oil as well as structurally related analogues is currently in progress. Methods for the detection of carriers of X-linked Duchenne muscular dystrophy continue to improve. Approximately 70% of known female carriers have elevated levels of serum creatine phosphatase. For the remaining 30% who have normal serum values, light and electron microscopic evaluation of biopsy material may prove helpful. Myopathic changes in the muscle of carriers may be extensive, minimal or absent.

The properties of dystrophic muscle continue to be elucidated. In tissue culture, muscle cells from dystrophic chickens differ in rate of fiber growth, rate of development of enzyme activity and strength of spontaneous contrations. It has been shown that muscle fibers of dystrophic mice have significantly lower resting potentials. The decrease for fast fibers is approximately twice as large as that for slow fibers. Finally, it may be assumed that present research on the molecular events in contraction and on the chemistry of cell and organelle membranes will soon include dystrophic muscle.

Bioengineering Studies of Muscle Dynamics

During the past decade there was a surge in activity initiated by the joint efforts of engineering, physical, biological, and medical scientists to develop suitable methods for the prevention, treatment, and management of disease.

The NINDS has played a catalytic role in this activity by providing research grant support during the developing years of biomedical engineering, a field which deals with the interaction between the engineering sciences in biology and medicine. The report of the research activities of such a multidisciplinary team is presented below.

This group of investigators includes engineers with considerable experience in experimental mechanics and in developing mathematical models; biophysicists knowledgeable in systems analysis; biomedical scientists well qualified in the field of nerve and muscle physiology; and a surgeon whose major research interest is muscular-skeletal physiology and orthopedic surgery.

The long-term goal of their research program is to determine what differences exist between the mechanical and control behavior of the limbs of normal subjects and those afflicted with various known neuromuscular disorders. The investigators' working hypothesis is that the neuromuscular skeletal system

can be represented by a mechanical plus control system model of muscle dynamics. In disease- or disability afflicted subjects part of this system, depending on the disease, will be functioning abnormally or not at all.

The model has undergone many modifications as experimental data have been collected to check its various details. Since the system under analysis is complete, the mathematical determination of the model constants is not a simple process. A digital computer program has therefore been written to process the experimental data and systematically determine the model parameters from them. The program modifies parameter values in order to minimize the error with which the model can predict the experimental data submitted to it. The mathematical scheme used in this program, called an identification scheme, has been treated extensively in control theory. The model is mathematically non-linear, however, so that the development of the scheme has not been a simple straight-forward procedure. The principal part of the successful reduction system the investigators are now using is a non-linear simplex method.

On the practical level, it appears that quantitative measures of neuromuscular function in human neuromuscular disorders are almost non-existant. For example, in connection with spasticity, it was pointed out a year ago that an objective, accurately reproducible and easily applied test for muscle spasticity has yet to be devised.

This group has developed control systems and mechanical models in the relatively neglected field of muscle dynamics. The first stage of the study was concerned with correcting, improving, and expanding conventional analyses with particular regard to the theoretical effects of heat production on the parameters of the model. Movement of the forearm around the elbow was studied for this purpose. Movements of the forearm against various types of loading in response to sensory signals were monitored both by measuring displacement parameters and by electromyography. The study was extended to other muscle systems of the body.

From the dynamic models of the system, the investigators hope to be able to localize the malfunctions, and use the quantitative information obtained as a basis for improved diagnosis and therapy.

The approach to this problem has two major components. First, the quantitative approaches of engineering--especially in the areas of human dynamic structural analysis, solid mechanics, and control theory--can now be applied to complex non-linear biological systems such as human muscle. Second, such an engineering analysis makes it possible to derive quantitative parameters.

To achieve this goal, the problem was attacked in four basic steps, three of which are now well in hand. In the analytical and modelling phases of the study, progress has occurred in three directions; analysis, model construction, and parameter evaluation. In the analytical work, the investigators combined current physiological understanding of muscle constitution and behavior with continuum mechanics. They have thus established a useable quantitative model for forearm movements including limb dynamics, muscle mechanics, and neural control. It provides numerical measures of both neuromuscular parameters and

muscle tensions. A series of simple non-invasive tests provides a normal data base.

Three types of studies designed to evaluate the parameters in the model have been performed on human subjects. The first, a series of static tests, demonstrates on the basis of the principles of structural mechanics that averaged electromyogram amplitude is directly related to muscle contractile force. This series of tests also provides numerical values for a number of parameters in the model and for the forces in the individual muscles. The second study, consisting of a set of constant angular velocity tests, independently evaluates these same parameters as well as a damping coefficient. The results are in agreement with those of the first study, and in addition show that damping is small. The third study, a series of "quick-release" tests, serves as an evaluation of the control parameters in the model. The results indicate that direct positional control is much more important for present experimental conditions than velocity control.

A model that includes central nervous system characteristics, local thermal effects, wave propagation effects, and the dynamics of the skeletal frame would be of considerable value to those physiologists, biophysicists, and engineers who are working in the field of control theory and system performance in other biological systems. This effort to provide a systematic account of how muscle systems function in situ should contribute basic information of ultimate value to many medical specialties.

The investigators plan to continue this line of research to refine both the structure and numerical constants in their model for the system so that it represents the physiological condition more accurately.

At this time a complete testing and evaluation facility is already functioning. Human subjects are being studies on a regular basis. The results obtained to date indicate that numerical values for parameters, separately identifying muscle properties and nervous system control properties, have been calculated from measurements during a very simple (for the subject) dynamical test. This means that changes in individual parameters in the neuromusclar systems of human subjects can be monitored quantitatively. Such a quantitative technique is much needed, not only for differential diagnosis, but also for monitoring therapy.

In addition, studies of patients with a well characterized disability, e.g., muscular dystrophy, are planned to determine the clinical usefulness of the model, thus leading to improved quantitative tools for diagnosis and clinical studies.

Convulsive Disorders

Patients who suffer from convulsive disorders often conceal this fact because it can adversely affect their social and economic status and their insurability. It is therefore difficult to estimate numbers of persons affected. Their number is estimated between 2 and 4 million. Conservatively, loss of earning capacity caused by this disease, costs the Nation more than a billion dollars a year. Much of this would be unnecessary if the general public, by better

understanding of the disorders, would make available to victims the social, educational, and employment opportunities now denied them largely because of ignorance and fear. Gnerally, persons with epilepsy have average or better-than-average intelligence and good earning potential. A majority of them can live normal lives because their convulsive disorders can be controlled with anticonvulsant drugs.

Epilepsy is a disease characterized by one or more of the following symptoms: (1) paroxysmally recurring impairment or loss of consciousness; (2) involuntary excess or cessation of muscle movements; (3) psychic or sensory disturbances; (4) perturbation of the autonomic nervous system. On the basis of origin, duration of seizures, clinical and electroencephalographic evidences, epilepsy is classified into four subdivisions.

In epilepsy known as "Grand Mal," in more modern terminology, "major motor seizures," the attack is most violent and convulsions last longer. The afflicted person may bite his tongue and invariably loses control of his faculties. If the area or areas of the brain can be located from which these abnormal electrical discharges emanate, the affliction is termed "Focal" or Jacksonian epilepsy. In "Petit Mal," a disorder of the young, the seizures are of short duration, and attacks occur more frequently. Seizures are characterized by myoclonic jerks and they generally start in the extremities and/or in one corner of the mouth. The affected part trembles violently. The trembling movement moves upwards. It either ends up in a minor convulsion or the individual loses consciousness in the same way he does in grand mal. "Psychomotor" epilepsy is caused by discharging neurons which exert their influence upon the mental processes as well as upon the muscle movements. A patient exhibits adversive or torsion movements, dreadful fear, flinging of arms aimlessly, smacking of lips, and other incoherent physical and mental behaviors. The last type is called "Autonomic" epilepsy. A person suffering from it experiences a sharp, acute, sudden pain in the stomach. Hypertension, perspiration and other visceral disorders are the common symptoms.

Epilepsy may be caused by several factors, such as brain damage, presence of a scar caused by a wound or an injury, drugs, congenital malformation, nutritional deficiences, metabolic abnormalities, fever (in infants), infectious diseases (encephalitis, meningitis), brain tumors and abscesses. In a recent study of the long term effects of administering a diet rich in median chain triglyceride (MCT) on seizure control in children indicated that a diet deriving 60 percent of its calories from MCT, when administered to a group of children with a variety of seizures refractory to conventional modes of therapy, was effective in controlling seizures, particularly myoclonic and akinetic types. A less sustained control was obtained in some older children with psychomotor, focal motor and grand mal convulsions. These studies provided very little information regarding the mechanism of the effect of the diet on seizure control. They only indicated that the diet produces hyperketonemia but no metabolic acidosis.

The role that heredity plays is as yet undetermined. If one twin develops epilepsy, the other one is likely to develop it also. However, patients with this disease do not necessarily have epileptogenic offspring. Comparative intelligence evaluations and electroencephalogram studies show that patients

with familial idiopathic epilepsy have lower intelligence quotients than their non-epileptic relatives. It is estimated that a significant portion of the U. S. population is epileptic with seizures that are symptomatic of a primary disorder or of unknown idiopathic etiology. Evidence has been developed indicating that at least a portion of the idiopathic epilepsy observed in man has a genetic basis, although the specific mode of inheritance is not well understood. Experimentally, using Beagle dogs with spontaneous, recurrent convulsive seizures similar to those observed in man, evidence is being provided indicating a strong genetic basis for convulsive episodes. Based upon the comparative studies of spike-and-wave EEG train in groups of patients suffering from febrile convulsions, focal EEG anomaly, or focal epilepsy, and in a sample of normal people, it is suggested that several of the epilepsies might have a common genetic factor in their etiology. A number of investigators have reported genetic involvement in other forms of epilepsy. These include photogenic epilepsy, idiopathic epilepsy, benign familial neonatal convulsions and partial epilepsy.

Although the exact mode of action of mechanisms underlying epilepsy is largely unknown, the experimental evidence based on electroencephalographic studies indicates that the neurons in the affected area build up a supply of electrical energy through their metabolic action. By repetitive activity of charge and discharge, the cells become overactive and start firing in synchrony. The firing pattern may spread to other areas of the brain resulting in an epileptic seizure. When the neurons start firing again in dysharmony, the seizure is over. Brain waves so monitored give no indication of the individual's intelligence, thoughts, or his mental health. However, they provide strong clues as to whether or not a person has epilepsy. An EEG recorded during a seizure is likely to show unusually high bursts of energy release. The pattern indicates the type of seizure an individual has suffered.

One of the most versatile electrical instruments used in the detection of electrical discharges from the nerve cells is an electroencephalograph (EEG) which picks up electrical discharges from the brain cells, amplifies them and records the impulses in the form of graphs. With its help, various areas of the brain have been scanned and, using microelectrodes, electrical energy discharge patterns in groups of cells or a single cell, both in vivo and in vitro, can be recorded.

