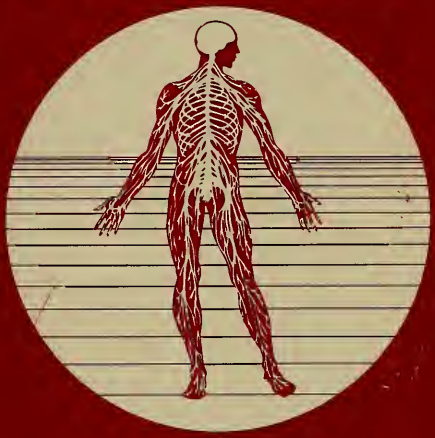
National Institute of Neurological and Communicative Disorders and Stroke

Intramural Research



Annual Report Fiscal Year 1984

U.S. DEPARTMENT
OF HEALTH
AND HUMAN SERVICES
Public Health Service
National Institutes of Health

National Institute of Neurological and Communicative Disorders and Stroke

Intramural Research

Annual Report
Fiscal Year 1984

U.S. DEPARTMENT
OF HEALTH
AND HUMAN SERVICES
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National Institutes of Health

ANNUAL REPORT

October 1, 1983 through September 30, 1984

INTRAMURAL RESEARCH PROGRAM
National Institute of Neurological and Communicative Disorders and Stroke

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* New Laboratory or Branch

** The Biometry and Field Studies Branch was incorporated into the Intramural Research Program as of September 12, 1984. BFSB was formerly the Office of Biometry and Field Studies, OD.

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Alphabetical Listing of NINCDS Intramural Principal Investigators

Alphabetical Listing of NINCDS Intramural Principal Investigators - (Cont'd)

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INTRAMURAL RESEARCH PROJECTS
Numerical Inventory

* This project was transferred from NIMH and is new for NINCDS

Intramural Research Projects - Numerical Inventory (Cont'd)

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National Institute of Neurological and Communicative Disorders and Stroke

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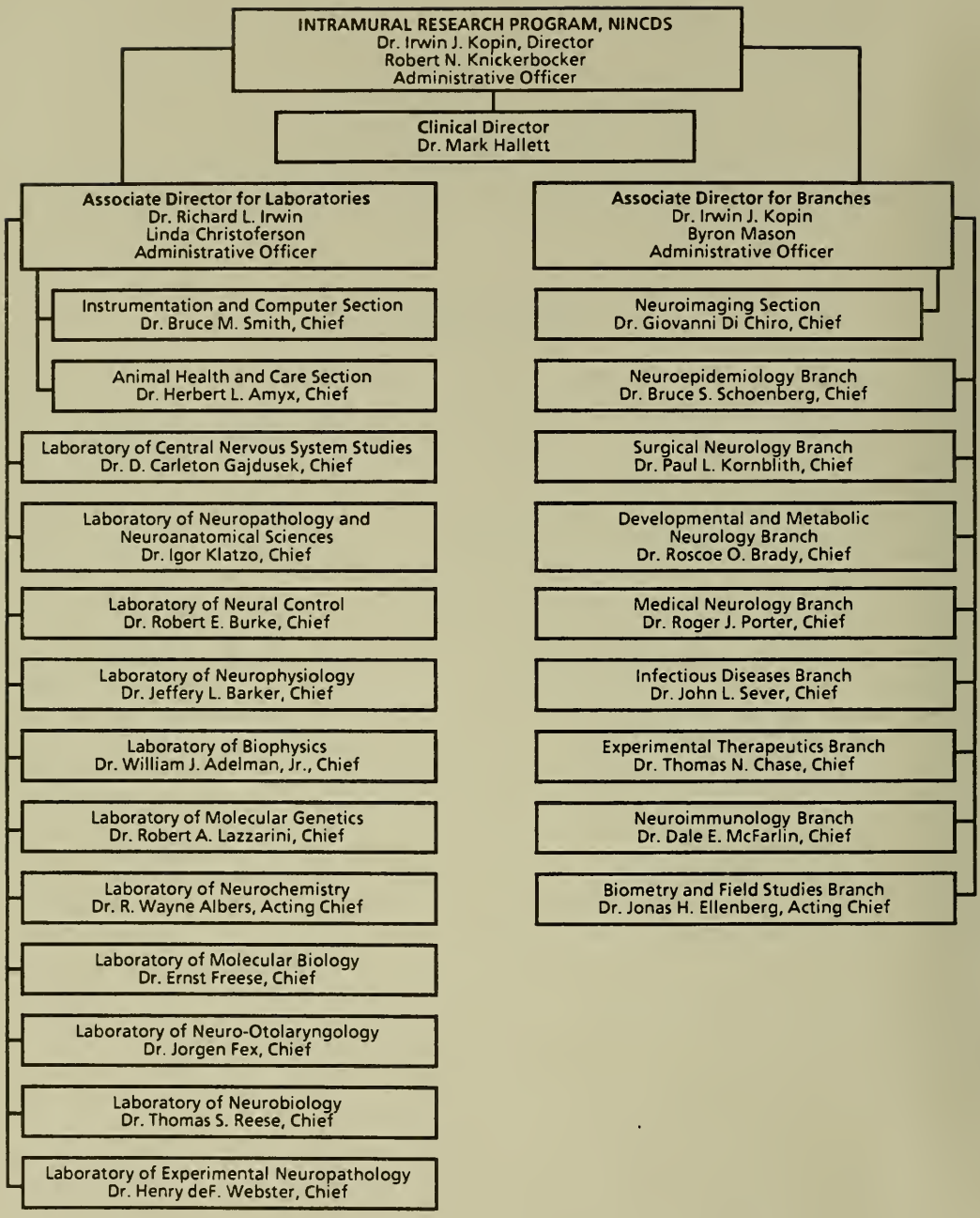

(Fiscal Year 1985)

Annual Report of the Scientific Director
of the
National Institute of Neurological and Communicative Disorders and Stroke
October 1, 1983 through September 30, 1984
Irwin J. Kopin, M.D., Scientific Director

The Intramural Research Program research efforts are conducted through direct operations of laboratories and clinics mainly at the NIH complex in Bethesda. In addition, a small portion of the research is performed away from Bethesda, at Fort Detrick in Frederick, Maryland, or at the Marine Biological Laboratory in Woods Hole, Massachusetts. In these facilities Federal Government scientists and their support staff as well as guest research workers continue to discover and produce new knowledge that aids our ability to prevent, ameliorate or cure neurological or communicative diseases. Ranging from chemical interactions of molecules to therapeutic interventions with new drugs in patients, the studies contribute significantly to the explosive growth of new knowledge in the neurosciences and diseases of the nervous system. The research projects are investigator-initiated and all projects relate to the main mission of the Institute and NIH: the advancement of biomedical research for the ultimate prevention or alleviation of human suffering from disease or injury.

During the last year, the Intramural Research Program largely emerged from a transitional managerial period and gained the stability that reflected the naming of a permanent Director of the Institute and subsequent appointment of a Director for Intramural Research. A number of important changes in the administrative structure and personnel have been effected to reflect more precisely program goals and individual contributions towards a more effective research program. Reasonably knowledgeable and efficient daily administration of a research endeavor which encompasses eight clinical branches and eleven laboratories, mainly divided between the Clinical Center and Building 36, requires a suitable division of responsibility. The IRP has been fortunate in having Dr. Richard L. Irwin serve as Associate Director for Laboratories. Dr. Irwin had previously served for about nine months as Acting Scientific Director of the IRP. His knowledge of the research program, institutional memory, wisdom, and understanding of both personnel and space issues have been of inestimable value in assuring an orderly, productive and expeditious transition of administrative responsibilities and implementation of necessary reallocations of resources.

The clinically oriented research programs are mostly carried out by the Branches. These are under the direct supervision of the Scientific Director in his role as Associate Director for Branches, whereas the basic research programs in the Laboratories are under the direct supervision of Dr. Richard Irwin in his capacity as Associate Director for Laboratories.

The major changes in personnel and structure of the IRP will be outlined in this Scientific Director's Summary of FY 84 whereas digests describing the major scientific advances by the Laboratories and Branches are included in the summaries provided by each of the Laboratory and Branch Chiefs.

The appointment of Dr. Mark Hallett as Clinical Director culminated a long interval during which several of the Branch Chiefs sequentially served as Acting Clinical Director, dividing their attention between the demands of this office and that of their large, active research programs. Dr. Hallett has had, even during the relatively short time since his appointment in January, a significant impact on improving the monitoring and general excellence of clinical care, clinical services, and the educational programs. The Office of the Clinical Director now includes neuropathological services, EEG, EMG, the neurological consultant service to other institutes, patient recruitment, and outpatient services. Dr. Hallett has also been appointed Chief, Human Movement Disorders Section which is included in the Medical Neurology Branch. The latter appointment permits Dr. Hallett to pursue his research on movement disorders and EMG.

The Medical Neurology Branch has been revitalized under the leadership of Dr. Roger Porter. Dr. Porter had been serving as Chief, Epilepsy Branch in one of the extramural programs and, as a guest worker, Chief of the Clinical Epilepsy Section in the Experimental Therapeutics Branch (ETB), IRP. Although the research program of the Medical Neurology Branch will include a Clinical Epilepsy Section (transferred from the ETB), the investigations in the Branch will also encompass other neurological disorders. Research on the autonomic nervous system and studies on Familial Alzheimer's disease are included in the Clinical Neuropharmacology Section which is headed by Dr. Ronald Polinsky. Dr. Polinsky is a well-trained neurologist who developed these clinical research interests while in the Laboratory of Clinical Science, NIMH, where he had been appointed to a tenured position. His transfer to NINCDS appeared appropriate to the Institute's research goals and brought a new perspective to

the clinical research program. As indicated earlier, Dr. Mark Hallett will head a Human Movement Disorders Section in the MNB. In addition to studies of muscle, peripheral nerves, and regulation of movement, this section will include the Speech Pathology Unit under the leadership of Dr. Christy L. Ludlow. A fourth section supporting basic research on neuronal excitability is being planned and will complement Dr. Porter's clinical research interests.

The Medical Neurology Branch will also include a section on Neuropsychology headed by Dr. Paul Fedio. This transfer will leave vacant the Clinical Neurosciences Branch. This designation will remain inactive until appropriate resources are identified and a new initiative, e.g. in degenerative disorders, is sufficiently mature to warrant branch status.

The Neuroepidemiology Branch was formerly in the Office of the Director, IRP, as a section. Its elevation to the Branch level reflects the importance of neuroepidemiology in our research program and recognizes the scientific stature of Dr. Bruce S. Schoenberg who has been so productive during his tenure as Chief of the Section.

The Biometry and Field Studies Branch, formerly the Office of Biometry and Field Studies in the Office of the Director, NINCDS, has been transferred, effective Sept. 12, 1984, into the Intramural Research Program. This transfer recognizes the necessity for adequate review of the investigator-initiated research in this group as well as their collaborative contributions to other portions of the IRP. A substantial fraction of the research productivity has been, and will continue to be, in association with the various extramural programs. With the retirement of Mr. William Weiss, Dr. Jonas H. Ellenberg has assumed the duties of Acting Chief of this Branch until a permanent chief is selected.

The Developmental and Metabolic Neurology Branch has continued its active clinical and basic investigations programs. Two conversions to tenure are currently in progress. Dr. Norman Barton, a pediatric neurologist-biochemist has been nominated for tenure as a collaborative clinical investigator and Dr. Edward Ginns has been proposed for tenure as an essential part of a molecular genetics team involved in cloning of genes for essential enzymes.

The Surgical Neurology Branch, under the leadership of Dr. Paul Kornblith has begun to define administrative units identified with the various aspects of its research programs. Dr. Elizabeth Grimm, an immunologist formerly with the Cancer Institute and Dr. Richard Youle, a biochemist from NIMH, have been recruited to establish independent research programs in areas related to tumor immunology and chemotherapy. Dr. James D. Bona has been recruited to assist Dr. Kornblith in administrative aspects of studies of in vitro assessment of tumor chemotherapeutic agents. The importance of Drs. Edward Oldfield and Donald Wright in the clinical research areas, as well as their essential roles in conducting surgical treatments, have been recognized in the appointment of Dr. Oldfield and the proposed conversion of Dr. Wright to tenured positions. Dr. Richard Burns, who has been actively studying a toxin which destroys nigrostriatal neurones and provides an animal model of Parkinson's Disease will be working in the Surgical Neurology Branch to collaborate in studies of the efficacy of brain transplants in reversing the toxin-induced neurological disorder. The Brain Imaging Section, formerly in the Surgical Neurology Branch has been transferred to the Office of the Scientific Director.

The Neuroimmunology Branch has been strengthened with the appointment of Dr. William Biddison as a tenured investigator. He will continue his research of molecular mechanisms of lymphoid cell interactions. Plans for renovation of suitable laboratories for Dr. Biddison have been completed and await implementation. A Section on Immunopharmacology under my direction (as a scientist and independent of my role as Scientific Director) has been planned, but space limitations have delayed implementation.

The Experimental Therapeutics Branch has, with the exception of the transfer of the Clinical Epilepsy Section to the Medical Neurology Branch as indicated earlier, remained unchanged. The investigators in this Branch have continued to be among the most productive in the institute. Dr. Thomas N. Chase has adequately reviewed the progress of this branch in his report.

With completion of the phasing out of the Collaborative Perinatal Project, the Infectious Diseases Branch has expanded its efforts in studies on SAIDS, the simian model of AIDS. Furthermore, this Branch is developing a deeper interest in molecular genetics but continues to exploit the simian model of human AIDS as well as other viral diseases of the nervous system. This evolution of an active research program reflects modifications of research goals to keep pace with latest advances.

A new Section on Neural Imaging has been created in the Office of the Director by transfer of this operation from the Surgical Neurology Branch. This Section, headed by Dr. Giovanni DiChiro, is now located in space donated by the Diagnostic Radiology and Nuclear Medicine departments in the Clinical Center. Its purpose is to provide a means for close cooperation with the Clinical Center departments in the research in animals as well as humans. Dr. Susumu Sato, head of the Clinical EEG unit, will also participate in studies designed to evaluate and/or develop magnetoencephalographic (MEG) techniques. This relatively new approach has been claimed to provide useful information about electrical activity in deep brain structures because magnetic fields, unlike electrical currents, are unaffected by tissue and thus brain is "transparent" to such fields. There is a considerable amount of testing required to define the usefulness and limitation of MEG in diagnosis.

As indicated above, Laboratories are under the direct supervision of the Associate Director for Laboratories. Since most laboratories carry out studies involving research in animals, overall animal care has been centralized in the Office of the Associate Director for Laboratories. In compliance with the standards for animal care and use in the NIH intramural program, Dr. Herbert Amyx has been appointed NINCDS Veterinarian and is responsible for animal care in this Institute. He is chairman of the Animal Research Committee and acts as advisor to the Scientific Director on all matters relating to animal care facilities and management practices. This will assure NINCDS accordance with the Guide for the Care and Use of Laboratory Animals necessary for meeting accreditation standards of the American Association for Accreditation of Laboratory Animal Care.

The emergence of important new research areas and the growth of independence and scientific stature of senior investigators must be considered if the research programs of the Institute are to maintain their superiority and compete with state-of-the-art science. Limited resources must be divided and/or reallocated to meet programmatic needs. Occasionally investigators with newly developed interests find that another laboratory provides a more appropriate environment for collaborative efforts, stimulation of related ideas, and/or application of different techniques. Under such circumstances the administration must be sensitive to the goals of the research program as well as the needs of the individuals involved. The chiefs of the laboratories and branches recognize this need and are generally supportive of efforts to maintain the excellence of the IRP. A number of changes in alignment of the resources in the laboratories has been effected on the basis of such considerations.

The Laboratory of Neuropathology and Neuroanatomical Sciences under the leadership of Dr. Igor Klatzo continues studies on edema, cerebral ischemia, and brain vascular function, but in recognition of the independence and excellence of two of its Section Chiefs, two new laboratories have been established. Dr. Henry deF. Webster has assumed the duties of Chief, Laboratory of Experimental Neuropathology. This laboratory presently is divided into two sections. The Section on Cellular Neuropathology pursues studies on mechanisms of virally induced demyelination in the central nervous system and on the mode of development of myelin as well as its structure. The Section on Neurotoxicology is headed by Dr. Richard Irwin. Studies in this section have focussed on potential neurotoxicity of food additives, modes of action of anticonvulsants, and mechanisms of neurotransmitter storage and release.

The second laboratory which has been established is the Laboratory of Neurobiology under the leadership of Dr. Thomas S. Reese. This laboratory is divided into two Sections, one of which, the Section on Structural Cell Biology, headed by Dr. Reese, is based mainly in Woods Hole whereas the other, the Section on Structural Plasticity, headed by Dr. Milton Brightman, is in Bethesda at NIH.

Several new appointments have strengthened and given more defined direction to three of the laboratories. Dr. Manfred Schubert was appointed to a tenured position in the Laboratory of Molecular Genetics, in accordance with recommendations of the Board of Scientific Counselors and as part of the expansion planned by Dr. Robert Lazzarini, Chief of this laboratory. Drs. Michael Martin and Robert Wenthold were appointed to tenured positions in the Laboratory of Neuro-otolaryngology headed by Dr. Jorgen Fex. Their expertise in pharmacology and biochemistry will complement that of neurophysiology and provide a multidisciplinary approach to studies of auditory functions. Similarly interdisciplinary expansion of the Laboratory of Neurophysiology, has been initiated by Dr. Jeffery Barker's recruitment of Drs. Claire M. Fraser and J. Craig Venter into his laboratory. Their expertise in immunology and receptors will complement the studies aimed at isolation and characterization of specific neuronal populations in tissue culture as well as providing opportunities for collaborative studies with other laboratories.

Dr. Ralph Nelson, a neurophysiologist recently recruited from the National Eye Institute, has been transferred from the Laboratory of Neurochemistry into the Laboratory of Neurophysiology. Although Dr. Nelson will continue collaborative studies with

investigators in the Laboratory of Neurochemistry, his research is more closely allied to that in the LNP and this administrative change may facilitate closer cooperation and more appropriate supervision. Dr. Janet Passonneau, Chief, Laboratory of Neurochemistry, had been ill during the last year and for much of the time Dr. Wayne Albers served as Acting Chief of that Laboratory. The Section on Neuronal Development and Regeneration has been transferred to the Office of the Associate Director for Laboratories until a more appropriate research environment for this group is identified.

The Laboratory of Central Nervous System Studies under the leadership of Dr. Carlton Gajdusek has been unchanged in structure, but resources for primate research are being realigned to reduce costs and more effectively utilize animal space. Dr. Herbert Amyx, who, as indicated above, has been appointed NINCDS Veterinarian, continues to assist the LCNSS in their research efforts at Frederick.

The Laboratory of Neural Control is awaiting completion of renovations pending its move to more consolidated space. There have been no significant administrative changes in this laboratory.

The tragic loss of Dr. Elizabeth Freese has been a severe blow to the Laboratory of Molecular Biology. Dr. Ernst Freese has carried on and implemented planned changes in programs which were suggested by the Board of Scientific Counselors in their recent review of the Laboratory. His new efforts in studying the molecular basis for differentiation in mammalian glial cells have already resulted in interesting observations consistent with his earlier work in yeast. Dr. Ernst Freese was recipient of the prestigious Alexander von Humboldt Award and spent three months in Germany to share his expertise with scientists in that country. The outstanding achievements of the scientific staff of NINCDS were recognized by awards to the following: Dr. Clarence Gibbs, Jr., and Dr. Roscoe Brady received Meritorious Executive Rank Awards. Dr. Robert Lazzarini received the DHHS Distinguished Service Award and Dr. Dale McFarlin the Distinguished Service Medal of the USPHS. Dr. Richard Quarles was cited with the PHS Superior Service Award and Dr. Jeffery Barker received the Meritorious Service Medal. PHS citations were given to Dr. Edward Ginns and Norman Barton.

It has been a source of gratification that during my first year as Scientific Director of the NINCDS, productivity has continued at a high level and that changes in allocation of resources have been possible with the cooperation and understanding of the staff and with minimal disturbance to ongoing research. It is a privilege to have the opportunity to participate in the functioning of this excellent research endeavor.