In order to understand the seizure mechanisms, epilepsy has been induced experimentally in laboratory animals. The methods of induction can be divided into four main groups. Methods based on electrically-induced seizures include generalized stimulation of the intact animal to produce either threshold (clonic) seizures or maximal (flexor-extensor) seizures, and local stimulation of selected areas of the brain or spinal cord. Methods based on the administration of chemicals induce seizures similar to the ones indicated for electrical stimulation. Although chemicals, such as strychnine, picrotoxin, methionine sulfoximine, and thiosemicarbazide, have limited value in inducing seizures for evaluation of anti-convulsant drugs, they nevertheless provide valuable tools for the study of seizure induction. The most important chemicals used for producing seizures are convulsant drugs and irritant agents. For example, irritant agents, such as penicillin, applied to the cerebral cortex or injected into various brain structures, result in an acute irritable

focus, impulses from which may rapidly spread to other areas of the brain and produce generalized seizures. Included in this section are the methods for producing chronic epileptogenic animals by inducing various forms of brain injury. Evidence linking acetylcholine to synaptic transmission in the central nervous system and especially to cortical arousal systems, plus the evidence that seizure activity can at times result from endogenously produced acetylcholine, has led several investigators to focus their attention on this agent with a hope to elucidate more general principles of epileptogenesis. The injection of alumina cream in the brain of monkeys appears to produce a reliable model which resembles human epilepsy. Investigations with iron hydroxide, magnesium hydroxide, zinc oxide, chromium oxide and mixed earth oxides showed that all these oxides or hydroxides are without epileptogenic effect when applied to the motor cortex of the monkey. On the other hand, nickel and antimony proved most epileptogenic only when implanted as pellets into the motor cortex. Mild effects were observed with bismuth, zirconium, tin, titanium, molybdenum and tungsten. Twenty-three more metallic powders have been tested on the sensorimotor cortex of monkeys. Some have shown very severe effects, while others remained ineffective. A third technique used in inducing experimental seizures is based on sensory stimulation, such as elicitation of audiogenic seizures in susceptible mice and rats and photic seizures in rabbits and baboons. Interest has recently been directed to baboons which show a high incidence of spontaneous photic seizures comparable to those observed in photic-sensitive epileptic patients. Lastly, seizures may be induced by metabolic deviations, including water and electrolyte alterations, CO_2 withdrawal, hyperthermia, endocrine ablation, or hormone administration. The aim of these studies is to elucidate the neural mechanism involved in the transition of a localized lesion in the brain to an epileptogenic zone. This type of experimental approach has contributed new methods of inducing chronic epilepsy in animals which provides an important new tool for the study of this disease. By applying EEG techniques to these experimentally induced epileptic animals, the efficacy of new drugs is being tested, epileptic loci (foci) have been determined, and new and improved surgical techniques for therapeutic application are being developed.

In a recent classification of the epilepsies, seizures elicited in the newborn have been described as showing partial discharges, susceptible to change from time to time, in both morphology and topography, from area to area and sometimes from one side to the other. In terms of epilepsy the maturing nervous system acquires with age, an augmented capacity for generalized seizures, an increase in the frequency, duration and spread of after discharges, and an overall reduction of seizure thresholds. The developing nervous system has thus been shown to be in an advantageous position in terms of protection from convulsive disorders. Using developing brain as an experimental model, greater insight into the pathophysiological mechanisms involved in the production of seizures is currently being investigated.

One of the experimental leads to the study of the ontogenesis of seizure mechanisms is based on the potassium shift across the nerve membrane. A large increase in the radioactive potassium surface efflux prior to, and during the early stages of seizures elicited by metrazol and electrical stimulation of both the hippocampus and neocortex of cats and rats, was observed. Using this as supportive evidence for the potassium accumulation

hypothesis, a model for the regenerative, all-or-none aspects of the initiation of seizure activity was proposed. Added strength was gained by a recent observation that Dilantin (diphenylhydantoin), one of the most widely used antiseizure drugs, despite its great clinical utility, has thus far not been shown to have any specific effect on the nervous system other than that of antagonizing seizures. For this reason it has been of little value in understanding the pathophysiology of epilepsy. However, in later experiments Dilantin was found to stimulate the rate of potassium uptake in an isolated lobster leg nerve preparation. This effect was completely abolished by 2, 4, di-nitrophenol. These observations, together with the finding that Dilantin was without effect in changing the potassium efflux rate constant, suggested that Dilantin may be stimulating the active uptake of potassium. The idea that Dilantin exerts its antiseizure effect by stimulating the so called Na-K pump is supportive of the hypothesis that accumulation of potassium contributes to the seizure induction. By stimulating the active uptake of extracellular potassium, Dilantin stabilizes the nervous system against excessive changes in potassium which, according to this hypothesis, is an effect that would be expected to diminish the likelihood for seizure activity.

Several physio-chemical differences between normal and epileptogenic brain tissues have been found. For example, tissue from the area giving rise to abnormal electric discharge did not bind as much acetylcholine as did the normal tissue. This metabolite has been shown to occur in high quantities in epileptic regions of the brain, which suggests that, when this substance accumulates, seizures result. Furthermore, the widely accepted idea that acetylcholine functions primarily as an excitatory transmitter in the central nervous system is supported by the fact that intracarotid, intraventricular or intracisternal injections, or the local application of this chemical to various exposed areas of the brain provokes grand mal seizures. This observation is further supported by the fact that inhibition of acetylcholinesterase led to the same effects on seizure threshold as did the exogenously applied acetylcholine. On the other hand, norepinephrine decreases seizure susceptibility and dopamine and serotonin appear to have anticonvulsant activity. Gamma-aminobutyrate (GABA) is of special interest in research on epilepsy because of abundant evidence that it may function as an inhibitory transmitter. Applied topically to the exposed cerebral cortex or injected into the carotid artery or the brain ventricles, it elevates thresholds for both chemically and electrically induced seizures. In this connection, a deficiency of vitamin B_6 (pyridoxine) has induced seizures by producing a deficiency of GABA. Also, a number of known convulsant toxins appear to interfere with the use of GABA in the body. Disturbance of electrolyte balance may be related to the common convulsion during fever in infancy. Information about the cause and correction of such disturbances may be applied to reduce the severity of recurrent epileptic attacks and to reduce the possibility of permanent brain injury which may be caused by such episodes.

Clinical solutions to the problem of epilepsy are concerned primarily with medical and surgical approaches. Before surgery is performed, it is necessary to ascertain that the seizures originate wholly or in part from an area of the temporal lobes that can be safely removed without causing serious neurological damage, the seizures occur frequently and are incapacitating, and presently available drugs are ineffective in controlling the seizures. Also,

the surgery must be accomplished so as to avoid creating a secondary scar which may in turn cause recurrence of the seizures. Recently, it has been found that barbituate and thiopental used in surgery can pinpoint areas of the diseased brain tissue responsible for epileptic seizures. Locating this tissue precisely permits it to be removed surgically.

Several investigators have confirmed the observation that sexual disturbances in man occur frequently in temporal lobe epilepsy and less so in other varieties of the disorder. The exception to this is the relatively rare instance in which anticonvulsants used in average therapeutic doses impair libido. It is widely held that anticonvulsants are often responsible for impotence. This may happen if the therapy is excessive. The majority of the patients with temporal lobe epilepsy and with hyposexuality regained their normal sexuality after operation. Attempts have been made to locate the precise region of the temporal lobe which may be disordered in the impotent patients. Several investigations have presented clinical evidence implicating the rhinencephalic structures and their connections in sexual disorders, and have produced convincing operative evidence that anterior temporal lobectomy may have a beneficial effect on sexual functions.

Hypothermia (90° - 92° F) as a tool in therapy of patients with acute cerebral lesions, such as cerebral contusions, cranial hemorrhage, tumors, and cardiac arrest has been tried. Patients that were in status epilepticus, or had intermittent seizures which could not be controlled by medication were completely relieved of attacks or at least were benefited by cooling. Local cooling of the epileptic brain resulted in the suppression of spiking activity which also facilitated its response to anticonvulsant drugs. From this study it was concluded that hypothermia is another treatment for problems of status epilepticus where drugs are not effective or are contraindicated. No complications were observed that could be attributed to the hypothermia.

The treatment of epilepsy has depended to a major extent on drugs. One of the earliest medications was a sedative called bromide. This was followed by another sedative, phenobarbital, which worked better, but caused drowsiness in some cases. About twenty years ago diphenlhydantoin (Dilantin) was introduced in the treatment of epilepsy. It has been widely used and has proved to be a valuable anticonvulsant drug. However, it was soon recognized that ataxia sometimes occurred as a complication of therapy with this drug. The ataxia may develop rapidly over a period of a few days, or insidiously over weeks or even months. One unexplained feature about this ataxia is the fact that occasionally a patient will rapidly develop ataxia in spite of having taken the same dose of Dilantin for several years. It has been thought that ataxia is a benign symptom only requiring a reduction in dosage or occasionally withdrawing of the drug. It was, however, later conclusively shown that cats subjected to Dilantin medication had developed severe loss of Purkinje cells in the cerebellum and cystic gliosis of the cerebellar white matter. A number of cases of permanent damage to the cerebellum, apparently due to this drug, have also been reported.

Further investigation on epileptic patients who had suffered Dilantin intoxication indicated a high level of CSF protein levels during the period of intoxication. All the patients improved when the drug was withdrawn, but

abnormal signs occasionally persisted for several months. Inhibition of the NA, K, Mg, ATPase enzyme system by Dilantin was observed which may be an important factor in causing neuronal damage. It is suggested that Dilantin intoxication may not be such a benign complication as was previously thought, but this still does not alter the fact that Dilantin is probably the most effective single drug presently available in the management of epilepsy. The need of potent drugs of low toxicity is still paramount, especially in psychomotor epilepsy. The use of Dilantin, phenobarbital, primidone, and mephenytoin (Mesantoin) has left a relatively large group of inadequately responding patients. Recently, considerable interest has been centered on the use of benzodiazepines in the treatment of epilepsy. Diazepam (Valium) I.V. has been proved to be effective in the interruption of epileptic seizures, particularly Status Epilepticus. Similarly, chlordiazepoxide (Librium) and especially nitrozepam (Mogadon) have been shown to be useful in prophylactic treatment.

Oxazepam (Serax) which is a metabolite of diazepam is easily absorbed from the intestines and is excreted quantitatively, or nearly so, as the glucuronide. Oxazepam has been widely used as an ataraxic because of the large safety ratio and the infrequent occurence of side effects (drowsiness, ataxia, skin rash, headache, etc.). Clinical trials made it clear that Serax is a potent drug in the treatment of psychomotor epilepsy and is of low toxicity compared to Dilantin and other anticonvulsant agents. The effect is seen not only in the reduction of seizure frequency, but also in the EEGs. The fact that his compound does not interact with Dilantin metabolism, facilitates its use in combination with Dilantin.

In order to establish relationships between biological activity and molecular structure, studies involving X-ray crystal structures are being undertaken on anticonvulsant drugs used in the treatment of grand mal epilepsy. Recent structural determinations of the clinically important anticonvulsants drugs, Dilantin and Valium, have shown that although these drugs are chemically unrelated, conformationally the two drugs have many similar features. It thus appears that their efficacies against grand mal epilepsy are primarily due to stereochemical features. Similarly the drugs, procyclidine and triexyphenidyl, newly important anticonvulsants, are being investigated by X-ray diffraction methods and their molecular structure is being compared in detail with Dilantin and Valium. Such biochemical and biophysical tools have provided new avenues for the synthesis of more efficacious and less toxic anticonvulsants.

Infectious Diseases

Infectious diseases of the nervous system include many types of illness caused by, or communicated by parasites, such as bacteria, protozoa, fungi or viruses. Studies of infectious diseases are primarily concerned with epidemiology and etiology of causative agents, their mode of transmission, host relationships, and possibly ways to control their propagation.

Methods most commonly employed for recognizing the presence of viral agents in cell cultures include; observations for cytopathic effects, hemaglutination, hemadsorption, and interference, fluorescence and electron microscopies. Immuno-fluorescent techniques are being used in diagnosing and studying brain

inflammation due to viruses. Other studies are concerned with experimental encephalitis, the epidemiology of Eastern equine encephalitis, the effects of parasites on the nervous system, testing vaccines for protection against arboviruses, and the possible role of viruses in acute neruological syndromes in children.

Criteria of the responses of the experimental animal to viral infection are established by careful monitoring of the physiologic behavior of the CNS following inoculation with tissue extracts and whole cells derived from brains of patients with chronic encephalopathies. The early events of the experimental disease in animals have been detected by electroencephalography because the EEG record becomes abnormal before any other signs of diseases appear. These techniques are being perfected for clinical application. However, at present it permits the selection of diseased animals for study during initial phases of the pathogenic process. Before drawing any definitive conclusions about the nature of the disease produced, especially about its relationship to the original human disease, it is well to remember that a CNS disease produced in animals may not be identical with the diseases of the patient from whom the tissue was obtained. A dramatic example of species difference in host response is the effect of simian virus B. In man it induces a disease that is severe, and usually fatal, whereas for monkeys this virus is innocuous.

A considerable amount of work is being done on (1) after effects of infections during pregnancy, where they may result in the offspring's brain damage and mental retardation; (2) analysis of viral polioencephalomyelitis in animals; (3) experimental measles encephalomyelitis; (4) mode and spread of a variety of neurotropic agents; and (5) so-called "slow virus" diseases of the nervous system. Several years ago a viral polioencephalomyelitis was identified in pigs. The virus has now been isolated from several different organs and has been shown to be an enterovirus quite unrelated to many other known viruses. All isolates but one produced polioencephalomyelitis in germ free pigs indistinguishable from naturally occurring infections.

Recently an agent causing paralysis of the CNS in rats has been isolated and is called hemorrhagic encephalitis of rats (HER). It produces acutely lethal encephalomyelitis when injected into suckling rats, including severe hemorrhagic lesions of the brain and spinal cord.

Subacute sclerosing panencephalitis (SSPE) is a degenerative neurologic disease of children and young adults. It is characterized by progressive mental and motor deterioration, myoclonic jerks, and coma. The patients become severely emaciated and die from intercurrent infections. The diagnosis established during the incipient stages often shows a personality disorder or mental retardation. At that time, the EEG shows slowing and dysrhythmia. However, high amplitude, low frequency synchronous waves do not develop until the patient exhibits myoclonic jerking. Spinal fluid proteins and cell counts remain normal or increase slightly during the entire course of the disease. Transmission of encephalomyelitis from humans to animals and further from animal to animals, producing symptoms typical of SSPE in the animal, has provided an important new lead in isolating and understanding the causative agent in SSPE. During the last few years, evidence of a relationship between SSPE and measles virus has been established. With the help of electron

microscopy, tubular structures have been seen in the brain with SSPE which resembled the nucleocapsides of measles virus. Further investigation showed a high or rising titer of measles antibody in serum, measles antibody in cerebrospinal fluid, and measles viral antigen in the brain of SSPE patients. On the basis of cytopathology, filtration, and serology, measles virus has been isolated from patients with SSPE. These patients had no history of clinical measles, but had received live measles virus vaccine; some had rubeola once in their life time. Several characteristics common to both measles virus and SSPE have been found. They include antigenic properties, host-range, cytopathogenic effects, temperature sensitivity, thermal inactivation and interferon production. Although basic similarities have been established, still unanswered are the questions of how and why the virus persists during the long period after the patient has recovered from measles and before he develops SSPE. Is the virus different in some way from regular rubeola? Will measles vaccine protect against SSPE? Is the isolated paramyxovirus a neuro-adapted strain of measles virus or a distinct but antigenically related virus? Is a second "helper" or "co-virus" necessary to produce disease? If two etiologic agents exist, how do they interact? How does virus reach the brain and spread within the CNS? How is it maintained for years if indeed it is measles virus? Are the unusually high serum measles antibody levels noted in most SSPE patients in response to viral antigen in brain and how is the virus protected from antibody? Does interferon play a role in limiting the disease? Is it possible to reactivate a latent or defective virus in the CNS?

Measles encephalitis is a disease characterized pathologically by demyelination. Evidence that the measles virus is directly responsible for the brain damaging effects has been inconclusive. This consisted primarily of the demonstration of cytoplasmic and nuclear inclusions, and small giant cells in brain material from fatal cases in various stages of the disease. Presently, research is directed towards the etiology of multiple sclerosis (MS) and the mechanism involved in its development. It seems likely that the measles virus is responsible for an acute demyelinating disease, known as "measles meningoencephalitis." It is postulated that MS may be a "slow" infection of the nervous system by measles virus, i.e., that post-measles encephalitis, SSPE and MS are different temporal manifestations of measles. It has been reported that MS patients have significantly higher serum measles antibody titers than patients with other neurological diseases or normal individuals. These are the only observations that provide a possible link between measles and MS at the present time.

The concept of slow viruses was originated by Sigurdsson in the year 1954 who recognized in Icelandic sheep a special category of diseases with prolonged incubation period, slow in clinical course, and restricted to a single organ and species. Rida (or scrapie), a chronic neurological disease, and maedi, a chronic pneumonia, were included. Their relevance to human diseases stems from the similarity of sheep scrapie to human kuru. Kuru and Creutzfeldt-Jakob Syndrome are two chronic human neurologic diseases. Kuru is a fatal neurologic illness restricted to the Fore people of New Guinea. It has been transmitted to chimpanzees. The transmissible agents involved have not been characterized and studies are limited because a susceptible small laboratory animal or tissue culture system has not been found.

Electron microscopy has revealed particles similar to viruses of the papova group in the brain from patients with progressive multifocal leucoencephalopathy. No evidence has been presented on the transmissibility of this disease. Visna virus is found in sheep and is also capable of rapid growth in tissue culture, but pathogenesis in vivo is slow. Aleutian disease of mink is also considered a slow virus.

Of all the slow viruses implicated in acute, subacute and chronic neuropathies, scrapie is the only virus studied extensively because of the relative speed and economy of using mice. Reliable criteria of its induction have been established. Mice in advanced scrapie, when suspended by the tail, characteristically clasp their hind legs together, whereas normal mice splay theirs outwards. During the developmental stages, mice exhibit a sequence of responses reflecting progressive central nervous system damage before the clasping reaction is manifested. These studies have also generated many of the seeming paradoxes of scrapie virus, such as its size relative to its molecular weight and apparent absence of any nucleic acid. Hypotheses explaining the special features of the scrapie agent include persisting tolerated infection; defective virus; special auto-immune response; and a replicating polypeptide, polysaccharide or membrane defect. Directly or indirectly these concepts have provided rational bases for research. For example, if scrapie is a defective virus, Sendai virus may be acting as a helper in the accelerated course observed. If scrapie represents a membrane defect, it may be visible by scanning electron microscopy. Scrapie virus has been successfully grown in brain cell cultures of sheep, mouse, and mink. All workers have observed differences in vigor and appearance between infected and normal brain cells in vitro. Biochemical differences associated with scrapie have also been reported. Increases in enzyme activity, such as glucosaminidase, offer the possibility of quantitative assay using florescent-tagged enzyme substrates and detecting the split produced with ultraviolet microscopy. Mice inoculated with scrapie virus showed increased DNA metabolism. Inhibitors of DNA metabolism (idoxuridine and hydroxyurea) did not alter the course of disease. Interferon has also been studied in scrapie. Extrinsic interferon, injected peripherally, had no effect. Other studies showed that scrapie neither induced interferon nor prevented its induction by viruses. Studies with poliomyelitis and a crude predecessor of statolon, an interferon inducer, showed that peripheral treatment was active against peripherally inoculated polio virus, but not against polio virus inoculated intracerebrally unless treatment was also given intracerebrally. Experiments currently in progress indicate that scrapie can be altered in vivo. Test materials have been selected for their relation to interferon, immunity, DNA metabolism, and antiviral or other therapeutic activity. No effect on survival was noted with adenine arabinoside, started the date before scrapie inoculation and continued throughout the course of disease. Tentative findings indicate that short-term amantadine treatment late in the disease did not reverse its neurologic effect. The results of cyclophoshamide treatment varied depending on the time of initiation. Recently, scrapie virus has been found in sheep brain inoculated with SSPE and MS infected brain tissue. Also, scrapie-like illness in mice inoculated with MS brain has been observed. Acceptance of the importance of these reports awaits clear evidence that an agent responsible for the disease in sheep and mice had its origin in the MS tissue and not in the experimental animals or their surroundings.

Current studies are directed towards the development of new methods of evaluating scrapie in vivo and in vitro, increased understanding of the disease and etiologic agent associated with scrapie, its related neurologic disorders, and the means of altering them.

Myxoviruses are medium sized, ether sensitive RNA viruses, which in man have the common effect of causing respiratory disease. Although they are not regarded as "neurotrophic" viruses in man, mumps is the most common virus causing central nervous system infection in rodents. In these studies occasional neurons are infected by the unadapted strain, and repeated passage has yielded a strain which infects parenchymal cells of the brain, causing acute encephalitis. However, mumps virus in hamsters has been shown to cause a clinically inapparent infection limited largely to ependymal cells and neurons, resulting in acute encephalitis.

Hydrocephalus may be caused by infectious agents contracted prior to birth. Suckling hamsters infected by mumps virus developed a narrowing of the aqueduct of Sylvius with subsequent hydrocephalus. The narrowing of this canal, responsible for draining the ventricles of the brain, is the most common cause of hydrocephalus in man.

The study was conducted with experimental models to determine the histopathological changes in the brain, aqueductal narrowing, and hydrocephalus induction. The signs of clinical disease developed only after the resolution of the acute infection (about fourteen days), during which the lining cells of the aqueduct had been almost destroyed. Of the suckling hamsters inoculated with mumps virus, ninety-five percent developed clinical hydrocephalus. These and other histological changes were shown to be specifically related to the mumps virus infection.

Although studies of experimental infections indicate that viruses may reach the nervous system by various routes, studies of naturally occurring infections indicate that the major pathway of virus spread to the nervous system is by the hematogenous route. The various humoral and cellular defenses which act as deterrents to limit the occurrence, intensity and duration of viremia, as well as subsequent spread to the nervous system, constitute a physiologic blood-brain barrier which must be overcome if infection of the nervous system is to occur. It has been shown that herpes simplex virus gains access to the central nervous system by the hematogenous route. Since blood contains both phagocytic and immuno-competent cells, the extent and duration of hematogenous dissemination of virus depends upon the outcome of interactions between host defenses and virus. The characteristics of herpes simplex virus are: (1) it is a DNA virus whose replication is susceptible cells is generally associated with formation of easily demonstrable intranuclear inclusions; (2) it is associated with viremia and central nervous system disease in both man and animals; (3) it can cause acute and subclinical infections; (4) it may persist in the tissue in occult form for prolonged periods, giving rise to exacerbations of disease following a variety of stimuli and; (5) it has been associated with chronic and recurrent encephalitis in the rabbit, suggesting that it may also be responsible for such illness in man. From these studies it has been ascertained that human white cells, specifically lymphocytes will support the replication of herpes simplex virus in vitro. This was the first DNA virus to be propagated in

human leukocytes. Viral replication failed to occur in the absence of phytohemagglutinin, suggesting that lymphocytes must be in a particular growth phase to be susceptible to infection with this agent.

Work with the Sindbis encephalitis virus in mice demonstrated a precipitous development of resistence during the second week of life, due apparently to a limitation in the spread of infection. No non-specific viral inhibitors could be detected. Studies on mumps virus encephalitis in hamsters showed that disease and death resulted from the infection of neurons which remained morphologically normal. It is suggested that this material may be useful in investigating the "slow" viruses which seem to act in a similar way.

In Schilder's encephaloclastic sclerosis, virus-like particles were identified by electron microscopy which are distinctly different from those observed in the case with herpes simplex encephalomyelitis, but are similar to those that occur in subacute sclerosing leukoencephalitis. The nature of these virus-like particles is being investigated.