TAB I.A -- INSTRUMENTATION AND COMPUTERS SECTION -- (ODIR/ICS)

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Instrumentation and Computers Section
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INSTRUMENTATION & COMPUTERS SECTION

National Institute of Neurological and Communicative Disorders and Stroke

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The Instrumentation and Computers Section provides technical support for investigators of NIMH and NINCDS IRPs by (1) assessing the instrumentation and computer needs of the investigator; (2) designing, developing and constructing special purpose electronic and mechanical instrumentation and systems not commercially available; (3) designing, specifying and managing laboratory computer systems for data acquisition and processing.

Additional services provided by the Section include consultation on measurement techniques, signal processing, noise and electro-magnetic interference in data measurement systems, and equipment purchases. Several formal and informal courses for investigators are taught by Section personnel; topics include electrical circuit theory, operational amplifier applications, digital logic design, and computer applications.

Due to manpower limitations and economic considerations, the Section is unable to provide the following services: repair of commercial instruments, duplication of off-the-shelf commercially available equipment, and fabrication of non-instrument items (shelves, bookcases, etc.).

When an investigator requires the services of the Section, he first meets with the Section Chief and other personnel as needed to discuss his requirements. On the basis of this meeting, a decision is made as to whether ICS (Instrumentation and Computers Section) will take on the project. If a commercially produced instrument will satisfy the investigator's requirements, he is advised to purchase it. If custom instrumentation is needed, ICS will accept the project unless we lack the appropriate expertise, or our current work backlog is excessive. In these cases the project may be contracted to a private firm, or the investigator may be directed to the Biomedical Engineering and Instrumentation Branch (BEIB).

When the Section Chief or the Assistant to the Chief agree to accept a project, the investigator submits a standard work request form (available from ICS), signed by his Lab Chief. This form will state the nature of the instrument or service requested, and will contain as many details and specifications as the investigator can provide.

The project is then assigned to an engineer, who will confer with the investigator to formulate a set of engineering specifications and a timetable and cost estimate for the project. The ICS does not charge for services, but the investigator will be billed for the cost of the components used. Upon delivery of the completed instrument, a memo is sent to the investigator listing the component costs and asking permission to have the Administrative Officer transfer funds from his CAN to the Section's CAN.

INSTRUMENTATION

The Section has a staff of five engineers and six technicians to design, develop, and fabricate electronic and mechanical instruments. The major effort is in the production of electronic instruments for basic neurophysiological research, and for clinical studies involving affective disorders. The following are brief descriptions of representative projects, chosen from a total of 253 projects undertaken this year.

(1) Patient Activity Monitoring System. The Section has continued to develop the Patient Activity Monitor (PAM) and the support hardware and software which forms the system.

(a) Monitor. Last year the PAM was redesigned to obtain a four-fold increase in memory capacity and a significant reduction in size. This year the major hardware efforts involved producing this new PAM in large quantities and redesigning the monitor's case to take full advantage of the reduced size of the circuitry. Forty-six new monitors were fabricated, tested and calibrated early this year. A second set of 60 monitors is now in the final phase of fabrication. In order to maximize the benefits of a smaller case, a contract with a local firm was awarded to design and produce a three piece injection-molded plastic case. The pieces are to snap together, with the main case and end cap forming a water-tight seal. Pre-production sample cases are now being evaluated. While the development cost of this case was high, production quantities of the case will be extremely inexpensive and readily available.

(b) Telecommunications. The PAM data telecommunications system project has resulted in the development of a remote readout terminal for the patient activity monitor. The hardware/software development phase is complete and a printed circuit board is now under development. An important facet of the development program was the incorporation of a new line of low power (CMOS) parts into the hardware design. As a result the power supply requirements were reduced by an order of magnitude resulting in a smaller package and less ventilation demands. The new hardware also eliminated the need for interface circuitry previously required to match the CMOS activity monitor to the HMOS hardware of the previous terminal design. The terminal has the capability to be used in the home of a subject or in an office/laboratory environment. Using a predefined software protocol, the terminal reads the contents of an activity monitor, dials a remote computer facility, sends the data read from the monitor, clears the monitor memory, and hangs up. Initially, the VAX computer managed by the Section will be the remote (recipient) computer. After receiving the data, the VAX will retransmit it over a phone line to the PAM minicomputer in Bldg. 10. The data can then be reformatted into standard activity files for further analysis.

(c) PAM Checkout Computer. This portable, battery-powered instrument was developed last year to provide a means of initializing and testing activity monitors right on the wards. The convenience of this instrument has improved the day-to-day management of the large number of monitors now in use. Nine checkout computers were fabricated this year.

(d) Software. The continuous file plotting program has been updated to enable users to plot outputs on both the Electrohome video display and the HP plotter located in Bldg. 10. Future updates will allow newer, inexpensive

dot-matrix printers to also be used. A second software update has expanded the PAM display program to allow users to calculate activity frequency distribution according to user-set parameters.

(2) Neurophysiological Data Preprocessor. A microprocessor system has been developed to replace the custom logic circuitry presently used by the Laboratory of Neurophysiology Data Acquisition System. The new preprocessor records the times of occurrences of 64 different events and 8 different pulses. This information is transmitted to the main processor (a PDP-11 minicomputer) through a parallel interface and the information is coded in such a form as to ensure compatibility with existing software that is used for analysis and display of the data. The preprocessor is built from industry standard cards and one custom printed circuit board which will allow for easy replication. The preprocessor decreases response time to events and pulses and it frees the main processor for experiment control. A software package has been written for the PDP-11 computer which enables users to add specific modules to present stimuli and/or control the experiment without the need for revising the basic data collection program.

(3) Pulse Generator System. A multi-channel timing instrument (pulse generator system) is a vital part of many neurophysiological experiments. Instruments used within the IRP that were purchased about 15 years ago are no longer manufactured and have become somewhat unreliable. Newer, commercially available units lack the flexibility and convenience of the older devices. ICS has developed a five-channel pulse generator system to fill this void. By employing both analog and CMOS digital design techniques, an instrument with both the required technical specifications and a high degree of operator convenience was realized. The base rate generator section provides a frequency range of .01 Hz to 110 kHz, plus manual and external triggering. Each of the five pulse generators provides a six decade range of pulse delay and pulse width with excellent linearity between ranges. The fifth pulse channel also has pulse train capability. Six of these new instruments were fabricated this year.

(4) EEG Amplifier System. A 32 channel EEG amplifier system was completed and is currently being used for several ongoing research projects including topographic brain mapping. The design incorporates state-of-the-art integrated circuit components and printed circuit board layouts to produce a reliable, compact, low-cost-per-channel unit. The system consists of a preamplifier and amplifier with an overall gain of 20,000. A flexible design and front panel switches allow the user control over signal bandwidth, sampling frequency into the computer, and external monitoring by a tape recorder and a 16 channel Grass polygraph.

(5) 30 Channel Electrode Array Amplifier System. A complete system for amplifying and processing signals from a micro-miniature array of 30 gold electrodes is being completed. The electrode array will be used in a variety of experiments to record cultured nerve tissue cell interactions. The signals from the nerve cells are first pre-amplified on a small unit right at the experimental set-up. A separate amplifying unit provides three selectable settings for overall gains of 100, 1,000 or 10,000.

For each electrode, the amplified signal is fed to a comparator with a front panel adjustable threshold level. The output of this comparator triggers a one-shot which is latched and eventually sampled by the computer. Additionally, a multiplexer is provided to display the amplified signal, comparator level and one-shot output on a single channel of an oscilloscope. Design of printed

circuit cards for both the pre-amplifier and amplifier/discriminator greatly simplifies the construction and increases the system reliability. A minicomputer system was also specified for this project and will sample each of the 30 latches, record and process the digital data.

(6) Oscilloscope Modification. Modifications to a dual channel B & K oscilloscope were completed which allows the investigator to use an inexpensive oscilloscope as a high speed, dual channel X-Y display. The horizontal and vertical axes, the intensity level, and the chopper frequency are placed under computer control which allows the user complete flexibility in using the oscilloscope for displaying neurological data. All the modifications were placed inside the oscilloscope. A single switch disconnects the circuit modifications and allows the unit to be used in its original oscilloscope mode.

(7) Microphone Amplifiers. Accurate recording of primate vocalizations in large outdoor enclosures required the design of a compact, reliable microphone amplifier to transmit the signal over long cables to the remotely located tape recorder. By utilizing new linear CMOS circuit elements (operational amplifiers, voltage converters, regulators and detectors) an extremely low power, low noise amplifier design was realized. Four of these amplifiers were fabricated; each provides a bandwidth-limited selectable high gain and over 300 hours of operation from a single 9-volt battery.

(8) LED Pulsing System. A light emitting diode system was constructed for experiments where a constant current, variable pulse width light stimulus is needed for retinal response studies. The requirements for this system specify an external input gate that enables a 1 kHz clock. The rising edge of this clock triggers a one-shot that allows a pulse width selection of 10 logarithmic intervals between 100 µsec. and 790 µsec. The selected pulse width is then used to generate a constant current drive for the LED. The constant current source is switch selectable in decades between 10 µA and 100 mA and was designed using a transistor and the LED in a feedback loop of a 741 op-amp.

(9) Microdensitometer. A standard split-viewing Zeiss compound microscope is being converted into a microdensitometer for use in the 2-deoxy-D-glucose autoradiographic method of studying functional brain anatomy. This instrument will produce a density reading from a small central spot (selectable as either .25, .63 or 1.6 mm dia.) within the 18 mm diameter viewing area. A linear photodiode/amplifier combination will convert the light transmission value within the spot into a proportional voltage for a microprocessor-controlled A/D converter. Corresponding to each transmission value, a logarithmic density value will be obtained from a memory look-up table. Transmission and density values will be simultaneously displayed, each in a four-digit format. Upon foot pedal command, the density value will be printed to facilitate recording of numerous successive readings. The split-viewing ability of the microscope will allow precise areas on the autoradiographic film to be identified by simultaneously viewing the film and a stained slide of the same brain slice section.

(10) Rodent Activity System. A system is currently under development which will monitor the running wheel activity of 72 rodents. The experiments are important in circadian rhythm studies involving light response on free-running hamsters. Six surplus tissue culture boxes will hold 12 cages each. The running wheel activity in each cage will be recorded with a simple microswitch and interface logic controlled by a 16-bit Plessey system 6100 laboratory computer. In

addition to the 72 running wheels, a fluorescent light source will present the programmed light stimulus and is located in the upper and lower chambers of each of the six units. The computer will monitor each of these lights by means of photodetectors to verify that the lights were on at the proper time. A total of 84 channels of digital data (72 running wheels + 12 light detectors) will be processed by the computer. The data will be stored on one 10 megabyte Winchester hard disc and also on two 1 megabyte floppy discs. In addition, data on each of the 84 channels will be continuously displayed in a printer/plotter using a strip chart simulator.

(11) Ambulatory Lux Monitor. An ambulatory data acquisition system (Vitalog PMS-8) is being used to monitor the temperature of manic-depressive patients. To allow simultaneous recording of the ambient light levels experienced by these patients, a small, micro-power lux meter is being developed as an input transducer for the PMS-8. A wide-angle photodiode with a special photometric filter will be combined with a CMOS logarithmic amplifier circuit to obtain a three decade dynamic range. The microprocessor data processing algorithms employed in the PMS-8 will be modified to handle the light intensity data.

(12) Eye Blink Detector. A device is currently under development to monitor eye blinks in subjects undergoing experimental exposure to light. The light will be administered with a gansfield dome. The device will be used to ensure and to document that the subject's eyes remain open during light exposure sessions lasting approximately one hour. Because light suppresses the pineal secretion of melatonin, it is important to record the total time the eye is open and receiving light. It is planned that a photodetector mounted on a modified eye glass frame will detect changing light reflections off the sclera when the eye is opened or closed. When the output of the detector crosses an adjustable threshold, a counter will record the total time the eye is open during the session. A panel light will also indicate to the person administering the test that the eyes are open.

COMPUTERS

Small computers are ideally suited for laboratory research in neurophysiology and psychology. They are used in the laboratory for on-line, real-time interactions, process control, and data acquisition. Recorded data may be stored, combined with other data, reduced statistically, transferred to larger computers for further analysis, transformed for presentation graphically or mathematically, and the results may be printed or plotted. Increasing use is being made of the small computer for processing the text of scientific papers and communications. Data base management is now available for the small computer, as are limited management information systems.

Techniques have been developed for image processing which are applicable to many diverse experimental systems, ranging from autoradiographs of brain tissue sections to the analysis of two-dimensional electrophoresis gels.

Larger minicomputers, the so-called super-mini's, have been reduced in price and are now available for functions formerly performed by larger time-shared systems. These systems allow applications in modeling, curve fitting and statistical treatment that would be prohibitively expensive on large systems.

Inexpensive personal computers are proving useful for dedicated applications. Many scientists are developing software for these computers, which they offer to the scientific community at low cost. PCs will become increasingly useful in the laboratory and their potential should be exploited.

Microcomputers incorporated in the design of biomedical instrumentation provide a savings in design and fabrication time for instruments, and a more flexible system than one based on discrete components.

The Instrumentation and Computers Section is actively involved in the applications of small computers in the IRP. By integrating the functions of biomedical instrument design and laboratory computer systems with software designed specifically for the research community, the Section offers computer support services for a broad range of scientific disciplines.

LABORATORY COMPUTERS

The design goal for the laboratory instrument computer is to provide maximum function, tailored to the specific experimental design, with minimum cost. ICS provides consultation on the specification and selection of laboratory computers for new applications; conducts systems studies in collaboration with the scientist; and helps the scientist in the procurement, installation and maintenance of the equipment.

In support of these efforts, ICS maintains two support computers, one in Bldg. 36 and one in the Clinical Center. These systems provide the more expensive equipment necessary for off-line data storage, efficient data processing, communications with DCRT computers, and plotting and printing of the data. The systems are run on an open shop basis and are used for program development, training, and testing the feasibility of new systems for the laboratories.

TRAINING AND SOFTWARE SUPPORT

ICS provides training for the scientist or support personnel who will be programming and maintaining the system. Personnel limitations make it impossible for ICS to provide applications programming, so such programming must be supplied by the laboratory. ICS computer personnel are always available for consultation, training and help in debugging, as well as assistance in the selection of part-time programmers or consultants. Commercial software packages or applications from other research labs are often available, and ICS will evaluate such systems.

ICS maintains a library of procedures which were written specifically for the laboratory computers used in the intramural community. These procedures are designed to be incorporated into the users' programs. In addition, ICS will aid the investigator in writing the difficult time and data dependent sections of real-time programs.

PERSONAL COMPUTERS

The Section is evaluating personal computers for potential use in both scientific and administrative applications. Potential scientific applications include data acquisition, experimental control, data analysis and display, graphics terminal emulation, and technical word processing. Potential administrative applications include terminal emulation, data storage and retrieval, spreadsheet analysis, and word processing. The Section has acquired several Apple Macintosh computers and is evaluating them for use with all of the above applications. The Macintosh is an advanced 32-bit design with many advantages over more primitive 16-bit personal computers.

PROGRAM MAINTENANCE

There are now more than 60 minicomputers in the program; many of these systems have been in use for years. The programs used on these systems were written by a number of people, many of whom are no longer in the IRP. Design of these programs is such that changes are usually required as the experimental protocol develops, so program maintenance is a continual and time-consuming function of the Section. Structured programming techniques and standardization of equipment have enabled the Section to provide these services without an increase in personnel.

MICROPROCESSORS

The Section also maintains a microprocessor development system for the software and hardware development of microprocessor-based instrumentation at both the chip and single board computer level. The system currently supports three common microprocessors; one 16-bit processor, and two 8-bit processors. These microprocessors and their associated peripheral chips are now available in CMOS low power versions. This development allows the design of both smaller, more reliable bench instruments and more intelligent portable instrumentation.

The performance of the development system has been enhanced by the addition of a 5M byte Winchester disc. This hard disc provides faster access time as compared to the floppy discs which it replaced and its greater storage capacity allows direct access to all system and commonly-used application software.

IMAGE PROCESSING SYSTEM

The Section on Instrumentation and Computers maintains a general purpose image processing system consisting of an Optronics rotating drum film scanner, a DeAnza image array processor, and a PDP-11/60 computer. Images to be processed may be obtained by scanning autoradiographs, x-ray film, or photographic negatives, or by using images generated by CAT or ECAT scanners. A camera station is available to generate color hardcopy using Polaroid SX-70 or 35 mm film.

Interactive, menu driven, software packages have been developed to provide an extensive and expandable repertoire of basic image processing functions. Special purpose functions can be developed to meet specific user requirements. The facility is useful for numerous applications involving evaluation and quantification of biomedical images. The two primary applications of the system are the densitometric analysis of autoradiographs of brain or tissue sections and the analysis of two-dimensional electrophoresis gels.

The Section is developing a prototype image processing system that will be capable of using these software packages, but will be smaller and less expensive to purchase and operate than the PDP-11/60 based system. It will be based on a PDP-11/23 computer and use a TV camera for digitizing instead of the rotating drum film scanner.

VAX COMPUTER SYSTEM

The Section manages a multi-user VAX-11/750 computer system that is available for use by all investigators in the IRP. The VAX is located in Bldg. 36, in space furnished by the Laboratory of Cerebral Metabolism, NIMH. Potential users in Bldg. 36 may request installation of hard wired cable connections, or the VAX may also be used on a dial-up basis.

A device independent graphics package has been developed on the VAX that permits plots to be generated on numerous display terminals and hard copy devices. A terminal emulation program is available which permits small PDP-11 laboratory computers to function as graphics terminals when using the VAX. This program also supports file transfers in both directions.

The SPICE2 circuit analysis program has been installed on the VAX and is being used by several investigators for modeling neuronal circuits. Programs have been written to generate graphical displays and hard copy plots of the output of the SPICE2 program.

ENGINEERING, COMPUTER AND FABRICATION SERVICES

This table shows the distribution of the Section's workload among the various laboratories and branches. We have listed only the major users.

LABORATORY OR BRANCH	HOURS	PERCENT
Neurophysiology, NIMH	2551	11.00
Clinical Psychobiology, NIMH	2444	10.55
Psychology and Psychopathology, NIMH	1910	8.24
Neurophysiology, NINCDS	1874	8.09
Neuropsychology, NIMH	1668	7.20
Cerebral Metabolism, NIMH	1340	5.78
Biophysics, NINCDS	1299	5.61
Neural Control, NINCDS	1086	4.69
Clinical Science, NIMH	1063	4.58
Adult Psychiatry, NIMH	970	4.18
Neuropathology and Neuroanatomical Sciences, NINCDS	840	3.62
Biological Psychiatry, NIMH	660	2.84
Preclinical Pharmacology, NIMH	633	2.73
General and Comparative Biochemistry, NIMH	497	2.14
Neurochemistry, NINCDS	426	1.84
Molecular Genetics, NINCDS	373	1.62
Neurobiology, NIMH	366	1.57
Surgical Neurology, NINCDS	345	1.49
Clinical Neuroscience, NIMH	272	1.17
Molecular Biology, NINCDS	233	1.01
Clinical Neuropharmacology, NIMH	180	.77
Experimental Therapeutics, NINCDS	155	.67
Neurochemistry, NIMH	116	.50

*NIMH	(Total)	14,777	63.30
*NINCDS	(Total)	6,704	28.61
*NICHD	(Total)**	1,875	8.09
		23,356	100.00

*These figures represent our total effort; they include time for labs not listed individually.