In 1958 there appeared the first report that free-living ameba could produce fatal meningoencephalitis in animals. More recently a death of a boy was reported who died five days after initiating the symptoms now being recognized as typical of amebic nemingoencephalitis (AME). It is believed that the invading ameba, Naegleria gruberi, enters the mouth and nose, possibly during swimming. The victim soon suffers flu-like symptoms followed by rapid deterioration and death. Unlike most of the parasites that live with man, Naegleria, as a free-living organism, requires adequate oxygen. This may explain why it has a great affinity for the oxygen-rich capillaries of lung and brain tissue. The disease appears to be fatal and is without response to anti-amebic drug therapy. Virus-like particles measuring about 100 mm in diameter have been observed in some strains of these invading pathogens and their morphological characteristics have been recorded. Preliminary experiments indicate that mice are killed by introducing axenic EG ameba either intracranially or interanasally. Other studies involving isolation, purification, characterization of the active agent are currently underway.

In 1943 and 1944 a virus was recovered from mosquitoes in the San Joaquin Valley of California. Until 1965 virtually nothing was known about its disease-causing potential in humans when the recovery of a representative strain of this virus was reported from the brain of a child with a fatal illness. It was apparent from serologic studies that many patients examined were infected with California encephalitis virus coincident with rather severe illnesses. These patients were studie very carefully to exclude other known causes of viral CNS diseases. All patients had encephalitic signs and symptoms different from those due to enteroviruses. In contrast to enteroviral illnesses, none of the patients in this study had a biphasic illness, none were associated with upper respiratory of gastroenteric signs or symptoms, and no illnesses occurred in siblings or other family contacts of the patients. Moreover, seizures, coma, and disorientation were more frequent in the patients in this study in comparison with children with CNS infection due to enteroviruses. Behavioral correlaries of patients suffering with this virus showed difficulty in receiving consistently meaningful, basic visual and auditory perceptual information. They had little or no difficulty in the higher language functions.

The personality of these children seemed to fit the "organic hyperkinetic syndrome." The result is a predictable behavior pattern seemingly associated with specific learning disorder.

Tumor C1300 has been in serial transplantation since 1940 at the Jackson Laboratory, Bar Harbor, Maine. This tumor arose spontaneously from the region of the spinal cord of a albino mouse and was diagnosed tentatively as neuroblastoma. Until recently no definite proof existed regarding its neuronal characteristics. One of the characteristic enzymes of nerve cells, tyrosine hydroxylase, is present in tumor C1300, confirming the diagnosis of neuroblastoma. Recently it has been demonstrated that cell-free extract (CFE) of neuroblastoma tumor induces a similar neoplasm in the dermis, mesentery, spinal cord and sympathetic chain after subcutaneous injection. A total of 70 percent of CFE-injected mice developed neuroblastoma tumors. Like tissue-transplanted tumors, CFE-induced tumors possess a similar amount of tyrosine hydroxylase and, in vitro, differentiate into neuron-like structures. Electron microscopic studies show that virus-like particles are present in CFE- and tissue-induced neuroblastoma. The viruses are doughnut-shaped and appear to bud from endoplasmic reticulum. Whether these viruses are causative agents in producing neuroblastoma by CFE remains to be ascertained. The following studies using partially-purified neuroblastoma viruses are in progress: (1) viral etiology of neuroblastoma; (2) induction of neuroblastoma using different routes of injection; (3) induction of neuroblastoma in different species; (4) deomonstration of the type of virus, DNA vs. RNA; (5) transformation study in vitro using different mammalian cell-lines; (6) selection of a cell-line which sustains viral multiplication without transformation; (7) exploration of the presence of viruses in human neuroblastoma; and (8) the induction of tumor using cell-free extract of glioma, medulloblastoma, astrocytoma and neurofibromatosis. The animals and human tumors will be used for this purpose.

FUNDAMENTAL RESEARCH IN THE NEUROLOGICAL SCIENCES

Introduction

The great importance of fundamental studies on neurological, sensory, and communicative components is frequently overlooked because they may have little or no immediate clinical application. However, the history of science shows clearly that improvements in the treatment of disorders are largely dependent on sound basic research.

It is generally accepted that the membrane around a nerve fiber plays an essential role in the transmission of the nerve impulse along the fiber. However, relatively little is known about the structure and mechanism of action of the nerve membrane or of living membranes in general. There is no doubt that research on membrane structure will contribute important information about how the nerve impulse is transmitted along the nerve fiber . There is a substance known as the nerve growth factor, the exact composition and source of which is still uncertain, which has a remarkable stimulatory effect on the growth of nerve fibers. The ultimate importance of the factor is still to be determined, but it surely will have important functions in nerve regeneration and it may well turn out to be useful in healing lesions and restoring function in the brain and spinal cord.

There are several anti-convulsant drugs that are relatively effective in controlling seizures in a good proportion of epileptic patients. Some of these drugs are quite unrelated chemically and there is no explanation why they are effective and why closely similar compounds are entirely ineffective. It has been suggested that the efficacy of a drug may depend upon a highly specific molecular structure rather than on the chemical composition of the molecule. Therefore, studies on molecular structure are now highly important and involve such sophisticated techniques as x-ray crystallography, electron paramagnetic resonance spectroscopy, and nuclear magnetic resonance spectroscopy. On the basis of such highly specialized information on the atomic interrelationships and the electron activity in the effective molecules, it will be possible to find or synthesize other molecules that are more effective in the treatment of disease. It may also lead to an understanding of how the present drugs work, which often is completely unknown now.

One of the great unknowns in neurophysiology has been the exact mechanism by which the nerve impulse is transmitted across the synapse from one nerve fiber to the next or from a nerve to a muscle or other end organ. In 1904 it was suggested that some nerves accomplished this by the liberation of epinephrine. In 1914 another investigator suggested that acetylcholine was the synaptic transmitter in some cases. These postulates were finally proven in 1921 by the classical experiment in which two frog hearts were connected by a small glass tube. When the beat of one heart was slowed by stimulating the vagus nerve, the beat of the other heart also slowed a few seconds later, demonstrating conclusively that some chemical was produced by the nerve in the first heart which was carried in the perfusion fluid and produced a similar effect in the completely separate second heart. It is now known that a number of compounds (acetylcholine, noradrenaline, dopamine, 5-hydroxytryptamine, gamma-aminobutyric acid, glutamic acid, glycine) may act as synaptic transmitters. However, the mechanism by which the transmitter may be produced and act very rapidly

(many times per second) and how the receptor nerve receives the stimulations is still largely unknown. As mentioned at the beginning of this Report, there are 10 to 13 billion nerve cells in the brain alone. Each then connects with from two to several other nerve cells. Therefore, the number of synapses is almost infinite and research on synaptic transmission is of the utmost importance. Also, this is a good example of why the application of basic research must be a time consuming process. The first evidence of the chemical transmission across synapses was obtained more than 65 years ago. However, the development of the area, even to its present unresolved state, had to await the discovery of highly sophisticated techniques such as electron microscopy and methods of chemical analysis sensitive enough to detect literally only a few molecules of a specific compound.

The Nerve Membrane

One team of investigators has used spin labels (paramagnetic free radical probes) to detect inside-outside conformational transitions of specific spin-labeled lipids and proteins in biological membranes. A variety of spin labels have been synthesized and they are designed so they may be directed toward a special region of the membrane or may serve as analogues of natural membrane components. It was found that in membranes, particularly nerve and erythrocyte, the motion of a series of spin-labeled fatty acid labels leads to a quantitative estimate of the amounts of gauche and trans conformations about carbon-carbon bonds of the fatty acid chains of phospholipids. Analysis of the magnetic resonance spectra showed that the preferred orientation of the amphiphilic labels I and II in nerve fibers was that in which the long molecular axis is perpendicular to the membrane surface. The use of spin-labels in nerves has led to the development of a new theory of anesthesia. The magnetic resonance spectra of lipid labels have revealed the fluidization of membranes by local anesthetics and the tightening of the membrane in the presence of cholesterol and antibiotics. ATPase of the cerebral cortex cell membranes is presumably part of the active sodium potassium pump of the membranes. The fact that biogenic amines such as serotonin and norepinephrine affect enzyme activity and that the ATPase is found primarily in the synaptic region suggests that, in addition to its function in maintaining sodium and potassium gradients, it may be involved in nerve impulse initiation.

Another project is working toward providing a mathematical physical-chemical theory of the activity of the axonal membrane and thereby to correlate observed membrane phenomena and predict new phenomena. Further objectives are to provide a theory of nerve activation by transmitter substances, to assist in understanding the action of pharmacological agents, and possibly to explain cyclical nerve cell activity such as is detected by the EEG. The project is designed to show that the excitability of membranes results from classical physical-chemical forces, and does not depend upon any unusual specialized structures, such as specific ion species channels, or special field-sensitive gates. Some years ago it was shown elsewhere that the activity cycle consisted first of all of a large increase in the inward Na^+ current followed by an increase in outward K^+ flow. However, no attempt was made to provide a theoretical foundation for the empirical equation obtained since this was felt to be beyond the available physical knowledge at this time.

The problem is two-fold; the calculation of the steady state ion distribution, and the transient. The first requires the solution of the total differential equation two-boundary problem, and the second, the PDE two-value boundary problem. The total DE solution gives the steady state, from which the activity cycle commences, and the PDE solution gives the activity cycle. Both problems were much more complex than the investigators anticipated. Much of the effort during the first two years was required to simply develop the necessary numerical methods.

In this "model" of an excitable membrane, it has been shown that the important factors are the height of the potential barriers for the ions to traverse the two interfaces, the effect of the local electric field on these barriers, and the blocking effect of absorbed CA++. A high negative charge concentration near the outer surface of the membrane was found to be of importance in giving the local field necessary at the interface. An interesting and suggestive finding was the existence of oscillatory currents following a disturbance. Their magnitude depends on factors yet to be investigated. However, it appears that some clue to the source of EEG rhythms may be found.

Nerve Growth Factor

More than two decades ago it was observed that a crude extract of mouse submaxillary gland could produce an explosive growth when applied to cultured ganglion cells. Several years later a protein was isolated and purified which possessed the characteristics of nerve growth factor(NGF). It was determined that the growth promoting factor was present in lower concentrations in a number of other tissues, and that the extract of mouse salivary gland contained a number of proteins other than NGF. Most notable among the latter is a protein that specifically stimulates the growth of epithelial cells. Still other growth factors may be identified in the future. The chemical and biological inter-relationships among the various substances isolated from the mouse submaxillary gland are both complex and uncertain since chemical constitution and biological activity are closely dependent on the isolation procedures employed. However, it is now generally agreed that the simplest NGF protein extractable from the gland is relatively small with a molecular weight of 25,000 and is very basic. A larger species with NGF activity is a combination of this basic protein with two other types of subunits, one of which is an esteropeptidase. Whether or not other preparations with NGF activity are actually distinct from these two, has yet to be determined. In any case biological activity as reflected by neurite growth in tissue culture can be obtained with most preparations at a concentration of 10^{-7} grams/mililiter.

The mechanism of NGF activity is being pursued intensively in a number of laboratories utilizing modern techniques of biochemistry and cell physiology. We may soon know whether the NGF protein acts on the nerve membrane or moves through it, how cellular synthesis of nucleic acid and protein is initiated by NGF and how NGF inactivates the mechanisms which control cell size, shape and volume. As may be expected a number of laboratories are investigating the effects of NGF on regeneration in the nervous system.