**NICHD loans the Section one position, and is thus entitled to 1700 hours of service.

TAB 2 -- LABORATORY OF BIOPHYSICS -- (LB)

ANNUAL REPORT

October 1, 1983 through September 30, 1984

Laboratory of Biophysics
National Institute of Neurological and Communicative Disorders and Stroke

Table of Contents

Annual Report
October 1, 1983 thru September 30, 1984
National Institute of Neurological and Communicative
Disorders and Stroke
Laboratory of Biophysics
William J. Adelman, Jr., PhD, Chief

INTRODUCTION

The research program of the Laboratory of Biophysics (LB) investigates molecular and cellular mechanisms responsible for excitation, membrane potentials, the generation of the nerve impulse, synaptic activity, axoplasmic and neuroplasmic transport, the biophysical basis for the functioning of simple nervous systems, and the cellular basis for such integrative neural functions as behavior and learning. The laboratory is composed of two units. One of these units operates on a year-round basis at the Marine Biological Laboratory in Woods Hole, Massachusetts. The Woods Hole Unit is composed of 2 sections: the Section on Neural Membranes (NM) and the Section on Neural Systems (NS). The Bethesda unit of the laboratory is made up of the Section on Molecular Biophysics (MB).

LB continues to focus on channel behavior as the basis for neuronal function and thus logically as the basis for the function of ensembles of neuronal cells or neural systems. This overall program of the Laboratory of Biophysics was broadened in the 1970's by applying approaches used to study axons and artificial bilayer membranes to the study of neural systems. Biophysical methods integrated with modern ultrastructural and biochemical techniques were adopted to study complicated mechanisms at fundamental levels. Organizational restructuring of LB in 1974, and the establishment of two sections of LB at the Marine Biological Laboratory in 1975 resulted. In the spring of 1984, LB began another change with the establishment of a neuronal tissue culture laboratory and a synaptic studies program in Woods Hole. Both of these new departures are within the Section on Neural Membranes.

At present, the Section on Molecular Biophysics in Bethesda studies individual channels and their unit conductances. This section also studies membrane conductances or the behavior of channels in ensemble. The Section on Neural Membranes in Woods Hole predominantly studies axonal and synaptic membrane conductances and axoplasmic transport mechanisms with a strong emphasis on structure at resolutions approaching the molecular or atomic level. Both skeletal and cardiac muscle systems are included within this program. The Section on Neural Systems in Woods Hole studies mechanisms by which simple neural systems process information with a major emphasis on learning mechanisms. The Section's main thrust has been cellular electrophysiology with lateral integrations to membrane conductances, microscopic anatomy, integrative behavior and neuronal biochemistry.

Thus, the Laboratory of Biophysics operates over a broad range of basic interests in neuronal function. The insights gained at the channel or molecular level give direction to the membrane studies and the membrane studies give impetus to the neurophysiological and behavioral investigations. These all receive strong input from the Laboratory's investigations in ultrastructure science and biochemistry. These interrelations are not strictly conceptual, as methods, techniques, equipment and personnel also develop in parallel and become

part of the direction of LB. It is hoped that the following summary of highlights of LB's recent accomplishments give evidence that this integrative approach is fruitful.

Section on Neural Membranes.

The Section on Neural Membranes uses electrophysiological, electron optical, mathematical, biophysical, and computer science techniques to investigate the function and structure of neural cells and tissues at limits approaching molecular levels. Model systems are derived, tested and used to simulate neuronal function under a variety of natural and experimental conditions. Subcellular structures supportive of axoplasmic transport and membrane ionic channel function are sought.

The Section has continued its studies on sodium channel gating mechanisms. A new technique, "voltage-activated-resonance", has been developed to resolve intermediate components in gating (asymmetry) currents and thereby determine individual voltage sensitive molecular transitions. Using sine waves at various frequencies, f, and sufficient amplitudes, the "global" gating kinetics have been set in periodic motion. The non-linear gating current response and its harmonic content have given insight into the molecular channel gating process. Several competing gating schemes or theories are being tested rigorously with this method.

The Section also examined the effects of two anti-convulsants on the gating and ionic permeability of sodium and potassium channels. Ethosuximide and valproate were shown to be highly specific in their effects and these effects depended on whether the drug was applied to the internal or the external membrane surface. Ethosuximide applied internally affects Na channel gating, but not as a channel blocker. When applied externally, this drug behaved as a Na channel blocker with no effect on channel gating. Valproate internally also affects both Na and K channel gating, but does not act as an open channel blocker. The ionic channel effects of these drugs are being evaluated in terms of the control of paroxysmal discharge and synchronous impulse generation in neural tissue.

The Section's studies on neuronal structure/function correlations have continued. Good correlation has been achieved between electron microscopical fine structure of axoplasm and the effluent and residual proteins (as determined by SDS-PAGE) in "chemical dissection" experiments involving extraction with physiological buffer, activation of a resident protease specific for neurofilaments, and trypsin treatment which cleaves microtubule-associated protein cross-bridges between neurotubules. The Section's capability in using video-enhanced differential interference contrast light microscopy has increased and has led to several new findings. Implementation of tomographic 3-dimensional electron microscopy of thick sections has been set into motion and further correlations between fine structure and living axoplasmic transport are expected. A model system using DMSO-treated glycerinated axons has been achieved for studying axoplasmic transport in squid axons.

A new program studying chemical transmission in the squid giant synapse has been started. Taking advantage of a novel method developed in the section for arterial perfusion of the synapse to achieve rapid pharmacological access to the synapse, the identification of the neural transmitter is now under way.

Making use of high pressure liquid chromatography, chemicals released from the presynaptic terminal into the perfusate during direct depolarization are being analyzed. Cyclic AMP or serotonin-induced enhancement of transmitter release is being studied using voltage clamp methods applied to the synaptic membranes.

With the establishment of a tissue culture laboratory in the section, there is promise of the culture of squid neurons suitable for both axoplasmic transport and structure studies and for eventual patch clamp electrical studies of single ionic channels. If successful, these cultured neurons would be used in at least three other projects now supported by the section.

One of these projects is concerned with comparing how channel activity leads to repetitive and rhythmical activity in heart and neuronal cells. This study has revealed that K channels and two potassium currents are important in this respect. The study has provided a framework for analyses of the effects of drugs (particularly antifibrillatory agents) on nerve and cardiac tissue. The antifibrillatory compound, bretylium tosylate, which produces a marked K channel blockade in squid axons, has figured prominently in these studies. While antifibrillatory compounds (bretylium, behamidine, meobentine) produce a marked block of K current in squid axons, primary antiarrhythmics (lidocaine, procainamide) do not produce a comparable effect and are not effective antifibrillatory agents. These results suggest a new focus on K channel blockade in the design and application of antifibrillatory drugs.

In a completely different direction, it now appears that internal fluoride produces a reduction of a 30K dalton membrane protein which is correlated with a reversible loss of K channel conductance. These experiments may lead to the identification of a specific membrane protein as involved in the potassium channel process.

Experiments are continuing examining the role of quaternary derivatives of lidocaine (QX572) in blocking sodium channels. The data now suggest that there is a new understanding emerging as to the nature of site specific interaction of such agents with sodium channels.

These brief highlights of the Section on Neural Membranes' activities indicate that the range of approach from channel molecular resonances to basic pharmacology is productive and that the structure/function relations important to neural function and dysfunction are beginning to be understood in a basic way.

Section on Neural Systems.

The Section has as its principle goal the study of mechanisms, whereby simple neural networks process information with particular emphasis on mechanisms of learning. The Section uses a variety of integrated techniques in this approach which range from electrophysiological studies of membrane currents to behavioral studies of whole animals. The Section has primarily devoted its attention to the marine invertebrate nudibranch, Hermissenda crassicornis, because of the small size and relatively simple organization of its nervous system.

The nervous system of Hermissenda crassicornis has proven to be a good model for information processing at several levels: sensory transduction by photoreceptors and hair cells, analysis of synaptic circuitry, changes in

synaptic circuitry produced by conditioning paradigms administered to intact animals, as well as to isolated nervous systems, membrane properties modified by conditioning, identification of critical developmental stages for the neural networks of interest, as well as stages critical for learning. Techniques employed thus far to pursue these questions include simultaneous intracellular recording from multiple neural elements, paired stimulation of the visual and vestibular pathways using a rotating table, iontophoresis of fluorescent dyes and electron dense materials, electron microscopy, automated behavioral monitoring of intact Hermissenda, voltage clamp of identified neural elements. Other methods include mariculture, subcellular fractionation, protein phosphorylation analysis, uptake of neurotransmitter precursors, phosphoprotein characterization and purification, and immunologic protein identification. Patch clamp of membrane fragments of idnetified neurons is also being combined with enzymatic regulation of specific channels changed by learning to determine molecular mechanisms for encoding associatively learned information. Analogous protocols are also conducted with brain slices from neuronal aggregates which mediate classical conditioning of the rabbit nictitating membrane.

Section on Molecular Biophysics.

The Section continues to focus on the molecular mechanisms underlying the behavior of membrane ionic channels and of drugs that interact with these channels. During the past year, the Section's interests have broadened to include a wider range of channels. Thus, in addition to studies on single channels in tissue-cultured cells, channels in plant cells and in egg cells have also been studied. This broadened interest has allowed the use of methods that have been developed for excitable cells to study a number of interesting questions regarding other cells. Thus, a broader perspective on the similarities and differences between ionic channels with different functions is being achieved.

Regarding tissue-cultured cells, the main efforts in the past year have been on the study of voltage-dependent potassium channels, GABA-activated inhibitory postsynaptic channels, and BTX-modified sodium channels. Long-lined potassium channels related to the slow potassium currents of neuroblastoma cells were observed. These channels are gated by voltage and appear to have many of the properties of the potassium channels which regulate pacemaker bursting frequency. One feature of these channels, which was first seen macroscopically by Moolenaar and Spector, is the slow inactivation similar to the process first observed in squid axon. Using patch clamp, slow decline in probability of single channel opening has been observed which corresponds to this phenomenon. By use of non-stationary stochastic analysis the Section is testing whether slow inactivation is qualitatively similar to Na channel inactivation. The GABA response is thought to be potentiated by the clinically important drug, diazepam. Noise experiments and iontophoretic experiments by others suggest that diazepam acts at an allosteric site to enhance GABA-induced channel opening in some way. Single channel experiments to date show that channel conductance and open-state lifetimes are not altered by diazepam, so that the pharmacological activity may reside in more subtle channel interactions.

Previous work in the Section on batrachotoxin-modified sodium channels in neuroblastoma cells determined detailed kinetic properties of the channels. In particular, the closing rate of these channels is an exponential function of membrane potential, closing faster for increasing hyperpolarization of the

membrane. The closing rates for single channels varied by about an order of magnitude from patch to patch. This raised the general question as to the source of variability of channel properties from patch to patch. Part of this general question has been addressed by considering patches with exactly two channels and determining how much variability there is between these two channels in the same patch. The data is consistent with the hypothesis that the closing rates of two channels in the same patch are equal. Because of experimental limitations, the possibility that the closing rates of the two channels differ by as much as a factor of 2 cannot be ruled out, but the large (tenfold) difference found between channels in different patches can be excluded.

Flickering voltage-gated channels in a myeloma cell line derived from lymphocytes has been observed. These channels are typical of gated channels observed in a wide variety of inexcitable cells. Current experiments are directed toward identifying the channel types and explaining their role in maintaining cell resting potential or in the immune response.

A new single-channel project involves wheat protoplasts - plant cells whose cell walls have been enzymatically removed. Although the protoplasts are considerably more fragile than typical tissue-cultured cells used in patch clamp experiments, these protoplasts have been patched and single-channel records indicating the presence of several different types of channels have been obtained. These include a voltage-dependent channel.

The Section also investigates the possibility that channels may be present in egg cells by examining the response of sea urchin eggs to insemination by sperm, and by comparing this response with the response to injection of sperm components. This is the first step in an attempt to understand the process by which sperm causes an increase in intracellular calcium (and, hence, many important biological processes triggered by this increase). Examination of the fertilization literature indicates that it is likely that channels in intracellular organelles are involved. Formation of a fertilization membrane similar to that formed by insemination following injection of the soluble fraction of homogenized, centrifuged sperm has been observed experimentally. The fact that injected sperm extract is effective indicates that the site of action is inside the egg.

Voltage-clamped squid giant axons were used to study the effects on sodium channels of a large number of analogs of the drug, yohimbine. From the structure-activity relationships of these analogs, tentative conclusions regarding various chemical groups of the drug molecule were drawn. For example, the nitrogen in position 4, the $COOCH_3$ group in position 16, and the OH group in position 17 appear to be important for the use-dependent effect.

In addition to the experimental work described above, mathematical modeling has continued so as to improve data analysis and to predict the behavior of hypothetical models for comparison with experimental data. An example of the former is the detection and analysis of single-channel square wave currents, distorted by noise and low-pass filtering. An example of the latter is the calculation of the expected time course of the rise and fall of intracellular calcium for a specific model involving the release of calcium from intracellular organelles following opening of channels in the membranes of these organelles by agonists.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 01950-13 LB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line betwaen the borders.)*
Excitable Membrane Characteristics: Voltage Clamp and Impedance Measurements.

PRINCIPAL INVESTIGATOR *(List other professional personnal below tha Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	W. J. Adelman, Jr.	Chief	LB, NINCDS
Others:	J. R. Clay	Senior Staff Fellow	LB, NINCDS

COOPERATING UNITS *(if any)*
University of Minnesota (J. Fohlmeister); Marine Biological Laboratory, Woods Hole, MA (C. Tyndale, R. Waltz); Emory University (L. DeFelice); Hamline University (J. Brennan)

LAB/BRANCH
Laboratory of Biophysics, IRP

SECTION
Section on Neural Membranes (located at MBL, Woods Hole, MA 02543)

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.6	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Voltage clamp experiments were performed to evaluate and compare the effects of the anticonvulsants ethosuximide and valproate on the gating and permeability of the excitable Na and K channels of the squid giant axon. The drugs were shown to be highly specific in their effects on channel gating and ion permeability with regard to membrane side of application. Both drugs, when applied internally, affect the Na channel activation gating in ways that lead to the conclusion that they do not also act as channel blockers. However, external ethosuximide is clearly a voltage-independent Na channel blocker with no effect on channel gating. On the K channel, ethosuximide appears to have a mixed action affecting both gating and the ion flux through open channels. However, valproate slows K channel gating without effect on flux through open-gate channels. The Na channel results were confirmed by gating current measurements. The dose-response curve of the effects is similar to that of ethanol, although the anticonvulsant data are for much lower concentrations. These results suggest important implications for drug control of paroxysmal discharge and synchronous impulse generation in neural tissue. Some of the quaternary derivatives of lidocaine (QX572) produce a differential block of squid axon sodium current depending upon whether they are placed internally or externally. External QX572 produces a tonic block of I_{Na}, whereas internal QX572 produces a phasic block. If it is true that local anesthetics have only a single blocking site, and that they must cross the membrane to reach that site, then the qualitative nature of the block should be independent of where they are placed. These preliminary results suggest that something different is occurring, although the exact nature of this process is still to be uncovered.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02087-11 LB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Function and Structure of Ionic Channels: Ion Interactions and Gating Mechanisms.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	W. J. Adelman, Jr.	Chief	LB, NINCDS
Others:	J. R. Clay	Senior Staff Fellow	LB, NINCDS

COOPERATING UNITS *(if any)*
University of Minnesota (J. Fohlmeister); Marine Biological Laboratory, Woods Hole, MA (C. Tyndale, R. Waltz); University of Maryland (M. Shlesinger).

LAB/BRANCH
Laboratory of Biophysics, IRP

SECTION
Section on Neural Membranes (located at MBL, Woods Hole, MA 02543)

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.8	0.2

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Voltage clamp experiments are employed to determine the functional and structural characteristics of ionic channels in the squid giant axon. Information concerning these characteristics of the ionic channels is gained by studying the interaction of ions which block the passage of normal charge carriers and by studying the effect of voltage upon the opening and closing ("gating") of channels. The sodium conductance was isolated by the use of potassium-free solutions and voltage-clamped with pulses containing three levels of depolarization. The conductance rapidly changed during certain repolarizing clamp steps in the gating range. The percentage change in conductance increased with time of depolarization from ∿ 0 to ∿ 25-30% at 7 ms for a potential step from +70 to -30 mV. Conductance steps were also observed for voltage steps from various depolarized levels to -70 mV. All observed shifts were in the direction of a decreased conductance. The conductance steps appear to be a weak function of the concentration of external calcium, which also acts as a voltage-dependent channel blocker for inwardly directed sodium currents. These results suggested a voltage- and time-dependent molecular process that does not itself yield open or closed channel conformations, but that affects the magnitude of the rate constants that do connect open and closed state conformations. The technique of "voltage-activated-resonance" in nerve membranes is being developed for the purpose of resolving gating (or asymmetry) currents into components corresponding to individual voltage-sensitive molecular transitions. The forcing functions are sinusoidal changes in the electric field generated by a voltage clamp. The output is the asymmetry current component of the dielectric displacement current that is generated at the stimulus frequency, f. For sufficiently large voltage amplitudes ($\gtrsim$ ± 30 mV) the "global" gating kinetics are set into periodic motion whose non-linear response (expressed as harmonic content) depends on kinetic feedback patterns generated by the molecular gating process. The actual gating process will thus be determined by a comparison with model simulations.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02092-11 LB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Subcellular and Extracellular Structure Associated with Nerve and Muscle.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: W. J. Adelman, Jr. Chief LB, NINCDS

COOPERATING UNITS *(if any)*
Marine Biological Laboratory, Woods Hole, MA (A. Hodge, R. Waltz, C. Tyndale); Case Western Reserve (R. Lasek); Dartmouth College (R. Allen); University of Toronto (C. Govind)

LAB/BRANCH
Laboratory of Biophysics, IRP

SECTION
Section on Neural Membranes (located at MBL, Woods Hole, MA 02543)

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.9	3.7	0.2

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this project is to examine the subcellular and extracellular structure of nerve and muscle and relate such structure to function. Electron microscopy in TEM, STEM and analytical electron beam probe modes, such as EELS and EDAX, determination of proteins contributing to these structures and structural modeling are methods used in this study. The following structures are probed: 1) Neuroplasmic lattice, 2) neurofilaments, 3) microtubules, 4) axolemma, 5) glial cell membranes, and 6) myofilaments. Methods developed and used in this study are: 1) Stereoscopic imaging, 2) optical autocorrelation, 3) fast Fourier transformation (FFT) of STEM video images, and 4) STEM video image filtering and image enhancement using reverse Fourier transformation. Video imaged light microscopy is used to study living neurons in dark field or differential interference contrast. In diffusion experiments using whole squid giant axons, the combined application of EM and EDAX has shown that ferritin molecules (∿ 110A diameter) penetrate the sheath complex (including the basement lamella) only after trypsin treatment, but not the intercellular spaces between Schwann cells, their cytoplasm, or the axoplasm. Good correlation has been achieved between the EM fine structure of axoplasm and the effluent and residual proteins (as determined by SDS-PAGE) in "chemical dissection" experiments involving extraction with physiological buffer, activation of a resident protease specific for neurofilaments, and trypsin treatment, which cleaves microtubule associated protein (MAP) cross-bridges between neurotubules. Video-enhanced DIC microscopy (VEDIC), in conjunction with electron microscopy of squid and lobster axons, suggest a close linkage of fast axonal transport (FAT) with the neurotubular component of the neuroplasmic lattice. Preliminary VEDIC observations indicate the feasibility of developing a glycerol/DMSO model system for FAT using squid axons.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02273-08 LB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

An Investigation of Electro-Mechanical Coupling in Excitable Tissues.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: J. B. Wells Research Physiologist LB, NINCDS

COOPERATING UNITS *(if any)*

Marine Biological Laboratory, Woods Hole, MA; State University of New York (D. E. Goldman).

LAB/BRANCH

Laboratory of Biophysics, IRP

SECTION

Section on Neural Membranes (located at MBL, Woods Hole, MA 02543)

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.6	0.6	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is herewith terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02606-01 LB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Chemical Transmission at the Squid Giant Synapse.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	E. F. Stanley	Visiting Scientist	LB, NINCDS
Others:	W. J. Adelman, Jr.	Chief	LB, NINCDS

COOPERATING UNITS *(if any)*
Marine Biological Laboratory, Woods Hole, MA (C. L. Tyndale).

LAB/BRANCH
Laboratory of Biophysics, IRP

SECTION
Section on Neural Membranes (located at MBL, Woods Hole, MA 02543)

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.6	0.5	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The squid giant synapse has served as a model for the understanding of the physiology of synaptic transmission but the transmitter acting at this synapse has not as yet been identified. The lack of pharmacological studies on this preparation is due primarily to the high diffusion barrier between the synapse and the bathing medium. In this study we are taking advantage of a novel method of pharmacological access to the synapse by arterial perfusion to identify the transmitter substance. First, we analyze, by high pressure liquid chromatography, chemicals released from the axon terminal into the perfusate during direct depolarization. Second, we compare the pharmacological action on the postsynaptic giant axon of putative transmitter substances identified in the perfusate with those of the endogenous transmitter. In addition, we are using arterial perfusion to examine the cAMP or serotonin-induced enhancement of transmitter release from the presynaptic axon terminal and the changes in ionic currents associated with this enhancement are explored by the voltage clamp technique.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02607-01 LB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Structure and Function of Tissue-Cultured Invertebrate Neurons.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	W. J. Adelman, Jr.	Chief	LB, NINCDS
Others:	R. V. Rice	IPA Fellow	LB, NINCDS

COOPERATING UNITS *(if any)*
Marine Biological Laboratory, Woods Hole, MA (J. Harrigan and R. Mueller); University of Hawaii (J. Arnold).

LAB/BRANCH
Laboratory of Biophysics, IRP

SECTION
Section on Neural Membranes (located at MBL, Woods Hole, MA 02543)

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.8	0.7	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The aim of this project is to culture neurons in the laboratory for use in studies of axoplasmic structure and transport. These cultured neurons are also to be used in connection with voltage clamp and patch clamp experiments of ionic channels and their conductances and gating mechanisms. A tissue culture (TC) laboratory has been established. A sterile tissue culture hood, CO_2 incubator, refrigerators and freezer all restricted to TC use is augmented by U.V. germicidal lights and sterile techniques. Plastic TC flasks, dishes, pipettes, filters, etc. are essential because of lack of proper TC washing facilities. Autoclaves and a sterilizing oven are available. Squid embryos are cultured up to hatching in a separate sea water table. Squid fertilized fingers are decontaminated with dilute bleach (0.6%) in artificial sea water (ASW), dissected from the egg jelly and chorions with iridectomy scissors and washed with ASW containing concentrated antibiotics in sterile leucocyte tubes under the hood. Standard TC medium is Hanks minimal essential amino acids and vitamins dissolved in ASW sterilized via 0.22 μm filters. Fetal bovine serum and chick embryo extract is added as needed for squid embryo cell growth and maintenance. Whole embryos remain alive in the medium for one to two weeks. The culture of neurons is still in the early phase but appears promising.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02608-01 LB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Comparative Aspects of Ionic Conductances in Nerve and Heart Cell Membranes.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: J. R. Clay Senior Staff Fellow LB, NINCDS

COOPERATING UNITS *(if any)*
Marine Biological Laboratory, Woods Hole, MA (R. Mueller, C. Tyndale); McGill University (A. Shrier); University of Maryland (M. F. Shlesinger); University of Minnesota (M. B. Bacaner).

LAB/BRANCH
Laboratory of Biophysics, IRP

SECTION
Section on Neural Membranes (located at MBL, Woods Hole, MA 02543)

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.4	1.3	0.1

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is based on a comparison of sodium and potassium ionic currents which underlie excitability in nerve and heart cell membranes. The experimental preparations used in this work include squid giant axons and embryonic heart cells. Measurements of membrane current kinetics and rectifier properties in these cells have been described. For example, the currents which underlie the repolarization phase of the action potential in embryonic heart cells have recently been determined. Two potassium currents are involved in this process. One component is similar to potassium current in nerve; the other component is similar to the inward rectifier of skeletal muscle. These results have been incorporated into mathematical models which have been used to simulate heart cell excitability. Potassium current kinetics have also been measured in squid axons with a particular emphasis on the effects of external potassium ions on potassium current kinetics. These basic studies provide a framework for analyses of the effects of drugs on nerve and cardiac tissues. For example, the mechanism of antifibrillatory drug action on the heart has recently been investigated. Antifibrillatory compounds, such as bretylium tosylate, produce a marked blockade of potassium current in squid axons. Other drugs, such as lidocaine, which do not produce a strong antifibrillatory action on the heart, do not produce a significant block of potassium current. These results suggest that modifications of the potassium component are important in the treatment of some cardiac arrhythmias.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02151-10 LB

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Information Processing in Simple Nervous Systems

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	D.L. Alkon	Medical Officer	LB NINCDS
Others:	J. Acosta-Urquidi	Visiting Associate	LB NINCDS
	R. Forman	Staff Fellow	LB NINCDS
	A. Kuzirian	Staff Fellow	LB NINCDS
	S. Naito	Special Expert	LB NINCDS
	M. Sakakibara	Visiting Fellow	LB NINCDS

COOPERATING UNITS *(if any)*
Marine Biological Laboratory, Woods Hole, MA 02543 (J. Harrigan, I. Lederhendler, J. Neary); Northwestern University School of Medicine (J. Disterhoft); Boston University Marine Program (D. Coulter)

LAB/BRANCH
Laboratory of Biophysics, IRP

SECTION
Section on Neural Systems (located at MBL, Woods Hole, MA 02543)

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
9.0	8.5	0.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The principal objective is to study the mechanisms by which simple neural networks process information with particular emphasis on mechanisms of learning. The nervous system of Hermissenda crassicornis has proven to be a good model for information processing at several levels: sensory transduction by photoreceptors and hair cells, analysis of synaptic circuitry, changes in synaptic circuitry produced by conditioning paradigms administered to intact animals, as well as to isolated nervous systems, membrane properties modified by conditioning, identification of critical developmental stages for the neural networks of interest, as well as stages critical for learning. Techniques employed thus far to pursue these questions include simultaneous intracellular recording from multiple neural elements, paired stimulation of the visual and vestibular pathways using a rotating table, iontophoresis of fluorescent dyes and electron dense materials, electron microscopy, automated behavioral monitoring of intact Hermissenda, voltage clamp of identified neural elements. Other methods include mariculture, subcellular fractionation, protein phosphorylation analysis, uptake of neurotransmitter precursors, phosphoprotein characterization and purification, and immunologic protein identification. Patch clamp of membrane fragments of identified neurons is also being combined with enzymatic regulation of specific channels changed by learning to determine molecular mechanisms for encoding associatively learned information. Analogous protocols are also conducted with brain slices from neuronal aggregates which mediate classical conditioning of the rabbit nictitating membrane.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02088-11 LB

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Function and Structure of Membrane Ionic Channels

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	G. Ehrenstein	Research Physicist	LB NINCDS
Other:	N. Moran	Visiting Associate	LB NINCDS
	K. Iwasa	Senior Staff Fellow	LB NINCDS

COOPERATING UNITS *(if any)*

Weed Science Laboratory - AEQI, Dept. of Agriculture, Beltsville, MD.
(C. Baire and C. Mischke)

LAB/BRANCH

Laboratory of Biophysics, IRP

SECTION

Section on Molecular Biophysics

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.8	2.6	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Summary:

Channels in two types of systems were studied. One system is the wheat protoplast - a wheat cell whose cell wall has been removed - and the other is the BTX-modified sodium channel in neuroblastoma cells. In the wheat protoplast, using single-channel techniques, we found several different types of channels, including a voltage-dependent channel. In our study of BTX-modified sodium channels, we examined patches of membrane containing two channels and found that the closing rates of two channels within the same patch are very similar, in contrast to our previous finding that the closing rates of channels from different patches differ by as much as an order of magnitude.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02091-11 LB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Mathematical Modeling

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

R. FitzHugh Research Physicist LB NINCDS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Biophysics, IRP

SECTION
Section on Molecular Biophysics

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.1	1.0	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Mathematical modeling of the following phenomena was done:

Signal detection and analysis of the square wave currents from single channels opening and closing in a membrane, distorted by noise and low-pass filtering.

The spread of the fertilization membrane, through release of calcium, over the surface of a spherical marine egg.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02218-09 LB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Effect of Drugs on Voltage-Dependent Ionic Conductance in Membranes

PRINCIPAL INVESTIGATOR *(List other professionel personnel below the Principal Investigator.) (Name, title, leboratory, and institute affiliation)*

D.L. Gilbert Research Physiologist LB NINCDS

COOPERATING UNITS *(if any)*
R. J. Lipicky, Food and Drug Administration; E. Wenkert, Dept. of Chemistry, UCLA at San Diego; H. Pant, National Institute on Alcohol Abuse and Alcoholism, ADMHA

LAB/BRANCH
Laboratory of Biophysics, IRP

SECTION
Section on Molecular Biophysics

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.8	1.5	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standerd unreduced type. Do not exceed the space provided.)*

The purpose of this project is to better understand how drugs affect the mechanisms of the ionic conductance in membranes which are voltage-dependent and excitable. These studies involve the use of the squid giant axon. In particular, we have studied the structure-activity-relationship of the use-dependent drug, yohimbine, which also exhibits a frequency independent effect or tonic effect. There appears to be at least two different receptors involved in these phenomena.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: ZO1 NS 02317-07-LB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Excitable Membranes and Ion Channels in Cultured Nerve and Muscle Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	H. Lecar	Research Physicist	LB NINCDS
	G. Redmann	Postdoctoral Fellow	LB NINCDS

COOPERATING UNITS *(if any)*

LN NINCDS; Tissue Transplantation Program Center, NMRI (S. Yeandle); Dept of Medicine, Wash. Univ. (S. Misler); Dept of Biology, Univ. of Ottawa (C. Morris).

LAB/BRANCH

Laboratory of Biophysics

SECTION

Section on Molecular Biophysics

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.8	1.4	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the spece provided.)*

Single-channel currents are measured in isolated areas of excitable-cell membranes using the patch electrode method. Stochastic activation of gated ionic channels is studied as an indicator of the molecular conformation changes underlying excitation in the nervous system. Inhibitory postsynaptic channels from mouse spinal cord neurons and electrically activated potassium channels from neuroblastoma and myeloma cells have been the main objects of study. Modification of channel gating by pharmacological agents and neurotransmitters is studied as a means of establishing a picture of synaptic integration based on the properties of membrane ionic channels.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02526-03 LB

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Gated Ionic Channels in Membranes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

R. E. Taylor	Research Physiologist	LB NINCDS

COOPERATING UNITS *(if any)*
Dept. of Physiology, UCLA, Los Angeles, CA (F. Bezanilla, J.R. Stimers and R.M. Torres) Marine Biological Laboratory, Woods Hole, MA

LAB/BRANCH
Laboratory of Biophysics

SECTION
Section on Molecular Biophysics

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.4	1.0	0.4

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The results of measurements of ionic and gating currents in the membrane of the squid giant axon treated with pronase to remove inactivation were analysed and a report was given at the Biophysical Society Meeting in 1983. A manuscript of these results has been submitted.

We are continuing the study of the effects of increased outside osmolarity. The results are impressive and we feel that they are due to improvement in the uniformity of the spatial control of the voltage clamp resulting from expansion of the space between the membrane of the axon and that of the Schwann cell.

In 1983 we were able to record sodium current fluctuations with good bandwidth using the cut-open axon and to extract functional channels and incorporate them into bilayers. This work will continue.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02609-01 LB

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Mechanism of Egg Activation Following Fertilization

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: G. Ehrenstein Research Physicist LB NINCDS

COOPERATING UNITS *(if any)*

Emory University, Atlanta, GA (L. DeFelice)
Stazione Zoologica, Naples, Italy (B. Dale)

LAB/BRANCH
Laboratory of Biophysics, IRP

SECTION
Section on Molecular Biophysics

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.6	0.4	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Fertilization membranes form around unfertilized sea urchin eggs after micro-injection of a soluble spermatozoa fraction isosmotic with seawater. This demonstrates that the spermatozoon contains a chemical that triggers an increase in cystosolic calcium, leading to exocytosis of cortical granules. It also demonstrates that the triggering mechanism does not require an externally-activated egg-membrane process. Further experiments show that the chemical trigger is not calcium.

TAB 3 -- LABORATORY OF CENTRAL NERVOUS SYSTEM STUDIES -- (LCNSS)

ANNUAL REPORT

October 1, 1983 through September 30, 1984

Laboratory of Central Nervous System Studies

National Institute of Neurological and Communicative Disorders and Stroke

Table of Contents

ANNUAL REPORT
October 1, 1983 through September 30, 1984
Laboratory of Central Nervous System Studies
National Institute of Neurological and Communicative Disorders and Stroke

The major accomplishments of our laboratory over the past year have been as follows:

We continued to define the world-wide problem of human disease caused by the zoonosis of hemorrhagic fever with renal syndrome (HFRS) which we have renamed muroid virus nephropathy. Previously our laboratory demonstrated that HFRS was the most important zoonosis and one of the most important virus diseases of all provinces of China and caused by the same virus as that of Japan, Korea, and Far Eastern Siberian USSR. In the past year we have demonstrated that the Hantaan virus causing the Far Eastern form of HFRS is present in urban rats of most American cities. We have isolated in laboratory rats and tissue cultures and characterized a new virus of the Bunyamwera group of viruses antigenically related to Hantaan virus. We called the new isolate Prospect Hill virus after the Frederick property where we found the type strain in indigenous, wild American rodents. The further clinical, virological and epidemiological elucidation of this world-wide problem and the extension of it to the Americas will occupy dozens of laboratories for the next several decades.

The Prospect Hill virus has yielded a nephropathy model with protein, urea, and nitrogen retention in inoculated chimpanzees and cynomolgus monkeys; many other species of monkeys have been inoculated to determine their susceptibility. This is the first nephropathy model of a Hanvirus of the Bunabunyaviridae. Prospect Hill virus has had its three single stranded RNA segments of its genome sequenced at the 3'-OH terminal for 15 to 20 nucleotides. It has thus proved to be a classical member of the Hanvirus group. We have adapted the virus of nephropathica epidemica of Scandinavia to tissue cultures and are passaging it serially in Mongolian gerbils as well as in laboratory-bred Clethronomys.

In work on kuru, our most significant new contribution has been the clear documentation of incubation periods of thirty years and more in human kuru and the identification of the contaminating episode for several dozen patients occurring in recent years. We discovered that the great majority, in fact over ninety percent, of the infants and children of women present at a contaminating event of cannibalism have already come down with kuru. Continued surveillance has revealed no alteration in the pattern of kuru, the disappearance of which emphasizes the artifical man-made nature of the epidemic; kuru virus clearly has no reservoir in nature and no intermediate natural biological cycle for its preservation except in humans.

On Creutzfeldt-Jakob Disease, our continued epidemiological work has made it clear that the one per million per annum incidence and death rate is approximately the same on all six continents in all nations and that high incidence foci are a real phenomenon. We have further demonstrated that in familial cases a single autosomal-dominant gene pattern of occurrence is indeed true in spite of the fact that the disease is caused by a virus. This is the first example in man of an autosomal-dominant single-gene inheritance controlling the appearance of an infectious disease.

The enormous resistance of the unconventional viruses causing kuru and Creutzfeldt-Jakob disease of man and scrapie in animals has resulted in altered procedures in all autopsy rooms, surgical theaters and clinics in the world. Our continued study of the inactivation and the physical properties of these agents is thus mandatory in order to set the proper standards for handling possible contamination.

The problem this resistance to inactivation may cause has reached enormous proportions with respect to the hepatitis B vaccine prepared from the hepatitis antigen in serum of human volunteers; some of these volunteers may be incubating the Creutzfeldt-Jakob dementia syndrome. Once this has been suggested, it is apparent that there is no assay procedure sufficient to declare the vaccine safe. Even a chimpanzee assay would require decades and still be uncertain, as shown by our newer work on variation in host range of human strains of Creutzfeldt-Jakob disease.

Our work with primates shows that peripheral routes of inoculation give irregular "takes" and, as expected, are associated with long incubation periods of perhaps one or more decades. We pointed out that an accident with this type of virus actually resulted in tens of thousands of cases of fatal scrapie in British sheep previously free of the disease when a formalinized louping-ill vaccine was contaminated with the scrapie virus. The moral, ethical and legal aspects of continuing to use the hepatitis B vaccine once this problem has been raised and appreciated are enormous.

Determining physical chemical structure of the unconventional viruses using both a mouse-adapted strain of CJD virus and hamster and mouse strains of scrapie virus has been the major target of our laboratory. Recent highly-publicized speculations on the possible very exotic nature of these viruses are based in large degree on our data. Those speculations are ideas we have voiced over many years, but they are all still unprovable. Our own recent data again confirm the absence of any immune response to purified, high-titer virus or any involvement of the immune system in patients with the natural diseases or animals with experimental diseases. We have also been unable to demonstrate a nucleic acid by transfection and annealing (hybridization) techniques. By ultrasonication studies we found the high level of association of the hydrophobic viral particles into aggregates of 1000 monomers or more; this finding invalidates most of the studies in which an extremely small size has been determined by physical means, including equilibrium sedimentation, and also invalidates conventional interpretations of radiation resistance and chemical and enzyme resistances as well. On the other hand, it is clear that a new group of microbes has been defined that challenge the basic tenets of microbiology. Exotic new possibilities suggested by the scrapie virus include abnormal templates for laying down of plasma membranes and neurofilament, small proteins free of nucleic acids which are derepressors of cellular genes responsible for their own synthesis, or the first example of a filamentous virus in mammals. As a major problem for basic medical science, the resolution of this enigma is an inescapable challenge. Our most recent observation of unique helical fibrils in extracts of brains and spleens of animals with scrapie, kuru, and Creutzfeldt-Jakob disease, but not in controls, opens a new and promising possibility that the pathogenic agents themselves have finally been recognized and are a new form of pathogens--"filamentous viruses".

Our epidemiological studies of scrapie in France and elsewhere have revealed that scrapie virus is nearly ubiquitous in butcher shops and restaurants of the world. That it may be responsible for occasional disease in primates has not been epidemiologically established. Yet we now know from our own inoculations that the human viruses of CJD or kuru can cause scrapie in goats, and that goat, sheep and mouse strains of scrapie can cause the Creutzfeldt-Jakob syndrome in several species of monkeys inoculated but not yet in chimpanzees. We have participated in the study of the transmissible scrapie-like agent affecting wild mule deer and moose in Colorado, and in the enormously intriguing demonstration that such infected mule deer develop amyloid plaques in great profusion, as do kuru victims and a portion of the CJD patients.

Our study of the auto-immune antibodies to 10-nm neurofilaments in human patients or experimental animals with kuru, CJD and scrapie, has been extended. Autoantibodies are specifically directed against the 200,000-dalton protein subunit and not to two other components of the 10nm neurofilament. This very specific autoantibody appears in about one-fourth of patients with many other gray matter diseases, as opposed to over one-half of the kuru and CJD patients, and with very much lower incidence in normal control populations or patients with other autoimmune disorders. Thus, much more work on the significance of this enormously specific autoimmune response is now necessary and in progress.

Using monoclonal antibodies developed in this laboratory to various cytoskeletal structures of postnatal and adult hamster brains, it has been possible to study the migrations and maturation of neural cells during neurogenesis. We are currently studying inborn errors of metabolism in specific genetic lines of animals with neurological deficits as well as animals born of "slow-virus" infected mothers.

Our work on the high-incidence foci of amyotrophic lateral sclerosis and Parkinson's disease has led to the further confirmation that in these places there is premature aging of the population with early appearance of neurofibrillary tangles in brain. We have now identified the pathogenic mechanism involved in these foci, which has been demonstrated at the epidemiological level to involve early life (in utero ontogenesis, infancy, childhood, adolescence) spent in environments enormously deficient in calcium and magnesium, in "primitive", isolated cultures with no outside food sources and from which the patients have never traveled. With the change in social and economic conditions after World War II in the Japanese Kii peninsula focus and among the Chamorro people on Guam, it is now clear that the calcium and magnesium deficiency no longer pertains and this accounts for the enormous decline in incidences of both diseases. No such decline has occurred in New Guinea, where the focus of both diseases is much more intense, except in one village; people in that village moved away from the region and changed their environmental exposure and economic status and were exposed to imported foodstuffs. This hypothesis is clearly substantiated by environmental analyses of soil, drinking water and foodstuffs. Using neutron-dilution analyses and electron probe x-ray activation spectrography, it has now been demonstrated that hydroxyapatites containing calcium and aluminum and other di- and tri-valent cations are deposited and remain in neurons, particularly in those that develop neurofibrillary tangles. Thus, early parathyroid adjustment required for life in the calcium-deficient environment renders the host vulnerable to heavy-metal intoxication with deposition of heavy-metals and calcium in neurons and seems to

lead to the premature aging of the brain (the appearance of neurofibrillary tangles), and degenerative disease syndromes of the CNS. The implications of these discoveries for the study of motor-neuron diseases, parkinsonism-dementia and of the aging process itself are enormous and have already influenced research.