Structural Studies of Drugs

By methods of single crystal structure determination, using X-ray and

neutron diffraction, one project is studying the structural details of the way in which barbiturate molecules associate with each other and with molecular species of biological systems, such as water, peptides and lipids. The use of barbiturates in neurological disorders is well known. It has been assumed that for effective action a barbiturate, initially in an aqueous medium, must be capable of associating with the lipids of a biological membrane to modify the normal metabolic cellular processes such as membrane transport. A related possibility is that the barbiturate may directly inhibit the complex enzymatic processes of oxidative phosphorylation. It is now believed that the multi-enzyme system in electron transport is effectively blocked by barbiturate in the area of a flavoprotein.

The detailed crystal structures of some 20 barbiturate derivatives have been determined and tested in chemical and/or biological systems. It was found that the barbiturate ring is planar, apart from slight distortions depending on the crystal environment. Exceptions are found in the "half chair" conformation of 5, 5-disubstituted barbiturates in which at least one substituent is hydroxyl. With one exception (alloxan), all potentially hydrogen bonding hydrogen atoms were found to be hydrogen bonded.

There was a strong preference for barbiturate imine groups to hydrogen-bond with carbonyl groups of other barbiturate rings rather than with C(5) substituent groups or water molecules. Almost all barbiturate structures thus exhibited a one- or two-dimensional framework of NH...O-C hydrogen bonded barbiturate rings which appears to be the dominant crystal structure determining activity. Although the nature of the C(5) substituents and the possible presence of water of crystallization determine the manner of ring hydrogen bonding to form this framework, their own hydrogen bonding requirements appeared to be of secondary importance. The latter may frequently be of the "bent" or "bifurcated" type, indicating relatively weak hydrogen bonding interactions.

The overall aim of another project is to determine the conformations of acetylcholine (ACh) when it becomes attached to its nicotinic and its muscarinic receptors. The approach was to synthesize analogues of ACh in which a degree of rigidity was introduced into the amino alcohol portion of the ester. Cyclopropane and cyclobutane rings were used to impart the desired rigidity in the ACh analogues and congeners. Free rotation was thus prevented, and the number of possible conformers was greatly reduced. The overall shape of the molecules and pertinent ranges of interatomic distances could then be estimated. This work is based on the premise that the conformation and molecular shape of ACh are critical factors in its actions at the two types of cholinergic receptors.

Some 50 analogues of ACh have been synthesized, particularly those in which the amino alcohol portion is a part of a cyclopropane or a cyclobutane ring system. Each of the enantiomers of trans-2-acetoxycyclopropyltrimethylammonium iodide has been prepared. The absolute configurations have been determined and have been correlated with biological data. A new approach to the synthesis of cis-2-acetoxycyclopropyltrimethylammonium has been developed which may permit higher yields of the compound.

In the studies on the cyclobutane series, cyclobutanecarboxamides have

been converted to cyclobutylamines with lead tetraacetate. 2-Substituted cyclobutylmethyl ketones have been prepared from 2-substituted cyclobutanecarbonyl chlorides, and these ketones have been shown to undergo a facile Baeyer-Villiger reaction to give high yields of cyclobutyl acetates.

Despite its relative chemical simplicity and its importance as a neurotransmitter, the mode of action of ACh is still unknown. A logical hypothesis is that the flexible ACh molecule assumes different molecular shapes and different interatomic distances at the nicotinic and the muscarine reptors, conditions which are imposed on the ACh molecule by the chemical and steric nature of the receptor area. With the synthesis and testing of ACh analogues, this project is trying to clarify the mode of stereo-chemical attachment of ACh at its nicotinic and muscorinic receptor sites and in this way truly explain the mechanism of action of ACh.

The Synapse and Neurotransmitters

The identification of synaptic transmitter compounds and the mechanisms by which lobster nerve cells regulate their accumulation has been studied extensively in one laboratory. Early work showed that gamma-aminobutyric acid (GABA) was the inhibitory transmitter at the neuromuscular junction, that it was the most active inhibitory substance present in nerve extracts, and was the only physiologically active compound selectively localized in inhibitory neurons. Glutamate was the only excitatory substance found.

It has now been shown that GABA is actually released when an inhibitory neuron is stimulated and the amount released is proportional to the number of stimuli applied. Like the release of chemical transmitters at other synapeses, it depended on the presence of calcium in the bathing fluid. Recently, it has been possible to localize the site of GABA uptake in lobster nerve-muscle preparations. Certain aldehyde fixatives will covalently bond amino acids to tissue components. With this method, using H^3 labeled GABA, the site of uptake was shown to be the connective tissue cells and Schwann cells that surround the nerve-muscle preparation.

Using single excitatory and inhibitory axons, enzymic studies showed that the pathway of GABA metabolism in the lobster was identical to that in the mammalian nervous system. The substrates, glutamate and alpha-ketoglutarate, were present in the same concentrations in excitatory and inhibitory axons, and transaminase activity was similar in both kinds of cells. Only decarboxylase (and GABA) was different, with about 100 times as much activity in the inhibitory axon. It was found that high concentrations of GABA could inhibit the decarboxylase. At the normal concentrations of GABA and glutamate in inhibitory axons, the synthesis of GABA approximately balanced its destruction. This led to a new mechanism to explain the selective accumulation of GABA in inhibitory axons, with the decarboxylase being the key to accumulation and the GABA inhibition of the decarboxylase the key to the final level to which GABA accumulated.

In recent years, glutamate has been thought to be the excitatory transmitter compound. After a prolonged series of experiments, it was possible to demonstrate a small (10%) release of glutamate after excitatory nerve

stimulation. The lack of adequate methods made it impractical to try to obtain the necessary controls of calcium dependence on release or the proportionality between frequency of stimulation and amount of glutamate released. Quantitative comparisons of glutamate in excitatory axon extracts and the amount of excitatory activity in glutamate units in the extracts showed that there is about 2 to 3 times more activity than glutamate. After much work, it was shown that most of the excess activity could be accounted for as asparate. Asparate alone is relatively ineffective, but it can add to the excitatory effect of glutamate, causing an apparent potentiation of the glutamate response.

Studies on the post-tetanic potentiation (PTP) and post-tetanic repetition (PTR) are particularly important because these responses disclose the activity of motor nerve terminals. By this mechanism, another laboratory has studied the effects of many compounds on the soleus motor nerve terminals (MNTS) in vivo. Using increasing doses of d-tubocurarine (dTC), it was found that PTR and PTP were progressively suppressed and finally obliterated. It is impressive that PTR and PTP were abolished by a curare (dTC) dose that had only a minimal effect on neuromuscular transmission. This showed that the MNTS were a highly sensitive locus of dTC action, but that this in itself was not the source of the neuromuscular blocking action of curare. Nevertheless, this effect suggests that the pre-junctional structure may still be important in the action of depolarizing neuromuscular blocking drugs.

Because of its importance in neuromuscular studies, Mg was investigated. It was found that Mg also depressed the PTP and PTR. This demonstrates that the action of Mg is on the MNTS. The pre-junctional effect of Mg, like the dTC action, preceded the effect on transmission. Also, it was found that transmission block and depression of directly stimulated muscle coincided. Thus, Mg depressed both muscle and MNTS, whereas dTC acted selectively on MNTS.

The local anesthetics, procaine, lidocaine, tetracaine, and debucaine had been shown by other investigators to suppress PTP and PTR. Studies on this project showed that the respective potencies of these drugs to depress MNTS correlate exactly with the order of their condution blocking action on peripheral nerve. This result is important because analysis of procaine action on neuromuscular transmission by in vitro quantal methods by other workers had indicated that the major action is curariform on the post-junctional membrane. No doubt these drugs, like Mg, will affect excitable tissues non-selectively, but at the neuromuscular junction the first effect, as dose is increased, is the paralysis of MNTS.

Diphenylhydantoin (DPH), a drug used in epilepsy, was known to suppress PTP of the monosynaptic reflex pathway. Therefore, its effect was studied on the hyperpolarization of MNTS that follows high frequency stimulation. Anticonvulsant doses were found to suppress MNTS PTR and consequently the muscle PTP. Apparently, DPH opposes the hyperpolarization of MNTS just as it does in the primary afferent endings in the 2N pathway. The DPH action on post-tetanic hyperpolarization indicates that the drug selectively suppresses after-potential production in the terminals.

All of this work has been done in vivo and, therefore, the results are

unquestionably relevant to clinical neuropharmacology. Also, the actions of the various drugs classically associated with modification of neuromuscular function have been seen in a new light and new understanding of their action has been developed.

With the use of electron microscopy and semi-quantitative microscopic spectrofluorometry, a team of investigators has been studying the function of neurohumors such as serotonin (5-HT), nor-epinephrine (NE) and acetylcholine (ACh). It was possible to demonstrate a relationship between the dense-core, or granular, nerve ending vesicles and a storage form of NE in the rat vas deferens. Vas deferens depleted of endogenous NE were exposed to NE or to one of its precursors, DOPA or dopamine, in the presence of an inhibitor of the conversion of precursors to NE. Only NE specifically restored the proportion of granular vesicles to nearly normal levels. Thus, the granular vesicles were identified as the intra-vesicular compartment of NE storage. In the presence of an inhibitor of monoamine oxidase iproniazid, exogenous NE accumulated in the extravesicular compartment, but did not enter the granular vesicles. Under these circumstances, the contractions of the vas deferens usually produced by stimulation of the nerve were markedly inhibited, presumably due to excessive NE in the extravesicular compartment. It is clear from these studies that the distribution of NE can be identified in its storage sites at the subcellular level and that the subcellular localization of the amine influences physiological and pharmacological effects.

This group has also studied neurohumors in the rat brain. They showed that gamma-hydroxybuturic acid lactone (GBL) induced anesthesia, but had no influence on 5-HT and gamma-aminobytyric acid (GABA) levels in the brain, and like all central nervous depressants, produced an increase in brain ACh. In regard to effects on brain catecholamines, neither gamma-hydroxybutyric acid (GHB) nor GBL altered brain NE levels, but they did selectively increase brain dopamine (DA) levels, occasionally as much as 100%. In addition gamma-methylparatyrosine, which blocks the synthesis of both NE and DA, caused about 100% increase in the sleep time of animals given GHB or GBL. This indicates that DA may be necessary for the arousal of an animal from a sleeping state. The increase in DA during GHB-induced sleep may be due to a reduced utilization of DA or to a compensatory activation of synthesis as a result of suppression of dopamine receptors by the GHB. Thus, a study of the ultramicroscopic localization of neurotransmitters may make important contributions to knowledge of sleep mechanisms, a problem of utmost importance in itself.

COMMUNICATIVE SCIENCES

In communication, language is the carrier of the vast amount of what we call culture. Knowledge of the past, techniques of food getting, science, and social rituals are all carried in language. The social psychologist tells us that language is important first as it relates to communication and secondly as it functions in the socialization of the individual, particularly in the development of his personality. Moreover, it carries for the person the social definitions of situations, the world of discourse and the whole range of culture content which impinges upon and shapes the individual. The National Institute of Neurological Diseases and Stroke is pleased to report many important advances in knowledge in the Communicative Sciences this past year.

Sensory Processes

A physiological and psychophysical investigation in three sense modalities: audition, touch and vision, complemented by analytical procedures involving mathematics, electrical network modeling and digital computers, has extended the analogy between auditory and tactile psychophysical characteristics to cover several aspects in the frequency, time and space domain at threshold, as well as at suprathreshold levels. The generalized functional relationship between the firing rate produced by sensory reception and stimulus intensity, which was discovered in the previous year and which seems to hold independent of sense modality and animal species, was further documented with the help of neurophysiological experiments on the Limulus lateral eye and the auditory organ of Southern Army Worm Moths. A theorem resulting from the mathematical formation of the relationship was confirmed by studying summation between sinusoid and a band limited random noise. In audition measurements of central masking characteristics have continued, and the psychophysiological theory of central masking developed in the previous year was further validated. In electrophysiological investigations of the auditory receptors of the Southern Army Worm Moth, it was possible to infer the principal site of mechanical stimulation. In tactile studies, it could be demonstrated that the slope of the subjective intensity functions is negatively correlated with the threshhold intensity and the density of innervation. Sensory studies continued to focus on the Limulus lateral eye. The neurophysiological recordings from single receptor units have brought out a nonlinear interaction between excitatory and inhibitory processes and have made it necessary to introduce a nonlinear correction factor into the Hartline-Ratliff equations.