Our collaborative work on the use of viral nucleic-acid probes for demonstrating by in situ hybridization the presence of genomic copies of viruses in neurons has led to an extremely important discovery. By In situ hybridization, copies of viral genomes were identified in neurons of control subjects, rather than in Guamanian ALS and PD and American ALS brain specimens. This finding casts a shadow over that whole methodological approach to all virology of chronic human diseases.

Our studies on the introduction of cysticercosis into previously virgin populations of Papua New Guinea and West New Guinea demonstrated a self-limited form of grand mal epilepsy in older children and adults, which is undoubtedly caused by the larval migrans phase of pig tapeworm infestation at a period before real cysts have developed in the brain. This self-limited disease requires no antiepileptic therapy, and the patients are left with no further seizures and no other obvious sequelae. We are now following the situation to determine which patients will later develop calcified intracerebral cysts, breakdown of cysts, and intractable epilepsy or other brain syndromes requiring neurosurgical treatment or elaborate anticysticercus chemotherapy. We have developed a sensitive ELISA test, now in worldwide use, for studying cysticercosis in man and animals, and have recently improved this by the analysis of the antigens involved and the preparation of purer antigens. We have demonstrated in Southeast Asian epilepsy clinics, in areas like Bali where cysticercosis is highly prevalent, that this newly-appreciated diagnosis is probably the cause of much of the self-limited new epilepsy seen.

Our work on male pseudohermaphroditism in a focus among the Anga people in the New Guinea highlands has established that the syndrome is similar to that in the Dominican Republic, resulting from hereditary deficiency of delta-H steroid reductase, which prevents the production of dihydrotestosterone.

Our associated study of psychosexual development in this New Guinea population of pseudohermaphrodites and of adjacent populations has influenced basic thinking on gender and role identification in man, and of the biological and psychological effects of diverse cultural patterns of psychosexual development. Patterns of permissive and promiscuous prepubertal heterosexuality, of similarly promiscuous and early homosexuality, and of total sexual abstinence in different adjacent cultures provide important natural laboratories for study of child development that have great impact on psychiatric thinking.

Physiological and growth and development studies in these isolated cultures over 30 years have revealed incredible patterns of premature aging in some populations and of enormous delay in puberty and menarche in others. Migration and sudden cultural change have resulted in these latter groups in enormous advance in the age of puberty and acceleration of the adolescent growth spurt. Thus, these situations now provide a fruitful source of study of factors related to the control of the age of puberty, one of the most important problems facing modern society.

Acquired immune deficiency syndrome has been under investigation for several years as a continuation of Dr. Gajdusek's investigations of a similar "AIDS" epidemic of interstitial plasma cell pneumonia in infants in Europe in the 1940s, '50s, and '60s which resulted in the first paper in English on P. carinii. Both P. carinii and cytomegalic inclusion disease were causes of death; the epidemic receded without the primary cause of the immune deficiency having been identified. In the current outbreak in the U.S., chronic encephalitis has been brought to our attention in the past two years. We have inoculated tissue from AIDS (and Kaposi's sarcoma) patients into many animals, including juvenile chimpanzees and monkeys, which are under long-term immunological surveillance. Tissue cultures and explanted tissues from AIDS victims are cocultivated in an attempt to grow a virus provoking primary immune deficiency. We are using our usual techniques to search for inapparent infections of the cultures. Pre-AIDS tissue specimens (i.e., gay lymphadenopathy syndrome) and specimens from controls in contact with AIDS patients have been inoculated into many species of subhuman primates and apes. Some of the pre-AIDS donors have subsequently developed AIDS.

We are also investigating the two most interesting viruses isolated from AIDS patients which are candidates for its cause--the human T-cell leukemia virus (Gallo et al.) and the French retrovirus of Montaigner--in inoculated primates, including newborn and in utero animals. The eight cases of AIDS of infants and small children in Newark, New Jersey, have developed a concomitant encephalopathy which is under investigation using brain biopsy and early autopsy. We are concentrating our efforts on contacts of AIDS patients who have developed immune deficiency and chronic lymphadenopathy before opportunistic investigations have intervened.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 01282-20 CNSS

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)* Neurobiology of Population Isolates: Study of Child Growth and Development, Behavior and Learning, and Disease Patterns in Primitive Cultures

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

D.C. Gajdusek, M.D., Chief, Laboratory of Central Nervous System Studies, NINCDS
Clarence J. Gibbs, Jr., Ph.D., Deputy Chief, LCNSS; David M. Asher, M.D., Paul W. Brown, M.D., and Ralph M. Garruto, Ph.D.
OTHERS: Michael Alpers, M.D.; Sina Bahmanyar, M.D.; Mario Barragan, M.D.; Francois Cathala, M.D., Kwang-Ming Chen, M.D.; Chen-ting Chin, M.D.; Millicent Coker-Vann, Ph.D.; Judith Farquhar, M.A.; Peter Fetchko, M.A.; Father David Gallus; Dmitry Goldgaber; Steven Ono, M.S.; Robert G. Rohwer, Ph.D., Andres Salazar, M.D.; Euan Scrimgeour, M.D.

COOPERATING UNITS *(if any)*

AUSTRALIA: Dr. Timothy Asch, Australian National University, Canberra; Dr. Cyril Curtain, CSIRO, South Melbourne; Dr. Eric French, Mt. Eliza; Dr. Chev Kidson, Queensland Institute of Medical Research, Brisbane; (continued)

LAB/BRANCH

Laboratory of Central Nervous System Studies, Intramural Research Program

SECTION

INSTITUTE AND LOCATION

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
12	8	4

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Studies of human biology of vanishing primitive societies focus on neurological development and learning patterns in diverse cultural experiments in the human condition found in such isolated groups. Opportunistic investigation of problems phrased by man in isolation is the basis of approach from which all our studies have evolved. Techniques of molecular biology, immunology, virology, endocrinology and biochemistry and field epidemiological, clinical, linguistic and behavorial studies in cultural isolates and genetic and/or geographically isolated primitive bands yield more easily interpretable data than in cosmopolitan societies. Data and specimens collected on expeditions to Micronesia, Polynesia, Solomon Islands, New Hebrides, New Guinea, Indonesia, South America, Asia and Africa are used. Studies on nutrition, reproduction, fertility, neuroendocrine influences on age of sexual maturation and aging, genetic polymorphisms, genetic distance, unusual and odd employment of the higher cerebral functions in language learning, cognitive styles, computation (calculation without words or numbers) and culturally modified sexual behavior elucidate alternative forms of neurologic functioning for man which we would be unable to investigate once the natural cultural experiments in primitive human isolates are amalgamated into the cosmopolitan community of man. Foci of high incidence of kuru, ALS/PD, epilepsy, spastic paraparesis, familial parkinsonism, other CNS degenerations, hysterical disorders, schizophrenia, neoplasms, goiter, cretinism, rheumatoid diseases, diabetes, asthma, chronic lung disease, malaria, filariasis, leprosy, cysticercosis, and other infections are investigated. Zoonoses such as hemorrhagic fever with renal syndrome in China, Japan, Korea, USSR, Scandinavia, and the Balkans are studied including these newly recognized Bunyamwera viruses in the U.S. Acquired immune deficiency syndrome studied by our group in 1950-1960 have been reinitiated. Human evolution and adaptability to high altitude, excessively wet or arid climes, variable food supply, mineral deficiencies, toxic exposures and responses to severe diseases or social/psychological stress are under investigation in appropriate population isolates.

COOPERATING UNITS: (continued)

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BOLIVIA: Dr. Mario Barragan, La Paz; Dr. Mario Michael Zamora, Department of Neurologic y Neurocirugia, La Paz.

BRAZIL: Prof. Helio L. de Oliveira, Universidade de Sao Paulo, San Paulo.

CANADA: Dr. Jack Hildes, University of Manitoba, Winnipeg; Dr. Otto Schaefer, National Health and Welfare, Edmonston.

CHINA: Dr. H.E. Ge, Hubei Province Medical School, Wuchang; Dr. C.C. Chin, Hubei Province Medical School, Wuchang; Dr. Chin-min Hsiang, Virus Research Institute, Hupeh Medical College; Dr. H.E. Kwei, Hubei Province Medical School, Wuchang; Dr. Hung Toa, Department of of Electron Microscopy, Peking University, Peking; Dr. Zhi-Yi Xu, Department of Epidemiology, Shanghai 1st Medical College, Shanghai; Dr. Zheng, Virus Research Institute, Hubei Medical College, Hubei.

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FIJI: Mr. Ron Crocombe, University of South Pacific, Suva.

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NETHERLANDS: Father Ben van Oers, Missiehuis, Tilburg; Dr. Jaap Goudsmit, University of Amsterdam, Dr. Fransje van der Waals, Amsterdam.

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PERU: Dr. Carlos Monge, Universidad Cayetano Heredia, Lima.

PHILIPPINES: Dr. John Cross, NAMRU-2, Manila; Dr. Benjamin Catubay, Provincial Health Officer, Ilocos Norte; Dr. Martesio C. Perez, Universtiy of Philippines, Manila; Lt. Commander William Schroeder, NAMRU-2, Manila; Dr. Virginia Basaca Sevilla, Ministry of Health, Manila; Dr. Elizabeth Zaraspe-Yoo, University of Philippines, Manila.

MADAGASCAR: Dr. Pierri Coulanges, Institute Pasteur de Madagascar, Antanarivo.

MAURITIUS: Dr. B. Gurburrum, Ministry of Health, Port Louis.

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SCOTLAND: Dr. Alan G. Dickinson, A.R.C. Animal Breeding Research Organization, Edinburgh; Dr. J.D. MacGregor, Shetland Health Board, Shetland.

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Nevada--Dr. Warren V. Huber, V.A. Medical Center, Reno; New Jersey--Dr. Karl Maramorosch, Rutgers University, New Brunswick; Dr. Richard Masland, Englewood; New York--Dr. Robert Glasse, Queen's College, Flushing; Dr. Shirley Lindenbaum, The New School, New York; Dr. Ralph D. Peterson, New York Hospital-Cornell Medical Center, New York; Dr. Roger D. Traub, IBM Thomas W. Watson, Yorktown; Ohio--Dr. Richard Feinberg, Kent State University, Kent; Dr. Frank P. Saul, Medical College, Toledo; Dr. Arthur G. Steinberg, Case Western Reserve University; Pennsylvania--Dr. Paul T. Baker, Pennsylvania State University, University Park; Dr. Napolean Chagnon, Pennsylvania State University, University Park; Drs. Werner and Gertrude Henle, Children's Hospital of Philadelphia, Philadelphia; Rhode Island--Dr. Terrence E. Hays, Rhode Island College, Providence; Dr. John Strom, Rhode Island Hospital, Providence; Texas--Dr. Heather D. Mayor, Baylor University Medical School, Houston; Dr. Steven Wiesenfeld, Southwest Allergy Service, Inc., Midland; Washington--Dr. Ronald DiGiacomo, University of Washington, Seattle; Wisconsin--Dr. G.R. Hartsough, Great Lakes Mink Association, Pittsville; Dr. Richard F. Marsh, University of Wisconsin, Madison; Dr. Gabriel Zu Rhein, University of Wisconsin, Madison.

YUGOSLAVIA: Dr. A. Terzin, Department of Microbiology, Faculty of Medicine, Novisad; Prof. J. Vesenjak-Hirjan, Sveucilistau Zagrebu, Zagreb.

Sub-Project I:	Study of the development patterning of the human nervous system (cybernetics of human development).
Sub-Project II:	Human evolutionary studies in isolated primitive groups.
Sub-Project III:	Studies of isolated Micronesian populations.
Sub-Project IV:	Studies of isolated New Guinea populations.
Sub-Project V:	Studies of Australian Aborigines.
Sub-Project VI:	Studies of isolated New Hebrides and Solomon Islands populations.
Sub-Project VII:	Studies of Central and South American Indians.
Sub-Project VIII:	Developmental, genetic and disease patterns in primitive and isolated populations of Asia, Africa, Indonesia, Melanesia, Micronesia, Polynesia, South and Central America, and the Arctic.
Sub-Project IX:	Experimental developmental neuropediatrics in infantile programming: a empirical approach to the language of information input into the nervous system.
Sub-Project X:	Ciphers and notation for the coding of sensory motor data for neurological information processing.

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

Sub-Project XII: Studies of high incidence of neurological disease in specific racial and ethnic groups and in primitive, or geographically genetically, culturally, or socially isolated group population studies.

Sub-Project XIII: Studies of high incidence of non-neurolgical disease in specific racial and ethnic groups and in primitive, or geographically genetically, culturally, or socially isolated group population studies.

Project Description: Neurobiology of Population Isolates: Study of Child Growth and Development, Behavior and Learning, and Disease Patterns in Primitive Cultures (are attached)

Publications: Listed on pages 20 - LCNSS/IRP through 32 - LCNSS/IRP

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 00969-20 CNSS

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Chronic CNS Disease Studies: Slow, Latent and Temperate Virus Infection

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
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OTHER: Herbert L. Amyx, D.V.M.; David M. Asher, M.D.: Sina Baymanyar, M.D.; Paul W. Brown, M.D.; Chen-ting Chin, M.D.; Marie-Claude Moreau-Dubois, Ph.D.; Ryo Fukatsu, M.D.; Ralph M. Garruto, Ph.D.; Jaap Goudsmit, M.D.; C.M. Hsiang, M.D.; Yasuo Kuroda, Ph.D.; Pyung-Woo Lee, Ph.D.; Maryellen F. Franko, Ph.D.; Carlo Masullo, M.D.; T. Nakamura, M.D.; Maurizio Pocchiari, M.D.; Pamela Rodgers-Johnson, M.D.; Robert G. Rohwer, Ph.D.; Akira Takenaka, M.D.;(continued)

COOPERATING UNITS *(if any)* AUSTRALIA: Dr. Byron A. Kakulas, University of Western Australia, Nedlands; Dr. Chev Kidson, Queensland Institute of Medical Research, Brisbane; Dr. Robert L. Kirk, Australian National University, Canberra; Dr. Ian MacKay, Royal Melbourne Hospital, Melbourne; (continued)

LAB/BRANCH
Laboratory of Central Nervous System Studies, IRP, NINCDS

SECTION

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
24	14	10

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Studies elucidate cause and pathogenesis of chronic degenerative CNS disorders with emphasis on MS, ALS, Parkinsonism-dementia, Parkinson's, Pick's, and Alzheimer's disease, Huntington's chorea, supranuclear palsy, other presenile dementias, spinocerebellar ataxias, epilepsy, chronic encephalitis with focal epilepsy, muscular dystrophies, chronic schizophrenia, autism, SSPE, PML, dialysis encephalopathy, and intracranial neoplasm. Even familial, apparently hereditary diseases may be slow virus infections. Subacute spongiform virus encephalopathies: kuru and Creutzfeldt-Jakob disease (CJD) of man; scrapie and mink encephalopathy are caused by unconventional viruses with unique properties posing important theoretical problems to microbiology and molecular biology; a major goal is elucidation of their structure and mechanisms of replication. Transmissible virus dementias are increasingly recognized worldwide causes of death: high incidence foci, transmission by corneal transplant or brain surgery, and occupational hazards from exposure to diseased or infectious brain. In order to determine the usual mode of infection with the virus, a worldwide epidemiological study of transmissible virus dementia (CJD) cases is underway with special attention to familial clusters of cases and with a quest for possible relationship of scrapie of sheep to the human disease.

Familial and nonfamilial dementia and the dementias of senility are studied. The autoimmune responses to specific brain antigens in CNS diseases are under intensive investigation. DNA in situ hybridization and electrophoretic focusing partition of proteins along with enzymatic and hybridoma immunofluorescence and many other techniques are used to try to identify viral subunits and partial genomes in tissues in chronic diseases.

Fransje van der Waals, M.D.; Richard T. Yanagihara, M.D.; Francoise Cathala, M.D., Dimitry Goldgaber, Ph.D.; Euan Scrimgeour, M.D.; Leon Epstein, M.D.

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AUSTRALIA: Dr. Colin Masters, University of Western Australia, Perth; Dr. Eric Shaw, Red Cross Blood Transfusion Service, Brisbane; Dr. Margaret Sabine, University of Sydney, Sydney.

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BELGIUM: Dr. A. Lowenthal, L'Institut Bunge, Antwerp.

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CHILE: Dr. Sergio Galvez, Institute de Neurocirurgia, Santiago.

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EGYPT: Dr. Harry Hoogstraal, Naval Medical Research Unit, Cairo.

ENGLAND: Mrs. Elisabeth Beck, Institute of Psychiatry, London; Dr. M.C. Clark, Agricultural Research Council, Compton; Prof. P.M. Daniel, Royal College of Surgeons, London; Dr. A.J. Davey, Royal College of Medicine, Institute of Psychiatry, London; Prof. George Dick, Regional Dean's Office, London; Prof. L.W. Duchen, The National Hospital, London; Dr. D.A. Haig, Agriculture Research Council, Compton; Dr. Gordon D. Hunter, Agricultural Research Council, Compton; Prof. W.B. Matthews, University of Oxford, Oxford, Dr. R. Kimberlin, Agricultural Research Council, Compton.

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INDONESIA: Dr. Budi Subianto, Public Health Department, Jayapura.

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NETHERLANDS: Dr. Jaap Goudsmit, University of Amsterdam, Amsterdam; Dr. Jan ten Brink, University Hospital of Amsterdam, Amsterdam; Dr. Jan van der Noordaa, Laboratorium voor GezondheidsLeer, Amsterdam.

NEW ZEALAND: Dr. R. W. Hornabrook, Wadestown.

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PERU: Dr. Luis Palomino, Hospital Santo Toribio, Lima.

POLAND: Dr. P.P. Liberski, Department of Neurology, Lodz

PUERTO RICO: Dr. Victor Mojica, Veterans Administration Center, San Juan.

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SCOTLAND: Dr. Alan G. Dickinson, A.R.C. Animal Breeding Research Organization, Edinburgh; Dr. Hugh Fraser, A.R.C. Animal Breeding Research Organization, Edinburgh; Dr. J.D. MacGregor, Shetland Health Board, Shetland;Dr. Robert A. Sommerville, University of Scotland; Edinburgh.

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USSR: Dr. Mikhasil Petrovich Chumakov, Institute of Poliomyelitis and Virus Encephalides, Moscow; Dr. Lydia L. Fadeeva, Ulitsa Valtera Ulbrichta, Moscow; Dr. Sophia Janovna Gaidamovich, Ivanovskii Institute of Virology, Moscow; Prof. Vera I. Il'yenko, All-union Research Institute of Influenza, Leningrad; Dr. Prokopii Andrevich Petrov, Iakut Ministry of Public Health, Iakutsk; Dr. Vanda V. Pogodina, The Institute of Poliomyelitis and Virus Encephalitides, Moscow; Dr. Peter Rytik, Bilorussioan Institute of Epidemiology, Minsk.

COOPERATING UNITS: (continued)

UNITED STATES: California--Dr. Kenneth P. Johnson, San Francisco; Dr. David E. Kohne, Center for Neurologic Studies, San Diego; Dr. Peter Lampert, University of California, La Jolla; Dr. David Lang, City of Hope Medical Center, Duarte; Dr. Michael N. Oxman, V.A. Hospital, San Diego; Dr. Linus Pauling, Linus Pauling Institute, La Jolla; Dr. Stanley Prusiner, University of California, San Francisco; Dr. Gunther Stent, University of California, Berkeley; Dr. Robert Terry; San Diego; Dr. W. W. Tourtellotte, V.A. Hospital, Los Angeles; Dr. Myron Varon, Amyotrophic Lateral Sclerosis Society, Sherman Oaks; Dr. Steven Waxman, Stanford University, Stanford; Dr. Leslie P. Weiner, University of Southern California, Los Angeles. Connecticut--Dr. P. N. Bhatt, Yale University, New Haven; Dr. G.D. Hsiung, V.A. Medical Center, West Haven; Dr. Elias and Laura Manuelides, Yale University School of Medicine; New Haven. Hawaii--Dr. Arwin R. Diwan, University of Hawaii, Honolulu; Dr. Hong-Yi Yang, University of Hawaii, Honolulu. Illinois--Dr. Raymond A. Classen, Presbyterian-St. Lukes's Hospital, Chicago; Dr. Raymond Roos, University of Chicago, Chicago. Indiana--Dr. Bernadino Ghetti, Indiana University School of Medicine, Indianapolis; Dr. Morris Pollard, Lobund Laboratory, Notre Dame; Dr. A.N. Siakotos, Indiana University, Indianapolis. Kentucky--Dr. Dan Tynan, V.A. Hospital, Lexington. Louisiana--Dr. William Greer, Gulf South Research Institute, New Iberia. Maryland--Dr. Dan C. Cavanaugh, Rockville; Dr. Theodore O. Diener, Agricultural Research Center West, Beltsville; Dr. Paul Hoffman, University of Maryland; Baltimore; Dr. Richard T. Johnson, Johns Hopkins University, Baltimore; Mrs. Meta Neumann, Bethesda: Dr. Robert Traub, University of Maryland, Baltimore; Dr. Charles Wisseman, University of Maryland, Baltimore; Dr. K.V. Shah, Johns Hopkins University, Baltimore; Mr. T.C. Rains, National Bureau of Standards, Gaithersburg. Massachusetts--Dr. Amico Bignami, Children's Hospital Medical Center, Boston; Dr. Bernard Fields, Harvard Medical School, Boston; Dr. E. P. Richardson, Jr., Massachusetts General Hospital, Boston; Dr. W.C. Schoene, Peter Bent Brigham Hospital, Boston. Minnesota--Dr. Ashley T. Haase, University of Minnesota, Minneapolis; Nevada--Dr. Warren V. Huber, V.A. Medical Center, Reno. New York--Dr. Samuel J. Ayl, The National Foundation March of Dimes, White Plains; Dr. Jordi Casals, Mt. Sinai School of Medicine, New York; Dr. Teresita S. Elizan, Mt. Sinai School of Medicine, New York; Dr. Scott Halstead, Rockefellar Foundation, New York; Dr. Asao Hirano, Montefiore Hospital, Bronx; Dr. John Hotchin, Department of Health, Albany; Dr. J. Moor-Jankowski, New York University Medical Center, New York; Dr. Imaharu Nakano, Montifiore Hospital and Medical Center, New York; Dr. Michael L. Shelanski, New York University Medical Center, New York; Dr. Roger D. Traub, IBM Thomas B. Watson Research Center, Yorktown Heights; Dr. James D. Watson, Cold Spring Harbor Laboratory, Cold Spring. Ohio--Dr. S.M. Chou, Cleveland Foundation, Cleveland; Dr. Maurice Victor, Metropolitan General Hospital, Cleveland.
Texas--Dr. Samuel Baron, University of Texas, Galveston; Dr. Steven Wiesenfeld, Southwest Allergy Service, Midland. Virginia--Dr. J. L. Hourrigan, Arlington. Washington--Dr. Ellsworth C. Alvord, Jr., University of Washington, Seattle. Chou, Cleveland Foundation, Cleveland; Dr. Maurice Victor, Metropolitan General Hospital, Cleveland. Washington, D.C.--Dr. Harold Booker, Veterans Administration Central Office, Washington; Dr. John Kurtzke, V.A. Hospital, Washington; Dr. Frederick C. Robbins, National Academy of Science, Washington;

UNITED STATES: (continued)
Washington, D.C. (continued): Dr. Fuller Torrey, St. Elizabeth's Hospital, Washington. Wisconsin--Dr. Richard F. Marsh, University of Wisconsin, Madison; Dr. Gabriel Zu Rhein, University of Wisconsin, Madison.

YUGOSLAVIA: Dr. A. Gligic, Institute of Immunology and Virology, Beograd; Dr. Miha Likar, Mikrobioloski Institut, Ljubljana; Dr. D. Terzin, Institute of Virology, Serajevo; Prof. J. Vesenjak-Hirjan, University of Zagreb, Zagreb.

Sub-Project I:	Attempts to isolate, identify and characterize transmissible agents from humans and animals with subacute degenerative diseases of the central nervous system: transmissible heredofamilial diseases, presenile and senile dementias of the sporadic and familial types and primary sclerosing and demyelinating diseases.
Sub-Project II:	Characterization and pathogenesis of kuru virus.
Sub-Project III:	Characterization and pathogenesis of Creutzfeldt-Jakob disease (transmissible dementia virus).
Sub-Project IV:	Scrapie: studies on the purification, physical and biological characterization and nature of the virus.
Sub-Project V:	In vitro cultivation of the viruses of the subacute spongiform virus encephalopathies in cell cultures.
Sub-Project VI:	Host range of susceptible laboratory animals to the viruses of the subacute spongiform virus encephalopathies.
Sub-Project VII:	Strain variations among the viruses of the subacute spongiform virus encephalopathies.
Sub-Project VIII:	Cell-fusing properties of the viruses of the subacute spongiform virus encephalopathies.
Sub-Project IX:	Resistance to radiation of the viruses of the subacute spongiform virus encephlopathies.
Sub-Project X:	Resistance to disinfectants of the viruses of the subacute spongiform virus encephalopathies.
Sub-Project XI:	Tissue and cell culture techniques used to unmask slow infection of man and animals using brain and viscera biopsy and early autopsy, bone marrow and peripheral leucocyte specimens.
Sub-Project XII:	The syncytium-forming viruses (simian and human foamy viruses).

Sub-Project XIII: Studies on transformed human brain tissue in vitro and characterization of associated virus.

Sub-Project XIV: Electron microscopic membrane studies of subactue spongiform virus encephalopathies.

Sub-Project XV: Characterization and identification of new herpes viruses from explant cultures of tissues from subhuman primates.

Sub-Project XVI: Studies on persistent asymptomatic cytomegalovirus infections of healthy rhesus monkeys.

Sub-Project XVII: Focal movement disorders in rhesus monkeys following experimental infection with a strain of tick-borne encephalitis virus.

Sub-Project XVIII: Fluorescent antibody studies on the intracellular localization and identification of virus antigens in vivo and in vitro in tissues from patients with subacute diseases of the central nervous system.

Sub-Project XIX: Isolation and characterization of adenovirus from the urine of chimpanzees.

Sub-Project XX: Development of serological and immunological test system for use in the study of slow infections of the central nervous system.

Sub-Project XXI: Immune responsiveness of multiple sclerosis patients to established viral antigens by detection of specific antibodies in serum and cerebrospinal fluids collected serially during remission and exacerbation.

Sub-Project XXII: Animal management and intercurrent diseases in subhuman primates on long-term studies of slow infections.

Sub-Project XXIII: Studies to determine the possible presence of cryptic viral genomes in human brain tissues.

Sub-Project XXIV: Sequential development of kuru-induced neuropathological lesions in spider monkeys.

Sub-Project XXV: Studies on the isolation, characterization, identification and pathogenicity of type C viruses from human and animal tissues.

Sub-Project XXVI: Biochemical studies of the etiology of amyotrophic lateral sclerosis and parkinsonism-dementia.

Sub-Project XXIV: Sequential development of kuru-induced neuropathological lesions in spider monkeys.

Sub-Project XXV: Studies on the isolation, characterization, identification and pathogenicity of type C viruses from human and animal tissues.

Sub-Project XXVI: Biochemical studies of the etiology of amyotrophic lateral sclerosis and parkinsonism-dementia.

Sub-Project XXVII: Study of mitrochrondrial mutants from scrapie-infected mouse brain cells.

Sub-Project XXVII: Study of mitrochrondrial mutants from scrapie-infected mouse brain cells.

Sub-Project XXVIII: Isolation and characterization of the etiological agent of Scandinavian nephro-nephritis epidemica.

Sub-Project XXIX: The pathogenesis of Korean hemorrhagic fever virus and the elucidation of its biological and physical properties.

Sub-Project XXX: Worldwide seroepidemiological evidence of antibodies in human populations to the virus of Korean hemorrhagic fever.

Sub-Project XXXI: Development of an enzyme-linked immunoadsorbent (ELISA) test for the diagnosis and epidemiology of cystercercosis-induced epilepsy.

Sub-Project XXXII: Studies on the cytochemical and morphological properties of neurons cultured in vitro.

Sub-Project XXXIII: Development of immunological markers for the detection of autoantibodies to neurofilaments in the sera of patients with subacute spongiform encephalopathies.

Sub-Project XXXIV: Studies to determine the neurophysiological changes of neurons in vitro infected with CJD.

Sub-Project XXXV: Effects of the subacute spoingiform viruses on nerve cells grown in vitro.

Sub-Project XXXVI: In vivo and in vitro studies to determine the etiology of myasthenia gravis, Viliuisk encephalomyelitis and ALS-PD in high incidence foci of the Western Pacific.

Sub-Project XXXVII: Neurophysiological study of animals experimentally infected with subacute spongiform virus encephalopathies.

Sub-Project XXXVIII: Studies on in vivo pathogenecity of the retroviruses related to AIDS: HTLV (Gallo); French LAV-LOISEAU virus (Montagnier)

Sub-Project XXXIX: Attempts to transmit or isolate *in vitro* an etiological agent from AIDS, from pre-AIDS patients with lymphadenopathy syndrome, and from encephalitis associated with AIDS.

Project Description: Chronic Central Nervous System Disease Studies (described fully on pages 1-LCNSS/IRP through 5-LCNSS/IRP).

The projects (I through XXXIX) listed herein, as itemized in the Project Reports of previous years, have continued throughout this year and have been expanded, as are reflected in the extensive list of publications. Contractural phases of this work are being conducted at Gulf South Research Institute, New Iberia, LA.

Publications: Pages 20-LCNSS/IRP through 32-LCNSS/IRP

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6. Brown, P. (1984) Biologic and chemotherapeutic forays into the field of unconventional viruses. In: "Targets for the Design of Antiviral Agents", E. De Clerq and R.T. Walker, editors. (Proceedings of NATO Advanced Study Institute, 19 June-2 July 1983, Les Arcs, France). Plenum Publications, New York, 1984, pp. 131-157.

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24. Gourmelon, P., Court, L. and Gibbs, C.J., Jr. (1983) La tremblante experimentale du hamster: analyse de lactivite electrique cereberle. Abstract No. 88 in Travaux Scientifiques des Cercheurs du Service de Sante des Armees, No. 4. Direction Centrale du Service de Sante des Armees, Clamart, France. pp. 195-197.

25. Hoffman, P.M., Robbins, D.S., Gibbs, C.J., Jr. and Gajdusek, D.C. (1983) Immune function among normal Guamanians of different ages. Journal of Gerontology, 38:4 (July), 414-419.

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CONTRACTS

University of Southwestern Louisiana
New Iberia Reseach Center
New Iberia, Louisiana

Contract #N01-NS-8-09931

$491,660.00

Program Resources, Inc.
(Administration by NCI)

Contract #N01-CO-75380

$420,000.00

TAB 4 -- LABORATORY OF EXPERIMENTAL NEUROPATHOLOGY -- (LENP)

ANNUAL REPORT

October 1, 1983 through September 30, 1984

Laboratory of Experimental Neuropathology

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ANNUAL REPORT
October 1, 1983 through September 30, 1984
Laboratory of Experimental Neuropathology, IRP
National Institute of Neurological and Communicative Disorders and Stroke

Henry deF. Webster, Chief

The Laboratory of Experimental Neuropathology (LENP) was created in 1984 and includes the Section on Cellular Neuropathology and the Section on Neurotoxicology. Each section has achieved significant progress in a number of important research areas.

Section on Cellular Neuropathology

Projects concerned with mechanisms of virally induced CNS demyelination included studies of the effects of herpes simplex virus type 2 (HSV-2) in the central nervous system (CNS) of mice infected by a natural route. The MS strain of HSV-2 used in these studies was isolated years ago from the brain of a patient with multiple sclerosis (MS). Following vaginal infection, the results showed that: (1) The spectrum of CNS disease includes three clinicopathological syndromes. In non-fatal infections, demyelinative disease or leptomeningitis occurs while mice with fatal infections have servere myelitis. Other mice have no evidence of CNS invasion by virus. This is the first study to demonstrate that demyelinative disease can occur following HSV-2 infection by a natural route. The study also defines the spectrum of CNS disease within which demyelination occurs. Together with earlier studies from this section, it suggests that human demyelinative disease could be caused by this agent. The results are the first to show that HSV-2 causes experimental aspetic meningitis; they also provide the first pathological evidence that in this syndrome, virus reaches the spinal cord by ascending along sacral sensory roots instead of via the blood stream, as has been thought previously. (2) Acute thymic atrophy develops in all mice with fatal infection as well as in a proportion of animals with not-fatal demyelinative disease or meningitis. Further, virus can be isolated from lymphoid tissues of mice with severe disease, but not from those animals with milder infection. This is the first report of an association between disease severity, lymphoid tissue infection and lymphoid tissue lesions. This discovery suggests that a virus-induced immunosuppression may occur with HSV-2 infection. This immunosupression could be an important determinant of CNS disease severity and of virus reactivation in the CNS. In human disease, virus induced immunosuppression could have an important role in producing the recurrent episodes of demyelination that are seen in MS. It could also help reactivate virus in the encephalitis caused by HSV-1 infection. (3) Serum neutralizing antibody responses to HSV-2 may develop slowly in mice. A substantial number of mice which were clearly infected remained seronegative for at least a year after infection. Together with reports of others, this new finding questions some important assumptions made in serological surveys of MS and other disease in which HSV-2 infection has been suspected to be of etiological significance.

In another project, both light and electron microscopic immunocytochemical methods are being tested and modified so they can be used to demonstrate HSV-2 antigen in semi-thin (light microscopy) and thin (electron microscopy) epon sections. In CNS lesions produced by experimental HSV-2 infection,

abnormal "virus-like" particles are observed frequently; the above methods and their use in studies of cultured cells and neural tissue at intervals after infection are needed to show whether these particles and other relatively amorphous intranuclear and/or cytoplasmic material have anti-HSV-2 immunoreactivity. They also will permit more detailed study of the topographic distribution of antigen during neural spread to the CNS after vaginal infection. This study still is in progress; preliminary observations show that HSV-2 positive cells are present in spinal cord lesions within five or six days after vaginal infection.

In demyelinating lesions of viral or immunologic origin, antigen presenting cells (APCs) may have important pathogenetic functions. Identification of APCs in CNS lesions of experimental allergic encephalomyelitis (EAE) has been the goal of a project still in progress. Phenotypic characterization of APCs is by their expression of cell surface markers, mainly class II major histocompatibility antigens (Ia antigens), in combination with certain consecutive detection of Ia antigens (using immunohistochemical techniques) and enzyme markers (using conventional histochemical techniques) on the same section of frozen tissue. EAE has been induced in adult male Lewis rats using guinea pig spinal cords in Freund's complete adjuvant. Individual cells and tissue structures express most of the markers examined, but very few cells express two markers together (for example: Ia antigens and the hydrolytic enzyme, acid phosphatase). Large numbers of inflammatory cells in the EAE enzyme lesions express Ia antigens (Ir gene products) but most are on small lymphocyte-like cells (of either the T or B cell lineages). Very little demyelination is seen in the lesions of the subacute form of EAE produced here. As a result, very few phagocytic cells (a sub-group of APCs) are seen within the lesions. The early stages in the induction of EAE have yet to be examined and the significance of the observations remains to be established.

Myelin-associated glycoprotein (MAG), which constitutes less than one percent of the protein content of CNS myelin that is isolated for biochemical study, has been important to study immunocytochemically for two reasons. First, its localization in the CNS is not well defined and its role in the formation and maintenance of myelin will remain poorly understood until its location is known. Secondly, previous work in this section showed that distributions of MAG and MS and EAE (a widely used animal model for MS) lesions differed. Since we discovered that MAG was located at inner, periaxonal margins of CNS myelin sheaths, that localization has been demonstrated repeatedly by light microscopy in many laboratories. But, resolution of the light microscope was, and still is, insufficient to define precisely where this immunoreactivity is located. Also, the effects of fixatives and other processing steps used in these light microscopic studies are not known. In our electron microscopic, post embedding, immunocytochemical experiments using both polyclonal and monoclonal antisera, the location of reaction products was different. It was found on all compact myelin lamellae, not just those located periaxonally. This result suggests that MAG is a myelin constituent. Even though this localization is supported by biochemical evidence, it is important to study it again using pre-embedding methods. Many variations designed to enhance penetration of immunoagents have been tested and in a few experiments, patches of reaction product have been detected on lamellae of adult compact myelin in rat CNS. Many more have been detected on lamellae of adult compact myelin in rat CNS. Many more tests (now in progress) will be needed to demonstrate convincingly that these patches are sites of specific anti-MAG immunoreactivity. In other experiments, tissue from additional cases of acute and more chronic cases of MS have been immunostained with antisera to MAG, myelin

basic protein (MBP, a known component of compact CNS myelin), and glial fibrillary acidic protein (GFAP, a known constituent of astrocytes). Preliminary results suggest that decreased anti-MAG immunoreactivity found earlier in normal appearing white matter around acute MS lesions is an infrequent finding when several antiserum concentrations are used in two different immunostaining methods. When decreased anti-MAG reactivity is found around MS lesions, it may represent an early myelin sheath change.

The goal of another project is to assess the developmental expression of the peripheral nervous system (PNS) myelin basic proteins P_1 and P_2 in Schwann cells during early stages of myelinogenesis. Immunocytochemical techniques have been used to compare relative amounts of P_1 and P_2 and to correlate their expression with the progression of myelination as assessed by electron microscopy. Preliminary results suggest that at birth, P_1 and P_2 are only expressed in Schwann cells that are starting to form myelin sheaths. For the next few days, when myelination is progressing rapidly, intensity of immunoreactivity increases and an increasing number of myelin sheaths stain positively. At seven days of age, the majority of sheaths are stained by antisera to both proteins. Staining is uniform along myelin internodes and Schwann cell cytoplasmic staining is not observed. Antisera to P_1 stain all sheaths intensely. Staining intensity with P_2 antisera is variable and is highest in the largest sheaths. Factors responsible for this variation are being assessed. They will help determine whether an axonal signal related to fiber size controls how much P_2 is expressed or whether expression is limited to a preselected population of Schwann cells that ensheaths neurites destined to become the largest axons.

Myelin is a highly ordered, multilamellar, paracrystalline alternating array of proteins and bilayers of lipids. MBP accounts for 30% of the protein in CNS myelin. Much is known about its antigenic properties and its capacity to induce EAE, an autoimmune demyelinating disease that has been used as a model for MS. Several different lines of evidence suggest that MBP also has an important role in the formation and maintenance of CNS myelin's compact multilayered structure. But, very little is known about the conformation of this important molecule and how myelin-forming oligodendroglia process it during myelin assembly and maintenance. Therefore, MBP amino acid sequences are being studied with a number of predictive algorithms. These studies have created the first detailed model of MBP conformation, a compact Greek-key-type-B-structure consisting of five B strands. Phosphorylation and dephosphorylation are thought to be important in folding of the nascent polypeptide and inserting it into the myelin membrane. Changes in the activities of essential enzymes or induction of enzymes with new specificities could seriously disrupt orderly synthesis and folding of MBP. Incorrectly folded and/or partially degraded polypeptides could become competitive inhibitors of nascent MBP phosphorylation, thereby further increasing the likelihood of erroneous folding. This vicious circle could create an "error catastrophe", severely restricting the synthesis of functional MBP. Lack of sufficient MBP for myelin maintenance might precipitate intracellular breakdown of performed myelin, further inhibiting MBP synthesis. According to this hypothesis, viral effects would not need to be cytopathic, nor would immunologically mediated damage need to be directly cytotoxic for demyelination to occur in diseases like MS. Rather, some demyelination might be initiated by relatively subtle changes in activities of protein kinases. These, when combined with inadequate proteolysis, could precipitate widespread myelin breakdown. Several aspects of this hypothesis are now being tested.