Another investigator, attempting to determine the extent to which evidence for the Stevens power law shows itself in the recorded potentials from sensory cells, determined that in numerous sense modalities the neuroelectric potentials can be shown to grow as a power function of stimulus intensity. The power function exponents for two modalities, vibration and hearing, have exhibited an interesting dependence on frequency.

Auditory Masking

In attempting to identify differences between sinusoid and noise masking,

an investigator developed a new hypothesis which accounts for some of the differences. The observer, faced with the problem of trying to detect a weak sinusoid in some masking stimulus, searches the frequency spectrum and listens primarily at the region in frequency where the signal - to - masker ratio is most favorable. In detecting a sinusoid in a white noise spectrum, there is no problem. The maximum signal - to - noise ratio occurs at the signal frequency, and asserting that the observer listens primarily at that frequency, is simply the statement of the critical band hypothesis. Detecting a gated sinusoid in a continuous sinusoidal masker is a different problem. The mere fact that a signal is turned on and off implies presence of energy in regions of the spectrum other than the signal frequency. The amount of this splattered energy depends on the exact shaping employed in the process of gating the signal. The ratio of signal - to - masker energy depends both on how the signal is gated and how much the masker is attenuated by the observer's auditory filter. The critical item is not the rise time of the gating filter, but the off-band attenuation rate of the auditory filter.

Vestibular Mechanisms

One group of researchers developed an analog equivalent electrical circuit of the cochlea that follows the principles of the electromechanical hypothesis for the function of the organ of Corti. The steady-state parameters of this heuristic electrical model were calculated from data about the impedance and potential differences that exist across the walls of the cochlear partition. The behavior of the model is such that it replicates many observations obtained during experiments on the cochleas of guinea pigs. The model affords a qualitative as well as quantitative approach to the study of the direction, magnitude, and source of electric currents flowing through the inner ear during acoustic stimulation. A series of experimental investigations on optokinetic nystagmus revealed that the oculomotor responses to optokinetic stimulation of rabbits, cats and man were qualitatively similar when studied by electronystagmographic methods. There were, however, some noticeable quantitative differences. The frequency of nystagmus increased as the velocity of the stimulus was made greater. For comparable velocities, the frequency of the nystagmus in the rabbit is smaller than that of the cat and of the "stare" nystagmus in man, but is larger than that of the "look" nystagmus in man. The greater effectiveness of the optokinetic stimulus when presented monocularly and moving in the usual field from lateral to medial direction depends on the existence of a large number of ganglion cells in the retina that are preferentially stimulated by visual targets moving in that direction.

Another group of investigators described the discharge characteristics of peripheral vestibular neurons innervating each of the semicircular canals in the squirrel monkey. The average resting discharge was about 90 spikes/second. All neurons responded to angular accelerations in one direction with an increase in the discharge and to oppositely directed accelerations with a decrease. The response was always consistent with the morphological polarization of the hair cells. Many units did not adapt and their response to constant accelerations resembled the response predicted by the torsion-pendulum model. This and two other studies on the physiology of peripheral neurons innervating the otolithic organs of the squirrel monkey produced findings that

reinforced to a certain extent the current theory on the mechanics of the semicircular canals (overdamped torsion-pendulum model) and also provided a basis supporting various observations in man and animals, which deviated or are not predicted from theoretical considerations. Further, these studies may help to interpret the response of semicircular canals when exposed to rotatory or caloric stimulation as practiced in the clinic.

Cochlear Mechanisms

The cochlear potentials (cochlear microphonics, summating potential and endocochlear potential) as recorded inside the cochlear duct showed much less variability than when these potentials were recorded by one investigator in the perilymphatic spaces. The magnitude of the cochlear microphonics recorded in the same place in the cochlear duct showed a range of less than ± 3 dB. around the mean. At the same time the magnitude of the cochlear microphonic for different species varied as a function of frequency in a manner that is different in different animals. Cats showed the greatest resolution in their tuning curve. The summating potential behaved similarly among different animals of the same species, but also showed quantitative differences among species.

Another investigator working on cochlear hair cell metabolism and cochlear potentials found that scala tympani resting pressure in the anesthetized guinea pig was usually slightly higher than atmospheric pressure, the greatest value measured being 10 mm Hg. with an average of 6 mm Hg. The scala typmani pressure rose markedly during breathing of low oxygen concentrations and the scala vestibuli pressure changed in the same direction as scala tympani pressure during anoxemia. Finally, the scala tympani pressure during anoxemia followed a rise of cerebral spinal fluid pressure. It was also noted that intravenously administered dinitrophenol caused an increase of cochlear endolymphatic dc potential, in some cases 15 to 20 percent.

Inner Ear

A group involved in microscopic studies of the inner ear reported pathological changes produced by age, disease, drugs, and noise. They found that interest tends to focus more and more on tissues, such as the stria vascularis and spiral ligament, that provide by their metabolic activity the special homeostatic controls that the organ of corti requires for its functioning, and the blood vessels supplying those tissues. The degenerative changes that occur in Corti's organ itself tend to follow a more or less stereotyped pattern, regardless of their cause, and the sequence of changes, especially as they affect the hair cells, is now well established. In guinea pigs treated with Kanamycin they found there is an increase in the number of avascular channels of the spiral ligament above Reissner's membrane, indicating a loss of capillaries. Kanamycin causes not only loss of capillaries but changes in the cells of the stria, especially the intermediate or chromophobe cells. Exposure to white noise for 6 to 30 hours at 120 dB. produces capillary vasoconstriction in the spiral ligament and in the vas spiral system, as well as vacuolization of Reissner's membrane. The hypothesis is that capillary vasoconstriction is the primary result of noise exposure associated with the temporary elevation of auditory threshold. Similar vascular changes are caused by quinine and

salicylates. If long continued, the ischemia causes hair cell loss and permanent threshold shifts.

Another group engaged in the chronological development and accumulation of acid mucopolysaccharide and various enzymes in the endochrondrol model of rodent temporal bones spent this year working out techniques which will allow them to assess more critically acid mucopolysaccharides, phosphatases, phosphorylase and non-specific esterase in the otic capsule. The specific activity of these enzymes are also being investigated with controlled chemical procedures including colorimetric determination of the various enzyme systems.

Hearing Disorders

Investigators concerned with determining the characteristics of very low frequency auditory sensitivity observed that the rate at which auditory sensitivity (as determined by microphonic potentials) decreases with the decrease of stimulus frequency below approximately 100 Hz is apparently species dependent. A combined anatomical and electrophysiological study was completed on four species: cat, guinea pig, chinchilla, and kangaroo rat. It was demonstrated that the sensitivity change below 100 Hz is 12 dB./octave for cat and chinchilla, while it is only 6 dB./octave for guinea pig and kangaroo rat. Concomitant low frequency cochlear microphonic phase differences were also seen. They have shown that it is the size of the heliotrema that is primarily responsible for the differences. The area of this opening is approximately ten times greater in the cat and chinchilla than in the other two species.

Comparative Hearing

A group of investigators working on the problem of sound conduction in the ear found that some problems could be solved by studying certain species of lizards. Previously, they found that some chameleons, which lack an external ear opening and lack the tympanic membrane, hear rather well. Some possess a substitute tympanic membrane in the form of a thin plate of bone (a modified part of the pteryoid bone). Recently, two additional species have been investigated; one (Chamaeleo dilepis) that has this substitute mechanism, and another (Chamaeleo ellioti) that lacks it. The hearing was found to be significantly better in C. dilepis. A second form of substitute for the tympanic membrane was encountered in Cophosourus texona. It consists of an air sac within the middle ear cavity with which the columella is in contact. This ear performs remarkable well in sound reception. A problem of central interest is the manner of stimulation of the hair cells, and the relative effectiveness of different structures and modes of operation. In a study of the basilar papilla of the crocodilian, Caniman crocodilus, researchers found that the papilla differs from that known in other reptiles and in vertebrates in general. The general morphology of the papilla bears some resemblances to that in birds, but the structure of the short hair cells and supporting cells appears to be unique among vertebrates in which the fine structure of the ear is known. The Chamaeleontid shows papillar structure suggestive of a cytologically unspecialized condition, somewhat reminiscent of that in turtles.

Echolocation

Investigators are pursuing the design of echolocation in bats and the processing of acoustic information by the bat brain by analyses of the "sonar" output of several genera of bats - Hyposideros, Mormoops, Chilonycteris, and others; some during goal directed flight and electrophysiological recording from the cochlea, auditory nerve, and inferior colliculus. Bats using pulses of long duration, Chilonycteris parnellii and Rhinolophic, are now known to use pulse duration, in effect, to separate two channels of information arriving via echo. The constant frequency portion is processed for some 25 to 40 msec. and presumably yields velocity information via Doppler shift while FM sweeps are actively held off until later to be processed separately, probably for position determination. The ultimate goal will be to understand how signals of given pitch, loudness, and timing can be processed by the brain into a form that reveals the position, velocity and the nature of objects in the surroundings.

Another group working in tissue transmission found that a topography of sound transmission velocities exists in the porpoise forehead that causes a focussing of sound within the fatty melon, either upon transmission or reception. Sounds transmitted into the fatty melon tend to be focussed very strongly into the right nasal plug lip. In general, velocities are slower than in either fresh or sea water except near the plug. In the sperm whale, the entire spermaceti organ is filled with waxy oil that transmits much faster than sea water and which is probably the homologue of the area adjacent to the right nasal plug.

Noise Induced Deafness

Interaural alternated speech with and without intervening noise and interrupted speech was presented to subjects with normal and impaired hearing. The results were found by a group of researchers to indicate little effect on speech discrimination among normal listeners for interaural alternated speech until intervening noise is introduced. Although there were marked individual differences in the presence of intervening noise, the difficulty in discriminating speech increases as the rate of alternation increases. For subjects with impaired hearing, the trend indicates that they perform all tasks more poorly than normals even though there may be little improvement of speech discrimination for continuous speech. Normal and hard of hearing subjects make equal loudness balance judgements with various bands of noise at the same and different center frequencies. The normal subjects sum loudness over a much narrower band width than do subjects with cochlear involvement. In a study on perception of complex auditory stimuli by the deaf it was found that coarticulation of the consonants and vowels of speech produces transitions in the frequency location of the vowel formants. The results suggest that sensorineural damage does not necessarily impair a persons ability to use brief, small transitions in formant frequency as cues for discrimination. It does seem to reduce the ability to find cues when they occur in a speech-like environment.

Speech

A group of researchers has been investigating the efficiency of function

of the reinnervated canine larynx and on in vitro and in vivo tests of their antilymphocyte serum. In addition, they have pursued other means of improving the chances of success after transplantation of the canine larynx by removing the offending cell, i.e., the small lymphocyte, and of accelerating reinnervation. They are making a serious attempt to tissue-type their dogs before transplantation of the larynx. Heretofore, no effort was made to match animals in regard to histocompatibility of antigens. They are using antilymphocyte serum (ALS) as the immunosuppressive agent of choice in their laryngeal transplantation. The problem of voice function of a transplanted larynx is closely related to that of voice function of a reinnervated larynx.