The controversy regarding the suggestions that food additives may cause behavioral problems in some children is still unresolved. However, in recent years, one of the major projects of the Neurotoxicology Section has been the investigation of whether food dyes are potential neurotoxins. The evidence that Erythrosin B, U.S.F.D and C. Red No. 3 (tetraiodofluorescein) is a potent inhibitor of various CNS processes in vitro is clear. In our attempts to clarify the in vitro neurotoxic actions of eryhthrosin B on neurotransmission at brain synapses we are examining age and genetic variations in sodium and potassium ion stimulated adenosine triphosphatase (Na,K-ATPase). This membrane bound enzyme plays an important role in the control of the ionic environment which underlies nerve activity. We have demonstrated that erythrosin B inhibits a variety of CNS functions associated with this enzyme. Despite age-related differences in Na,K-ATPase in rat cortex, we have found no difference in the inhibitory potency of erythrosin B when these actions are compared in tissues from perinatal vs. young adult rats. We are examining a rat strain with a mutation for obesity, characterized by hyperphagis, hyperinsulinemia, and defective energy utilization mechanisms, to determine whether these mutants have altered brain Na,K-ATPase which we could exploit as a useful tool to investigate the actions of erythrosin B on brain Na,K-ATPase activity. Although erythrosin B is a photo-active compound its in vitro neurotoxic actions are demonstrable in samples protected from light, as well as those which are illuminated. Erythrosin B appears to be an in vitro toxic substance for a variety of physiological processes, in general, and not a specific inhibitor of a form of Na,K-ATPase exclusive to brain. On the other hand, the manner in which it interacts with cell membrnaes awaits clarification.

In addition to the mechanisms of actions of neurotoxic compounds, this section also studies endogenous neuroactive substances and therapeutic agents. Patients with Parkinson's disease have decreased dopamine in the basal ganglia and also decreased brain levels of the neuropeptide, cholecystokinin (CCK). The fact that the efficacy of L-dopa therapy decreases with time indicated that dopamine replacement alone is not sufficient to correct the neurochemical deficit(s) of Parkinson's disease. Although cholecystokinin is present in high concentrations in the brain, little is known about its functional role in the CNS. Prompted by the possibility that a therapy combining cholecystokinin with L-dopa would be an improvement over the efficacy of therapy with L-dopa alone, we have been studying the in vitro effects of cholecystokinin on dopamine D_2 receptors in rat striatal membrane preparations. Despite earlier reports, we have not found a consistent, dose-dependent interaction between cholecystokinin and striatal dopamine D_2 receptors in vitro.

Seizure disorders are a major cause of neurological dysfunction. We are investigating the possibility that clinically used anticonvulsants exert their effect by binding to central adenosine receptors. We have used in vitro assays to measure the effects of the anticonvulsant carbamazepine on adenosine receptors in rat and guinea pig brain. Carbamazepine is clearly an antagonist at the stimulatory A_2 receptor, however, the nature of its interaction (agonist, partial agonist, antagonist) at the inhibitory A_1 receptor is unknown. Present data suggest that interaction of carbamazepine with central A_1 adenosine receptors occurs at therapeutic doses, while equivalent interactions at A_2 receptors would require four fold higher concentrations. The relationships between adenosine receptors and the anticonvulsant activity of carbamazepine require further investigation. These studies will promote a

better understanding of the convulsant and anticonvulsant properties of drugs, and clarify directions for further biomedical research and therapeutic improvements.

The chromaffin cell provides a well-characterized system for investigating molecular and cell-surface mediated mechanisms of neurotoxin action. Since several neurotoxins of interest to neurology are divalent cations (lead, manganese, copper, etc.) and since the storage vesicles of these cells, the chromaffin granules, containing high concentrations of calcium, these preparations have been investigated to determine the effect of toxic cations on calcium-mediated storage and release processes. We have been testing the hypothesis that the neurotoxicity of heavy metals, such as lead and tin, may be due to interference with the calmodulin control of calcium-dependent processes. Recent results from other laboratories demonstrate that the chromaffin granule membrane contains several calmodulin binding proteins. We have shown that the calcium-promoted fusion of artificial lipid vesicles to chromaffin granule membranes can be placed under calmodulin control. Intestinal epithelium goblet cell cultures show changes in their calmodulin binding proteins when exposed to reserpine. However, no such changes could be detected in chromaffin cell cultures.

The storage vesicles of chromaffin cells, chromaffin granules, accumulate large concentrations of catecholamines and ATP *via* carriers linked to the granule membrane Ca^{2+}-ATPase. Granule membranes contain an F_1 ATPase subunit which is highly similar to that of mitochondria. The catecholamine carrier is inhibited by reserpine while the ATP carrier is inhibited by atractiliside. The tricyclic antidepressants imipramine and chlorimpramine were examined for their effect on ATPase activity. While both drugs inhibited the activity of whole mitochondria, sub-mitochondrial particles and solubulized F_1-ATPase, they had little effect on whole granule or granule ghost enzyme activity.

Release of neurotransmitters and neuromodulators from their storage organelles takes place by exocytosis, a process in which the influx of calcium into the cell or nerve terminal triggers the fusion of the storage granule with the cell plasma membrane. The membrane fusion events can be modelled by studying the calcium-promoted fusion of artificial or biological membranes with each other.

Chromaffin granules will aggregate and fuse in the presence of calcium. We have been exploring the molecular basis of these activities. Granule-granule recognition and aggregation is mediated by intrinsic membrane proteins; however, these labelling studies indicate that these proteins contain no free sulfhydryl groups. Fluorescent labelled lipid probes have been successfully inserted into chromaffin granule membranes *in vitro* without altering the storage properties of the particles. Resonance energy transfer studies of calcium-promoted fusion of these membranes show that, unlike artificial phospholipid vesicles, fusion runs 5-10 fold slower than aggregation. These results imply that substantial rearrangement of the proteins and lipids of the membrane is required for fusion to occur.

A multichannel, computer controlled stopped-flow rapid mixing spectrometer has been constructured to study these reactions and tested on a variety of artificial and biological membranes. Various proteins and polypeptides can catalyse fusion of artificial vesicle membranes. Some of these proteins have known functions in biological systems (e.g., the spike protein from Semliki Forrest virus). SFV is closely related to rabies virus; therefore *in vitro*

studies of SFV fusion mechanisms may have clinical relevance. Polylysine will fuse small unilamellar vesicles under conditions similar to SVF spike protein-mediated virus/cell membrane fusion. Recent stopped-flow studies indicated that polylysine-mediated fusion is aggregation rate limited. Furthermore, the aggregation rates themselves approach the diffusion controlled limit. This implies that polylysine binds rapidly to the membrane surface(s) and that almost every collision of activated particles results in fusion. Similar experiments using SFV spike protein as catalyst are planned.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 01995-12 LENP

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Morphological Studies of Myelin Formation, Breakdown and Regeneration

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	H. deF. Webster	Chief	LENP, NINCDS
Others:	G.L. Stoner	Senior Staff Fellow	LENP, NINCDS
	R.I. Craggs	Guest Worker	LENP, NINCDS
	G. Georgsson	Visiting Scientist	LENP, NINCDS
	A.F. Hahn	Guest Worker	LENP, NINCDS
	J.T. Favilla	Biologist	LENP, NINCDS

COOPERATING UNITS *(if any)*

Department of Biochemistry, McGill University, Montreal, Canada (Drs. P. Braun, D. Frail); Neurological Institute, University of Vienna, Vienna, Austria (Dr. H. Lassmann)

LAB/BRANCH

Laboratory of Experimental Neuropathology

SECTION

Section on Cellular Neuropathology

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
7.1	3.3	3.8

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The long range goal of this project is to combine immunocytochemical methods with light and electron microscopy to study cellular mechanisms of myelin formation, breakdown, and regeneration. The main findings in current studies are: (1) Electron microscopic immunocytochemical observations using a different pretreatment and another chromophore have confirmed localization of myelin-associated glycoprotein (MAG) on compact CNS myelin. (2) Examination of immunostained sections from additional acute and chronic cases of multiple sclerosis (MS) indicates that decreased anti-MAG immunoreactivity found earlier (Itoyama et al., 1980) in normal appearing white matter around MS lesions is an infrequent finding when several antiserum concentrations are used in two different staining methods. (3) Antigen presenting cells (APCs), characterized by expression of cell surface markers (Ia antigens) and cytoplasmic enzymes (acid phosphatase), are present in early EAE lesions. They include small lymphocyte-like cells of either the T or B cell lineage and macrophages, the latter serving in the effector arm of this demyelinating process. (4) At birth, myelin basic proteins, P_1 and P_2, are only expressed in Schwann cells that are starting to form myelin sheaths. Anti-P_1 stains all sheaths intensely and uniformly. Staining intensity with anti-P_2 is variable and is highest in the largest sheaths. Factors responsible for this variation are being assessed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02264-08 LENP

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 charecters or less. Title must fit on one line between the borders.)*
Animal Models of Neurological Disease

PRINCIPAL INVESTIGATOR *(List other professionel personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Sally M. Anderson	Expert	LENP, NINCDS
Other:	Roger Weir	Guest Worker	LENP, NINCDS
	Martha Knight	Staff Felllow	ETB, NINCDS
	Otho E. Michaelis, IV	Research Nutritionist	CNL, USDA
	John W. Daly	Chief	LBC, NIADDK

COOPERATING UNITS *(if any)*
Experimental Therapeutics Branch, NINCDS; Carbohydrate Nutrition Laboratory, Beltsville Human Nutrition Research Center, USDA; Laboratory of Bioorganic Chemistry, NIADDK

LAB/BRANCH
Laboratory of Experimental Neuropathology

SECTION
Neurotoxicology Section

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this project is the investigation of basic mechanisms associated with naturally occurring or artifically neurotoxin-induced neurological diseases through the use of animal models and in vitro experiments. Interactions of various neuroactive drugs and neurotoxins with neurotransmitters in the central nervous system have provided the focus for combined behavioral and neurochemical studies emphasizing basic mechanisms of action of proposed neurotoxins. Two major interests of this project are: A) to define populations of individuals that may be at increased risk to neurological disease resulting from exposure to neurotoxins and B) to use naturally occurring variability in central nervous system function, anatomy and/or neurochemistry, to elucidate mechanisms of actions of neurotoxins. Several different projects have been investigated this year. (1) Interactions of the artificial food color, erythrosin B, with neuronal membranes and neurotransmissions have been studied. Erythrosin B has been demonstrated, by several different criteria, to be a potent inhibitor of ATPase activity in brain and other tissues. Its inhibitory potency can be enhanced in vitro by exposing the tissue-erythrosin B complex to light. Studies are in progess to elucidate a possible "ligand-receptor" interaction between ATPases and erythrosin B. (2) Genetic and age variation in brain Na,K-ATPase are being investigated because they present a potential tool for elucidating the actions of erythrosin B on brain Na,K-ATPase. (3) Neuronal interactions between neuropeptides and dopamine D2 receptors in basal ganglia are being studied to increase our understanding of the functional significance of dopamine defects in patients with Parkinson's disease and the therapies necessary for alleviation of their symptoms. (4) We are studying the effects of anticonvulsant drugs on adenosine receptors to promote a better understanding of the actions of convulsant and anticonvulsant drugs. This new information should point out new directions for further biomedical research and lead to therapeutic improvements for these diseases.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02525-03 LENP

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Exocytosis Modelling: Kinetics of Membrane Aggregation and Fusion

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Stephen J. Morris	Expert	LENP, NINCDS
Others:	Paul D. Smith	Visiting Scientist	BEIB, DRS
	Carter C. Gibson	Electronics Engineer	BEIB, DRS
	Diane Bradley	Chemist	LENP, NINCDS
	Wendy Weiger	Biologist	LENP, NINCDS
	Robert Blumenthal	Section Chief	MSF, LTB, NCI
	Anne Walter	Staff Fellow	MSF, LTB, NCI
	Duncan H. Haynes	Univ. of Miami Medical School	

COOPERATING UNITS *(if any)*
Pharmacology Department, University of Miami Medical School

LAB/BRANCH
Laboratory of Experimental Neuropathology

SECTION
Neurotoxicology Section

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.6	0.9	0.7

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Neurotransmitter and neuromodulator release takes place by exocytosis; the influx of calcium into the cell or nerve terminal triggers the fusion of the storage granule with the cell plasma membrane. The membrane fusion events can be modelled by studying the fusion of artificial or biological membranes with each other. Our previous stopped-flow mixing studies have shown that the kinetics of aggregation of small vesicular structures (artificial lipid vesicles, neurotransmitter storage granules, etc.) can be described as the sum of at least two bimolecular rate reactions. A new multichannel, computer controlled stopped-flow rapid mixing spectrometer has been constructed to study the kinetics of these reactions. Using stopped-flow mixing and our new fluorescence assay for fusion, we have extended this work to investigate the fusion of these particles.

Small and large unilamellar vesicles composed of phosphatidylserine: phosphatidylethanolamine (1:1) rapid-mixed with calcium show identical aggregation and fusion rates, demonstrating that the rate-limiting step for fusion of these vesicles is aggregation itself. Small unilamellar vesicles with a high radius of curvature leak profusely during fusion while larger vesicles with less radical changes in surface curvature do not. We ascribe this to defects in the packing structure of the membrane phospholipids. This is supported by results from a stopped-flow study of cobalt ion transport across these membranes.

Various protein and polypeptides can catalyse fusion of artificial and biological membranes. Some of these have known functions in biological systems, e.g., the spike proteins from rhabdoviruses; therefore in vitro studies of these fusion mechanisms may have clinical relevance. Polylysine will fuse small unilamellar vesicles under conditions similar to spike protein-mediated virus/cell membrane fusion. Stopped-flow studies indicated that polylysine-mediated fusion is not aggregation rate limited and resembles that seen for in vitro fusion of chromaffin granules.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02550-03 LENP

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biochemical and immunologic mechanisms in virally-induced CNS demyelination

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	G.L. Stoner	Senior Staff Fellow	LENP, NINCDS
Others:	H. deF. Webster	Chief	LENP, NINCDS
	S.J. Morris	Expert	LENP, NINCDS
	C.F. Ryschkewitsh	Medical Technologist	LENP, NINCDS

COOPERATING UNITS *(if any)*

Department of Biochemistry, McGill University, Montreal, Canada (P.E. Braun); Department of Medical Microbiology, University of Wisconsin, WI (D. Walker)

LAB/BRANCH

Laboratory of Experimental Neuropathology

SECTION

Section on Cellular Neuropathology

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The goal of this project is elucidation of the molecular mechanisms of demyelination in the CNS, especially in viral diseases, and in demyelinating diseases of unknown etiology such as multiple sclerosis. Two approaches have been utilized: (1) Role of immunity to herpes simplex virus (HSV) infection of the CNS: A model was developed in which immunization of C57BL/6 mice intraperitoneally with HSV-1 or HSV-2 allowed study of the influence of immunity on subsequent CNS demyelination following intracerebral challenge with the virus. Unfortunately, a switch in the supplier of the mice was necessitated by infection in his colony, and the new C57BL/6 mice behave differently. The problems presented have not yet been overcome, and that aspect of this project has been temporarily suspended. (2) Structure of myelin basic protein (MBP): The structure of this essential myelin component has been predicted from its known amino acid sequence. The model has an α/β molecular organization, with two α-helices between the five strands of an antiparallel β-sheet. The β-sheet faces may be loci of interaction with phospholipids on the cytoplasmic surface of the myelin membrane. This new model of a protein, previously thought to be largely disordered, is one of the most detailed protein structure predictions ever attempted. It leads directly to testable new predictions, as would be expected of a detailed model. Unexpectedly, it also leads to an entirely new biochemical mechanism for virally-induced demyelination. A key threonine phosphorylation site in MBP, which precedes the triproline sequence, is closely mimicked by certain viral proteins, including the large T antigen of the human papova virus, JC virus. JC causes the devastating demyelinating disease of immunocompromised patients known as progressive multifocal leukoencephalopathy (PML). Phosphorylation of the Thr residue, which the model indicates would be a key step in MBP processing, could be competitively inhibited by the presence of the viral protein. Whether a similar mechanism is in any way involved in the etiology of demyelination in MS is not yet known.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02549-03 LENP

PERIOD COVERED: October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Herpes Simplex Virus Type 2 Infection, CNS Demyelination, and Multiple Sclerosis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J.R. Martin	Senior Staff Fellow	LENP, NINCDS
Others:	H. deF. Webster	Chief	LENP, NINCDS
	G. Georgsson	Visiting Scientist	LENP, NINCDS

COOPERATING UNITS *(if any)*: Department of Ophthalmology, Johns Hopkins School of Medicine (W.R. Green)

LAB/BRANCH: Laboratory of Experimental Neuropathology

SECTION: Section on Cellular Neuropathology

INSTITUTE AND LOCATION: NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.9	1.4	2.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project seeks to define determinants of CNS demyelination in experimental herpes simplex virus type 2 (HSV-2) infection, and to refine and test a hypothesis which relates HSV-2 infection to the human demyelinative disease, multiple sclerosis (MS). Our previous studies suggest that major features of HSV-2 epidemiology and pathology are consistent with a hypothesis that HSV-2 is etiologic in MS.

During FY 1984, studies published or in press have provided evidence which further defines the spectrum of experimental CNS disease produced by HSV-2. These studies provide insights into human disease which this agent is known to cause, and suggest how it could produce MS. Specifically, they show for the first time that:

1) Virus is occasionally found in axons in acute demyelinative lesions, which may explain the distinctive tract-associated topography of some demyelinative lesions we have previously described in experimental HSV-2 infection. These lesions are similar to those which have been described in MS.

2) When infected by a natural genital route, some mice develop an acute, non-fatal demyelinative disease of the CNS, while others develop other clinically recognized neurological syndromes, including non-fatal meningitis and fatal panmyelitis. Other mice have no detectable CNS lesions. In man, similar mechanisms could produce non-fatal CNS demyelinative disease in genital HSV-2 infection.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02451-04 LENP

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 charecters or less. Title must fit on one line between the borders.)*
Cellular and Molecular Approaches to Neurotoxicology

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Stephen J. Morris	Expert	LENP, NINCDS
Others:	Robert Blumenthal	Section Chief	MSF, LTB, NCI
	Duncan H. Haynes	Professor, Pharmacology Dept., Univ. Of Miami	
	J. David Robertson	Chairman, Anatomy, Duke University	
	M. Joseph Costello	Assoc. Professor, Anatomy, Duke University	
	Gerald W. Feigensen	Professor, Biochemistry Section, Cornell Univ.	
	Martin D. Caffrey	Postdoctoral Fellow, Biochem., Cornell Univ.	
	Diane Bradley	Chemist	LENP, NINCDS

COOPERATING UNITS *(if any)*
Pharmacology Department, University of Miami Medical School; Anatomy Department, Duke University Medical School; Biochemistry Section, Cornell University

LAB/BRANCH
Laboratory of Experimental Neuropathology

SECTION
Neurotoxicology Section

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.4	0.9	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The calcium-regulated activities of chromaffin cells provides a well-studied system for investigating molecular and cell-surface mediated mechanisms of neurotoxin action. The storage granules of these cells, chromaffin granules, accumulate large concentrations of catecholamines and ATP which are eventually released by exocytosis. Isolated chromaffin granules will aggregate and fuse in the presence of calcium. We have been exploring the molecular basis of these activities. Rapid freeze/fracture electron microscopic studies and fluorescent probe studies have demonstrated that membrane-associated proteins redistribute themselves as a result of aggregation and prior to fusion.

Fluorescent-labelled lipid probes have been successfully inserted into chromaffin granule membranes in vitro without altering the storage properties of the particles. Resonance energy transfer studies of calcium-promoted fusion of these membranes show that, unlike artificial phospholipid vesicles, fusion runs 5-10 fold more slowly than aggregation. These results support the previous findings that substantial rearrangement of the protein and lipid components of the membrane are required for fusion to occur. This in vitro fusion is inhibited by both organic and inorganic monovalent anions and cations and is insensitive to the presence of Mg-ATP.