Another group found that in humans, when background talk reaches a level where it is just mildly disruptive to intelligibility for the normal hearer, it can be a serious masker for the individual with sensorineural hearing loss. They showed that competing speech causes a pathological increment in masking, and suggest that the traditional measurements of hearing loss, such as threshhold shift and discrimination loss, as defined by reduced intelligibility in quiet, should be supplemented with specification of the increase in the masking efficiency of competing speech and other background sounds. In a test of 24 subjects, measuring nonaurally discrimination of monosyllables against competing sentences, findings showed that a third dimension of handicap is imposed by sensorineural pathology. Such pathology not only changes threshold and impairs intelligibility in quiet, but also disturbes the ability to resist masking in complex environments containing background noise, particularly speech.

Olfactory Communication

A team investigated the olfactory connections of the limbic system and hypothalamus in the rodent. The tertiary olfactory contribution to a myelinated bundle in the lateral hypothalamus previously identified in the rat has now been found in both hamster and mouse. In all three species, lesions which include the olfactory tubercle cause degeneration of fibers which travel in the far lateral region of the medial forebrain bundle to the posterior hypothalamus border of the midbrain. In the rat, many regions of the olfactory cortex project to the lateral preoptic area, mediodorsal nucleus of the thalmus, and lateral hypothalamus. Other secondary olfactory zones (medial and cortical amygdaloid nuclei) do not show this pattern of projection. Lesions of the medial nucleus cause degeneration of fibers reaching the bed nucleus of the stria terminalis through its post commissural component. Other work included electrophysiological evaluation on hormone effects with urethane-anesthetized male rats. Comparing recorded results from normal and castrated male rats to study the effects of testicular androgens on the activity of neurons in the preoptic area, a hypothalamic region which receives a strong olfactory input, it was found that some units in the preoptic region reliably change their ongoing discharge rate when the cortical EEG shows sudden transitions between activation and synchrony characteristic of the urethanized rat preparation. Many more neurons in the normal male than in the castrate show this correlation with the EEG. Preoptic units which increase their discharge rate during EEG activation tend to be those which show excitatory responses to odors and tend to change activity before the EEG change in transition from activationary to synchrony but not vice versa.

Sonic Booms

Sonic booms may damage the apical turn of the cochlea causing weakening which in time leads to hearing loss according to another team of investigators. Guinea pigs were exposed to 1,000 bursts of 130 dB. lasting 2, 4, 5, or 125 msec. and were then retested for the Preyer reflex. Histological examinations revealed damage to hair cells of the apical turn of all test animals.

Otitis Media

Incidence of deafness, otitis media, and perforation of the eardrum in Guam populations is the highest reported anywhere. In school children hearing loss was found to be four times that in most North American cities. Selective Service examinations of young men in Guam showed similar incidence rates. Perforation of the eardrum accounted for 10% of Guam young men being disqualified for military service as compared with .01% of the Selective Service population of the U.S.

Rubella

Another research team reported that many pre-school children may have had congenital rubella and are likely to remain undiagnosed until a specific complaint develops, such as a language or hearing defect. The findings indicate that such conditions are recognized too often only after severe academic retardation and emotional suffering have occurred. Therefore, they suggest a two-part screening program as a model for identifying children with unrecognized communication problems so that treatment or special training can begin as early as possible. This team cooperated with Montgomery County Health Department in a program in which 136 children were located by radio, TV, newspaper notices and letters to the local medical society. Children with possible communication disorders were given a clinical examination which included evaluations of growth, speech, language, hearing, vision, and auditory memory. Blood specimens were obtained from 114 children and rubella hemogglutination inhibition (H.I.) antibody titrations were performed on the sera.

Aphasia

In an out-patient clinical research facility for aphasic involvements of children, studies on visual sequencing performance demonstrated that aphasic children are generally inferior to normal children on various types of sequencing tasks. A modality study by the same group of investigators has produced preliminary data which indicates that aphasic children make more errors in judging non-equivalent forms than equivalent forms, and they also had longer latencies for an intramodal haptic condition than for an intramodal visual condition. Another continuing study in this out-patient facility is a series of directionality investigations progressing from gross visual forms to discrimination of letters such as p and b, b and d. Findings show that aphasic children who failed directional discrimination on the initial pre-test, were able to complete this task after they had completed the program. Studies are continuing to determine which of the tasks in the progression of discrimination difficulty are essential for improved performance.

Another group of investigators has been working in a multidisciplinary effort on aphasia and related problems of brain function. They have reported that diagnosis and lesion site have a predictable relationship to pattern of work comprehension and production, with respect to semantic class, word frequency, and picturability. Scaling of syntactic tasks reflecting levels of agrammatism is currently in progress by this group. In this area of non-linguistic neuropsychological research, these investigators have presented evidence that the left parietal lobe is found to be more critical than the right in cross-modal transfer, but less critical for short term visual memory. Neuroanatomical studies have suggested an anatomical basis for cerebral dominance based on the larger average size on the left than on the right planum temporal in man. This same team of investigators has demonstrated that aphasic patients not only vary in their ability to comprehend what they hear, but do so according to at least four distinct patterns of auditory comprehension. They showed that five diagnostic classes of aphasic patients differed in these factors: (1) breadth of vocabulary; (2) auditory sequential pointing-span; (3) comprehension of directional prepositions; (4) recognition of correct grammatical usage of prepositions.

APPENDIX A

RESEARCH GRANTS AWARDED IN FY 1971 BY DISORDER CATEGORY

(Dollars in Thousands)

DISORDER CATEGORY	NO.	AMOUNT	% of $
TOTAL ALL DISORDERS	1256	$ 53,645	100.0
1. NEUROLOGICAL DISORDERS			
A. Neurological Disorders of Early Life	172	$ 6,200	11.6
B. Neurological Disorders of Aging	59	2,810	5.2
C. Cerebrovascular Disorders	80	5,350	10.0
D. Epilepsy and Related Paroxysmal Disorders	52	2,500	4.6
E. Sclerosing Disorders	63	2,360	4.4
F. Muscular and Neuromuscular Disorders	144	4,690	8.7
G. Infectious Diseases	10	250	0.5
H. Trauma and Injury	68	3,120	5.8
J. Tumors of Nervous System	26	590	1.1
M. Neuroendocrine Studies	86	3,050	5.7
N. Neural Aspects of Learning and Behavior	43	1,690	3.2
P. Nervous System Studies - Normal Function	140	5,040	9.4
TOTAL - NEUROLOGICAL DISORDERS	943	$ 37,650	70.2

APPENDIX A

RESEARCH GRANTS AWARDED IN FY 1971 BY DISORDER CATEGORY (Contd.)

(Dollars in Thousands)

DISORDERS CATEGORY	NO.	AMOUNT	% of $
2. SENSORY AND PERCEPTUAL DISORDERS			
A. Disorders of Hearing and Equilibrium	145	$ 6,605	12.3
B. Disorders of Speech and Other Higher CNS Functions	36	1,890	3.5
C. Disorders of Other Senses	108	3,750	7.0
TOTAL - SENSORY & PERCEPTUAL DISORDERS	289	12,245	22.8
3. MULTI-CATEGORICAL	20	$ 3,660	6.8
4. CONFERENCES	4	$ 90	0.2

ANNUAL REPORT
July 1, 1970 through June 30, 1971
Extramural Programs
Training Grants and Awards Branch
National Institute of Neurological Diseases and Stroke

The primary aim of the Training Grants and Awards Branch is the specialized training of skilled professional and scientific personnel for careers in the research and teaching aspects of the prevention, diagnosis, and treatment of neurological and communicative impairments. To accomplish the Institute's objective, two general types of awards are made, training grants and fellowships. Included in the Training Grants category are (1) awards to superior training institutions; (2) awards to institutions which wish to develop programs or strengthen existing weak programs; (3) Special Traineeships; and (4) Teacher-Investigator Special Traineeships. Included in the Fellowships category are (1) Postdoctoral Fellowships; (2) Research Career Development Awards; and (3) Research Career Awards. The methods of applying for support and the review of applications vary among the types of programs. Institutions apply for Training Grants and Developmental Training Grants; institutions also apply for Research Career Development Awards, except they apply for these awards on behalf of a specific candidate. (New Research Career Awards are no longer being made.) Special Traineeships, Teacher-Investigator Special Traineeships, and Postdoctoral Fellowships, are awards made directly to individuals.

To support the NINDS training activities in FY'71, the House-Senate Conference Committee recommended an allowance of $17.754 million which became the appropriation. However, only $17.082 million were allocated to the Institute which provided $14.3 million for Training Grants and Special Traineeships and $2.782 million for RCDA's and Postdoctoral Fellowships. The training grant funds enabled the Institute to support 219 training programs and 201 special trainees; the fellowship funds were used to continue 12 Research Career Awards and to award 76 Research Career Development Awards and 91 postdoctoral fellowships.

In FY'71, the Institute made training grant awards to 55 institutions that submitted renewal applications and to 9 institutions that submitted new applications. In view of the large number (27) of renewal applications that were not funded the previous year and the fact that no new awards were made, it was anticipated that a number of institutions would submit amended applications. In this regard, of the 55 renewal applications that were funded, 11 were amended applications and of the 9 new applications that were funded, two were amended applications. Thus, it was advantageous to 13 program directors who had previously approved programs to submit amended applications. Four other amended renewal applications were recommended for approval but again were not funded because the merit ratings they received were too low.

Following the March meeting of the Council, when recommendations were made concerning the funding of new and renewal applications, ten programs which

had been supported for a number of years had merit ratings too low to fund. These have been either already terminated or have been awarded phase-out support. Eight of these programs were in neurology and two were in communicative disorders. In FY'68, the latest year for which information is available, these ten programs provided full support to 36 trainees and partial support to 20 trainees. In addition, 16 individuals were supported for a two or three month period in order to give them an exposure to neurology or the communicative disorders. The following table identifies the institutions which were recommended for approval in FY'71 but not funded and it indicates the number of trainees in FY'68 who received full, partial, or short-term stipend support from grant funds:

Grant Number	Institution	Trainees Stipended		
		Full	Partial	Short-term
T01 NS 5049	Northwestern University	4	1	2
T01 NS 5099	University of Miami	-	14	-
T01 NS 5109	State University of New York, Downstate	-	-	2
T01 NS 5166	Wayne State	6	-	2
T01 NS 5182	Mayo Foundation	13	4	8
T01 NS 5201	University of Miami	1	-	-
T01 NS 5262	University of Illinois	5	-	-
T01 NS 5409	University of North Carolina	-	-	-
T01 NS 5573	Mt. Sinai School of Medicine	1	-	-
T01 NS 5574	Purdue University	6	1	2
		36	20	16

RESEARCH CAREER DEVELOPMENT AWARDS

Having made no new or renewal Research Career Development Awards in FY'70, FY'71 was a banner year for this program. Of 36 applications recommended for approval, 22 were funded. With only one exception, these 22 applications were all new; nine were amended applications submitted in behalf of individuals for whom support was sought the previous year.

In evaluating applications for renewal support, grantee institutions were asked to provide a special justification for needing additional support for the candidate. In addition, they were asked to (1) indicate their permanent plans for the candidates; (2) state specific accomplishments of the awardees during the initial award period; and (3) signify the need for additional periods of training and supervised experiences. For the most part, the institutions did not make strong cases for needing additional support and the general philosophy of both the training committees and the Council was to not recommend continued support for individuals who had already received five years of RCDA support unless special justification was provided. These candidates were considered independent investigators and as such they had achieved the objective for which the award was initially made.

New guidelines adopted this year, governing the Research Career Development Award program, provide that any application terminating after June 30, 1972, will not be eligible for renewal support. Thus, the RCDA becomes a single five-year award with no possiblity of being renewed. Another change concerns the evaluation of the applications. The initial review will be made by the appropriate DRG Study Section. The National Advisory Council has requested that all applications also be reviewed by an NINDS training review committee.

TEACHER-INVESTIGATOR SPECIAL TRAINEESHIP PROGRAM

FY'71 was the second year of the Teacher-Investigator Special Traineeship program. This program aims to recruit and prepare future teacher-investigators of the highest caliber for academic careers in disciplines or areas of the neurological, neurosensory, or communicative disorders. The award is the most competitive and prestigious training and development award made by the Institute to an individual.

During FY'71, 19 teacher-investigator applications were submitted. Fifteen of the applicants were interested in the neurological sciences and four were interested in communicative disorders. Eleven applicants were invited to Bethesda on February 27 to be interviewed by an NINDS Special Ad Hoc Interview Committee.

The following individuals were selected to receive Teacher-Investigator Awards:

AWARDEE	SPONSOR AND INSTITUTION	DISCIPLINE
Irving K. Arenberg, M.D.	Richard Torack, M.D. Professor, Pathology and Anatomy Department of Pathology Washington University School of Medicine St. Louis, Missouri	Oto-neuropathology
Ira B. Black, M.D.	Fred Plum, M.D. Professor and Chairman Department of Neurology Cornell University Medical College New York, New York	Neurobiology
Mary A. Guggenheim, M.D.	C. Henry Kempe, M.D. Professor and Chairman Department of Pediatrics and James Austin, M.D. Professor and Chairman Department of Neurology University of Colorado Medical Center Denver, Colorado	Neurology-Virology

Mark E. Molliver, M.D.	David Bodian, M.D. Professor of Anatomy and Guy M. McKhann, M.D. Professor of Neurology Johns Hopkins University School of Medicine Baltimore, Maryland	Child Neurology Neuroanatomy
Donald J. Woodward, Ph.D.	Paul Horowicz, Ph.D. Chairman, Department of Physiology University of Rochester School of Medicine Rochester, New York	Developmental Neurobiology

D. C. UNIVERSITY CONSORTIUM: ACADEMIC NEUROSURGERY

During FY'71, the staff held meetings with neurosurgery representatives of the D. C. universities and other neurosurgeons in an attempt to stimulate the development of a Ph.D. postdoctoral program in neurosurgery as a multi-university Consortium undertaking. The organizational unit for the program would be the D. C. Consortium and the objective of the program would be to develop a program in the Washington area utilizing several of the local facilities to train neurosurgeons for careers in research and academic medicine. The meetings were attended by the following individuals:

Dr. Jesse B. Barber, Jr.
Howard University

Dr. Calvin Early
National Naval Medical Center

Dr. Ludwig Kempe
Walter Reed Army Medical Center

Dr. John Luessenhop
Georgetown University

Dr. Hugo V. Rizzoli
George Washington University

Dr. John M. Van Buren
National Institute of Neurological
Diseases and Stroke

The advantage of utilizing the Consortium is that the administrative unit already exists and any facility in the Washington area could be utilized to provide for a training program which would be unique to the specific needs of the trainees.

It is anticipated that a formal application requesting support for this new program will be submitted prior to October 1, 1971

FRONTIERS IN RESEARCH IN TEACHING IN NEUROSCIENCE: A MINORITY TRAINING PROGRAM

In FY'71, the Institute made an award to the Marine Biological Laboratory, Woods Hole, to advance training in the neurosciences primarily for minority group individuals at the postdoctoral level who are seeking opportunities for further experience in research and who desire to strengthen their capacities as teachers of future neuro-scientists. The program is designed to enable trainees to carry out a personalized program in one or more areas of neurobiology.

An award to the MBL provided support for approximately 6 trainees. Although these individuals would be selected on a competitive basis, it was agreed that for this pilot effort the program would be experimental in design and it would be generally publicized with specific publicity aimed at institutions that are composed mainly of minority ethnic groups.

Should the project be successful during the pilot period, the Marine Biological Laboratory would submit an application for a similar grant for long term support.

STATUS OF OTOLARYNGOLOGY TRAINING PROGRAMS IN THE UNITED STATES - 1969

During FY'71 a report was prepared by the Committee on Education, Society of University Otolaryngologists, on the status of otolaryngology training programs in the United States in 1969. The data for this report were collected by Dr. Dean Lierle and the study was supported by an NINDS grant.

The survey included 103 programs which were approved by the American Medical Association and the Review Committee for Otolaryngology. Sixty-nine programs were in university teaching centers; of these, 36 were independent departments while 33 were sections of general surgery. Thirty-four were in government or private institutions.

The report calls attention to the critical manpower situation. In 1969, there were 253 first-year residency positions whereas there was a need for a minimum of 500 such positions. There should be one certified otolaryngologist for every 40,000 people in the United States and at least 500 residency positions should be available to develop adequate health care for the public, and provide the teacher-investigators required for academic positions.

The formation of new medical schools (eight in 1969) added a few residency positions, but not nearly enough to keep pace with increased needs. The manpower situation cannot improve unless there is an expansion of many of the residency programs. In addition to the lack of professional staff there is also a lack of technicians in otolaryngology. The report points out that paramedical personnel, properly trained, could be of value in lessening the workload of faculty and residents. There appeared to be a fairly good supply of audiologists and speech pathologists.

In the 103 institutions surveyed, a minimum of 150 more full-time teachers are needed to improve the quality of teaching and properly supervise the clinical and research areas. It is practically impossible for one full-time staff member to attempt to administer, teach, and conduct research, even in the smallest department of otolaryngology.

The report concludes by making the following recommendations to alleviate the manpower situation and improve the quality of otolaryngology training in the United States:

1. Increase the number of beds, the budgets and the faculty, in otolaryngology, particulary in smaller departments, thus providing more residency positions and improving the quality of teaching.

2. Utilize many available, competent young private practitioners in areas adjacent to medical school and hospitals. These young men could contribute greatly as part-time teachers and should be well compensated by the institutions.

3. Train more paramedical personnel in otolaryngology to help relieve the work load of residents and staff.

4. Make all departments and divisions of otolaryngology independent, particularly for budgets, in-patient beds, staff, access to the dean's office, and membership on medical school committees.

5. Require clinical clerkships for undergraduate medical students for a minimum period of two weeks; also, time should be provided for electives in otolaryngology.

6. Consider postgraduate courses designed particularly for family physicians, pediatricians, and other disciplines.

7. Provide more continuation courses for otolaryngologists in private practice.

NEUROLOGY: A MEDICAL DISCIPLINE TAKES STOCK

In 1962 the Institute awarded a contract to Columbia University to evaluate the status of training for research and service in the neurological sciences and to formulate recommendations for strengthening and improving university and non-university activities related to such training. The study was carried out by Dr. Aura Severinghaus and during the period of the study the following three publications by Dr. Severinghaus appeared:

Distribution of Graduates of Medical Schools in the United States and Canada According to Specialties, 1900-1964. J. Med. E., 40:721-736, 1965.

A Medical Discipline Takes Stock. Arch. Neurol., 17:461-470, 1967

Neurology and Neurological Sciences Research and Training Study. Arch. Neurol., 17:471-483, 1967.

Dr. Severinghaus's study has been completed and the Institute has received his final manuscript which is being put into final form for consideration for possible publication.

APPENDICES

Following are four appendices which show how support for the Institute's training programs is divided among the various training areas.

Appendix A is the anticipated number of grants and the training grant funds awarded for FY'71 according to the Institute's areas of responsibility. This is an increase of 1 award over FY'70.

Appendix B is the anticipated number of Special Traineeship Awards and the amount for FY'71 divided among the Institute's areas of responsibility. This is an increase of 40 awards over FY'70.

Appendix C is the distribution of nine Teacher-Investigator Special Traineeships among the Institute's areas of responsibility. This is an increase of four awards over FY'70, the initial year of this training activity.

Appendix D is the anticipated number and the amount awarded for RCA's, RCDA's, and Postdoctoral Fellowships in FY'71. This is an increase of five RCDA's and 19 Postdoctoral Fellowships over FY'70. The number of RCA's (12) remains unchanged from FY'70.

APPENDIX A

Distribution, by Scientific Fields,
of Training Grants Awarded in FY 1971

Field	Number	Amount
		$
Audiology	6	368,700
Cerebrovascular	2	68,600
Child Neurology	15	613,900
Communicative Disorders	7	533,100
Neuroanatomy	5	151,200
Neurobiology	1	20,800
Neurochemistry	3	102,000
Neurological Sciences	6	225,400
Neurology	59	4,197,800
Neuropathology	14	513,900
Neuropharmacology	3	166,100
Neurophysiology	13	731,700
Neuroradiology	10	250,300
Neurosurgery	24	917,400
Neurovirology	1	47,500
Otolaryngology	44	2,680,400
Sensory Physiology	3	128,300
Speech Pathology	3	162,900
TOTAL	219	$11,880,000

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APPENDIX B

Distribution, by Scientific Fields, of Special Traineeships Awarded in FY 1971

Field	Number	Amount
		$
Audiology	5	53,500
Basic Neurosciences	3	48,400
Biochemistry	8	81,200
Biophysics	1	16,700
Cerebrovascular	10	106,900
Child Neurology	46	500,600
Communicative Disorders	1	16,100
Immunology	3	35,600
Neuroanatomy	3	31,300
Neurobiology	6	74,700
Neurochemistry	6	65,100
Neuroendocrinology	5	49,000
Neurology	9	114,900
Neuropathology	16	171,700
Neuropharmacology	6	63,200
Neurophysiology	29	293,700
Neuroradiology	17	198,900
Neurosurgery	5	41,600
Neurovirology	4	44,900
Otolaryngology	4	47,500
Sensory Physiology	3	37,200
Speech Pathology	2	28,100
TOTAL	192	$2,120,800

April 29, 1971

APPENDIX C

Distribution, by Scientific Fields,
of Teacher-Investigator Awards Granted in FY 1971

Field	Number	Amount
		$
Neuroanatomy	1	19,500
Neurobiology	1	15,500
Neurology	2	35,000
Neurophysiology	2	35,000
Neurosurgery	1	22,200
Neurovirology	1	21,500
Sensory Physiology	1	15,500
TOTAL	9	$164,200

APPENDIX D

Distribution, by Scientific Fields, of Fellowships Awarded in FY 1971

Field	Research Career Awards		Research Career Development Awards		Postdoctoral Fellowships	
	No.	Amount $	No.	Amount $	No.	Amount $
Audiology	1	29,600	3	81,700	5	38,100
Biochemistry	-	--	4	78,700	4	35,500
Biophysics	-	--	-	--	1	8,500
Cerebrovascular	1	29,800	-	--	-	--
Communicative Disorders	-	--	1	20,300	1	7,000
Immunology	-	--	1	22,000	1	9,500
Neuroanatomy	2	26,200	5	103,100	13	96,700
Neurobiology	-	--	1	15,500	14	105,300
Neurochemistry	-	--	8	185,100	5	40,700
Neuroendocrinology	1	29,500	2	51,100	2	16,000
Neurology	2	60,900	4	107,600	2	14,300
Neuropathology	-	--	4	95,800	3	28,500
Neuropharmacology	2	59,300	9	190,100	7	59,300
Neurophysiology	1	30,500	19	426,200	25	200,500
Neurosurgery	-	--	2	46,000	-	--
Neurovirology	-	--	6	125,500	1	9,500
Otolaryngology	1	32,400	2	51,700	-	--
Physiological Psychology	1	30,800	1	29,800	2	16,700
Sensory Physiology	-	--	4	96,800	4	32,400
Speech Pathology	-	--	-	--	1	7,500
TOTAL	12	$329,000	76	$1,727,000	91	$726,000

April 29, 1971

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