A soluble, calcium-specific protein (synexin) isolated from chromaffin tissue or liver enhances the ability of calcium to aggregate chromaffin granule membranes. However, we have demonstrated previously that synexin has the same effect on mitochondrial membranes, microsomes and negatively charged artificial membranes. We have recently isolated a second protein (synexin II) from adrenal medulla and liver with entirely different molecular weight, protease susceptibility and peptide fragments. Synexin II also has entirely different aggregation kinetics than synexin I, showing a long lag period before a very rapid rise in aggregate size, suggesting that some activation step is required.

TAB 5 -- LABORATORY OF MOLECULAR BIOLOGY -- (LMB)

ANNUAL REPORT

October 1, 1983 through September 30, 1984

Laboratory of Molecular Biology
National Institute of Neurological and Communicative Disorders and Stroke

Table of Contents

Annual Report
October 1, 1983 through September 30, 1984
Laboratory of Molecular Biology
National Institutes of Neurological and Communicative Disorders and Stroke

Ernst Freese, Chief

Many microorganisms and some (germ cells) in higher organisms start to differentiate when their nutrition becomes scarce. The laboratory has shown that in both the prokaryote Bacillus subtilis and the eukaryotic yeast Saccharomyces cerevisiae this differentiation can be specifically induced by the partial deprivation of guanine nucleotides but not by the deprivation of pyrimidine nucleotides. This indicates that in the course of evolution the intracellular signal recognizing a nutritional deficiency has been retained. In B. subtilis, the probability of cells to enter differentiation spontaneously can be separately increased by ethionine, which is converted to S-adenosylethionine and thereby competes with methylation reactions. The results can be combined into a model. Other experiments have investigated cellular changes related to early differentiation such as transport and septation phenomena. Later in development, new proteins are synthesized, the control of one of which, glucose dehydrogenase, was examined using the cloned gene.

Mammalian cell studies have shown that the induction of β-adrenergic and other cell surface receptors by 5-azacytidine is not due to an undermethylation of DNA because the same induction can be achieved by other compounds that do not cause DNA undermethylation. In these cell lines, the laboratory has discovered a previously undescribed transport system whereby tricyclic antidepressants and adrenergic antagonists, but not catecholamines, are actively taken up. This transport requires a proton gradient which is maintained by a Mg-ATPase located in the plasma membrane.

1. Control of meiosis and sporulation in Saccharomyces cerevisiae. In analogy with the results earlier obtained in B. subtilis, the laboratory has shown that the meiosis and sporulation of diploid yeast can be initiated by partial deprivation of carbon, nitrogen, phosphorus, or sulfur sources. Under all these conditions the concentrations of GTP and S-adenosylmethionine (SAM) decreases whereas the concentration of other nucleotides increases in some and decreases in other cases. This differentiation can also be initiated by the partial deprivation of methionine (but not other amino acids) in appropriate auxotrophs or of guanine nucleotides either in a guanine auxtroph or by the addition of virazole (ribavirin, which has to be added in rather high concentration to pentrate the yeast cell wall). The methionine deficiency causes a decrease of SAM and, by an

unknown mechanism, also of GTP. Conversely, a GTP deficiency also causes a decrease of SAM. However, a small amount of methionine can maintain the concentration of SAM while GTP decreases, and the cells can still sporulate. Therefore, the controlling compound seems to be GTP. Further experiments will have to determine this in more detail by the use of mutants altered in the uptake of methionine or deficient in the two SAM synthetase activities.

To obtain molecular information about changes that shift the cellular machinery from mitotic to meiotic chromosome division, a rapid procedure for isolating yeast nuclei and nuclear matrices free of other cellular material was developed. About 320 polypeptides were found in nuclei and 100 polypeptides in the matrix. Some of these clearly change during the transition from mitosis to meiosis and are being further investigated. The same techniques will be used to investigate the nuclear properties of differenting astrocytes.

2. Control of sporulation by GTP, methylation and other reactions involved early in sporulation of B. subtilis. The fact that specific deprivation of guanine nucleotides causes massive sporulation of B. subtilis has been reported earlier. Deprivation of pyrimidine nucleotides does not cause this effect. Furthermore, all studies using stringent and relaxed strains have shown that the sporulation caused in stringent strains by amino acid deprivation is not due directly to the deficiency of the amino acid or the increase of ppGpp but rather due to the ensuing decrease of GTP. This stringent response can also be prevented by very small concentrations of antibiotics, some interfering with protein synthesis and others with other macromolecular syntheses, presumably because they prevent the uncharging of tRNA. Sporulation can be restored by addition of decoyinine which specifically inhibits GMP synthetase and causes a decrease of GTP regardless of the presence of the stringent response.

The frequency with which cells enter sporulation during a cell cycle can be greatly increased by addition of intermediate concentrations of ethionine or seleno-methionine both methionine analogs. Because ethionine is very toxic to normal Bacilli, this can be done only in mutants (genotype eth) which are partially resistant to the methionine analog. The increase in the sporulation frequency was observed only if ethionine could be converted to S-adenosylethionine, an analog of SAM. If this conversion was prevented by a mutation in SAM synthetase, no sporulation induction was observed. The eth mutation enabling this induction has pleiotropic effects, including a relaxed property (no ppGpp made upon amino acid starvation) and a change in DNA methylation. The latter was shown by the use of special phages and indicates that the DNA methylase activity of the eth mutant enables the methylation of the internal C of a GGCC tetramer that is part of a large recognition sequence. These are the first studies which indicate that methylation reactions control the frequency of differentiation in bacteria. Similar studies in higher organisms have shown

that the undermethylation of DNA increases the frequency with which cells switch to a differentiated cell type.

Asymmetric septation of Bacilli must somehow involve lytic enzymes. To investigate this involvement, several lyt mutants and well as rod mutant unable to form rods at high temperature were examined. It had been assumed that these mutants had deficient lytic enzymes. However, the laboratory found that the deficiency of cell wall lysis could be overcome by a change in the salt concentration. For example, the lyt-15 mutant had been assumed to be unable to turnover cell wall, but it started to turn it over when the salt concentration was increased to 0.2 M NaCl. Apparently, the structure of the cell wall is different in these mutants. The temperature sensitive rodB strain was unable to sporulate at elevated temperature. This indicates that the rod shape of the bacteria is essential to enable the formation of asymmetric septum and the production of a forespore.

3. Cloning and functional analysis of a developmental gene. Whereas the mechanisms controlling the enzyme induction and repression needed for cell duplication (vegetative growth) are reasonably well understood, it is not known how cells prevent the expression of developmental genes until they are needed at the particular stage of differentiation. The gld gene for glucose dehydrogenase of B. subtilis is such a developmental gene; it is transcribed and translated only during sporulation and only in the forespore cell compartment, which is located inside the mother cell and is surrounded by two membranes with opposite polarity. The laboratory has cloned the gene together with its control (promoter, operator) region in various plasmids. All plasmids able to grow in E. coli produced GlcDH in E. coli. However, the plasmids able to grow in B. subtilis behaved like the chromosome itself in not producing GlcDH until three hours after differentiation had started. By removing various portions of the 4 kb gld-containing DNA the location of the structural gene has been determined, and it was found that removal of a particular 0.5 kb DNA region caused constitutive expression of GlcDH in B. subtilis. Two alternatives are now being investigated: First, the 0.5 kb region may contain a terminator so that a promoter ahead of it enables transcription of the gld gene and thus expression of GlcDH. Second, there may be a repressor in B. subtilis which prevents the expression of GlcDH during vegetative growth by attaching to a operator in the 0.5 kb region. The investigation has also been helped by sequencing the 4 kb DNA (using the dideoxy method in phage M13). The location of the structural gene was affirmed by N-terminal amino acid analysis of GlcDH. The promoter does not have the normal combination of bases and its location is now being determined.

To map the gld gene on the bacterial chromosome, the 4 kb region was introduced into a plasmid containing a chloramphenicol resistance (cat) marker. After selecting for chloramphenicol resistant transformants, the location of the cat marker in the chromosome was determined by transduction

analysis and was found to be close to a mannitol (mtl) marker. The location of gld next to mtl could then be affirmed directly by using the original lambda charon phage carrying the 4 kb region. In transformation experiments it was shown that this lambda DNA also contained the mtl marker. Using the knowledge of the genetic location it was then possible to isolate mutants deficient in GlcDH activity. A combination of in vitro and in vivo experiments are now being used to determine how the gld gene is controlled and to isolate the presumptive repressor.

4. Control of membrane properties in mammalian cells. The previously reported synergism between induction of β-adrenergic receptors by butyrate and 5-azacytidine was further investigated in order to see whether undermethylation of DNA might be involved. It was found, however, that several other compounds, including nucleosides (6-azacytidine and cytosine arabioside) that do not cause hypomethylation of DNA, also had the synergistic effect. These results show that the compounds induce receptor formation by a mechanism different from DNA undermethylation. The coupling of the β-adrenergic receptor to the adenylate cyclase has also been investigated further and it was found that butyrate induces qualitative changes in the adenylate cyclase regulatory component (N or G/F).

It was discovered that several mammalian cell lines contain an active transport system for tricyclic anti-depressants and β-adrenergic antagonists which does not take up catecholamines. This transport system had not been found in the past because the cellular uptake had been interpreted as a binding to cellular receptors. The uptake is clearly energy-dependent and depends on the maintenance of an electrochemical proton gradient across the plasma membrane. These studies have also demonstrated that not only intracellular organelles, but also the plasma membrane, contains a Mg-ATPase which supplies the energy for the proton export. The amine transport system resembles that found in chromaffim granules, synaptic vesicles and platelet organelles, but it has a different specificity in being unable to take up β-adrenergic agonists.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02527-03 LMB

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Role of Methylation in Differentiation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

E. Freese, Chief, Laboratory of Molecular Biology, NINCDS

COOPERATING UNITS *(if any)*

Dr. A. T. Ganesan - Department of Genetics, Stanford University Medical Center

LAB/BRANCH

Laboratory of Molecular Biology

SECTION

Developmental Biology Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.0	2.5	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A mutation (eth) conferring resistance to ethionine, an analog of the amino acid methionine, in Bacillus subtilis has been studied. The eth mutation is pleiotropic, causing ethionine-resistance, increased spontaneous sporulation at 33°C, conversion of relA mutants from high to low serine-sensitivity, protection of phage phi105 grown in an eth strain against hsrR restriction and relaxed RNA synthesis as well as absence of ppGpp synthesis upon amino acid starvation. B. subtilis strains carrying the eth mutation continually enter sporulation at a much higher rate in the presence of 2mM DL-ethionine than in its absence. The fact that sporulation is caused by ethionine in a relaxed background suggests that in these mutants the onset of sporulation bypasses the GTP drop believed to be the initial event when sporulation is initiated by nutrient deprivation.

In contrast to Escherichia coli, B. subtilis produces S-adenosylethionine (SAE) upon ethionine addition. Inclusion of a mutation causing a deficiency in S-adenosylmethionine (SAM) synthetase activity (metE1) to the eth background abolishes the increase in sporulation upon ethionine addition and prevents the synthesis of SAE. This finding suggests that ethionine causes sporulation via SAE by interfering with the methylation or causing the ethylation of some cellular component.

In the eth mutant, DNA sites specific to the hsrR restriction endonuclease, are more methylated than in the standard strain. The DNA modification activity influenced by the eth marker is likely the methylase normally induced during competence for DNA uptake and subsequent transformation.

Changes in methylation of the genetic material occur during differentiation in mammalian cells. Our studies are the first to show a similar phenomenon in the differentiation of a microorganism which is accessible to the full range of tools of molecular genetics and biochemistry.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 01886-14-IMB

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Control of Meiosis and Morphogenesis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
E. B. Freese, Biologist, LMB, NINCDS
Sanford Silverman, Senior Staff Fellow, LMB, NINCDS

COOPERATING UNITS *(if any)*
NONE

LAB/BRANCH
Laboratory of Molecular Biology

SECTION
Developmental Biology Section

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	3.5	0.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Meiosis and sporulation in the eukaryotic yeast Saccharomyces cerevisiae can be initiated by the partial deprivation of purine nucleotide and most effectively by the deprivation of guanine nucleotides (GTP).

This differentiation can also be caused by partial deprivation of sulfur or methionine which in turn results in the decrease of intracellular concentrations of S-adenosylmethionine (SAM), methionyl-tRNA^{MET}, and GTP. Experiments are underway to determine which of these compounds control sporulation.

We have also begun to determine some structural and genetic changes which are involved in meiosis. A method has been developed to isolate large numbers of nuclei and nuclear matrices rapidly and cleanly. Two-dimensional electrophoresis was used to determine qualitative and quantitative changes in nuclear proteins and in DNA associated with the nuclear matrix.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 01244-20 LMB

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Control Mechanisms and Differentiation

PRINCIPAL INVESTIGATOR *(List other professionel personnel below the Principal Investigator.) (Name, title, leboratory, end institute effiliation)*

E. Freese, Chief, Laboratory of Molecular Biology, NINCDS

COOPERATING UNITS *(if any)*

Dr. Stuart Rudikoff	NIH
Dr. N. Vasantha	Genex Corporation, Gaithersburg, MD
Dr. R. Ramaley	Univ. Nebraska Medical Center, Omaha, NE

LAB/BRANCH

Laboratory of Molecular Biology

SECTION

Developmental Biology Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
7.0	6.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the spece provided.)*

Sporulation in the prokaryotic Bacillus subtilis can be initiated by partial deprivation of amino acids, which causes a "stringent response". Certain antibiotics prevent this induction with almost no inhibition of growth. Their effect was counteracted by decoyinine which specifically inhibits the synthesis of guanosine monophosphate (GMP). The stringent response also inhibits the uptake of guanine and uracil, but only the latter is also inhibited by addition of decoyinine alone. Thus, the increase of ppGpp controls some reactions directly and others indirectly, via the decrease of GTP.

To understand the mechanisms controlling the synthesis of a typical developmental protein, the gene for glucose dehydrogenase of B. subtilis was cloned. In the non-differentiating E. coli, the plasmid caused production of active glucose dehydrogenase during vegetative growth. However, a low copy plasmid shuttle vector produced essentially no glucose dehydrogenase activity in B. subtilis, and a high copy plasmid produced a low amount of enzyme; this suggests the titration of a repressor. In vitro, the gene could be transcribed into RNA, but only by a minor component of RNA-polymerase of B. subtilis which may play a special developmental role. The relevant DNA has been sequenced.

The cell wall turnover deficiency of B. subtilis lyt mutants was found not to be due to the lack of peptidoglycan hydrolases as previously clamined by others. Turnover deficiency in one of these strains (lyt-15) can be prevented by 0.2 M NaCl (other cations are less effective) and is due to a yet unidentified change in the wall. Sporulation of a rodB strain, which grows as a rod at 30°C and as a sphere at 45°C, was at 45°C unable to form a prespore septum. This suggests that the topological information provided by the cell shape is required for sporulation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02365-06 LMB

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Cellular Responses to Hormones and Neurotransmitters

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
R. C. Henneberry, Chief, Molecular Neurobiology Section, Laboratory of Molecular Biology, NINCDS

COOPERATING UNITS *(if any)*
Developmental and Metabolic Neurology Branch, NINCDS

LAB/BRANCH
Laboratory of Molecular Biology

SECTION
Molecular Neurobiology Section

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
5.0	3.0	2.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The major goal of this project is to better understand the responses of individual mammalian cells to extracellular signals. We have previously described modulation of hormone/neurotransmitter receptors on cultured human cells by manipulation of medium components and induction of receptor synthesis and expression by short-chain fatty acids. During this reporting period we have shown that induction of receptor synthesis does not correlate with DNA hypomethylation. We have also described a novel amine transport system with a previously undescribed specificity in several cultured cell lines, including C-6 rat astrocytoma cells; beta-adrenergic antagonists, but not agonists, are taken up at a site clearly distinguishable from the beta-adrenergic receptor. We have also found this amine transport system in a rat pituitary cell line which does not have beta-adrenergic receptors. Amine transport depends on the maintenance of an electrochemical proton gradient across the plasma membrane, which in turn depends on the activity of a MgATPase which appears to reside in the plasma membrane.

TAB 6 -- LABORATORY OF MOLECULAR GENETICS -- (LMG)

ANNUAL REPORT

October 1, 1983 through September 30, 1984

Laboratory of Molecular Genetics

National Institute of Neurological and Communicative Disorders and Stroke

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ANNUAL REPORT
October 1, 1983 through September 30, 1984
Laboratory of Molecular Genetics
National Institute of Neurological and Communicative
Disorders and Stroke

Robert A. Lazzarini, Chief

During fiscal year 1983, the Laboratory of Molecular Genetics pursued the five research projects that were initiated during the previous three year period, consolidated its gains, and greatly expanded and developed these five programs. In last year's Annual Report, we described the isolation and characterization of a small recombinant DNA clone that contained information for myelin basic protein (MBP). This clone was employed in the current reporting period to isolate five additional clones covering different regions of the MBP mRNA sequence. At present, virtually all of the mRNA has been represented in cDNA. These clones have been sequenced and the amino acid sequence of mouse small myelin basic protein has been deduced. This sequence agrees with the partial sequence that has been obtained by direct amino acid sequencing of the protein, but greatly extends the sequence information to regions not previously explored. These cDNA clones are also being used to study the structure of the mouse chromosome in the vicinity of the MBP gene. We know from our earlier work as well as from the work of others that the MBP gene is very large (about 50,000 base pairs long), yet only 2,300 bases appear in MBP mRNA. This sequence of 2,300 bases is not represented as a contiguous sequence within the gene, but is believed to be derived from five widely spaced regions (exons) of the gene. By using the cDNA clones, we have already localized one of these five regions within the gene and are well on our way to locating all five regions. The completion of this work will give us a detailed map of the MBP gene, the point of departure for a study of the molecular mechanisms controlling MBP expression in the oligodendrocyte.

Finally, we used the MBP clones to track the time of appearance, cellular localization, and relative abundance of MBP mRNA in cultured oligodendrocytes. To accomplish this feat, we first refined the existing procedures for oligodendrocyte culture so that sufficient quantities of cells could be easily produced. We then adapted published procedures for in situ hybridization so that they could be applied to oligodendrocytes cultured on glass coverslips without destroying the morphological details of the cells. This proved to be an important advance because the characteristic morphology of oligodendrocytes can be used to identify the cell in mixed cultures. Ultimately, we invented a procedure for double-labeling cells using immunolabeling to positively identify the variety of cell types in the culture and in situ hybridization to locate and quantitate mRNA. This entirely new technique allows us to identify MBP mRNA in cells long before myelin basic protein accumulates to sufficient concentrations to be detected by the immune reagents.

Our second major sphere of interest has been the molecular virology of the negative strand RNA viruses. This family of viruses, which includes measles, mumps, rabies, influenza, and VSV, share a number of morphological features and use the same general replicative strategy during the infectious process. Some of these viruses cause a variety of severe neurological diseases, many of which

are characterized by a slow persistent pace. During this past year, we carried out studies in three areas of molecular virology of negative strand RNA viruses. In the first, we studied the structure and function of the giant RNA polymerase protein. This protein is responsible for both the synthesis of viral mRNA and the replication of the viral chromosome. In previous studies, we had isolated and characterized five overlapping cDNA clones which span the entire gene coding for the RNA polymerase protein. This year, we spliced these clones together to form a single large recombinant DNA gene coding for the entire protein. This gene, approximately 6,400 bases long, represents more than 60% of the entire virus chromosome. We positioned this gene in appropriate expression vectors and introduced it into uninfected cells. Under these conditions, the VSV RNA polymerase protein is synthesized in sufficient amounts to enable us to demonstrate that the protein is functional and will rescue viruses that are defective in their RNA polymerase. We have now begun a series of experiments in which predetermined mutations will be introduced into the recombinant DNA gene in order to define the critical regions of the gene and to dissect the various functional domains of the protein.

The second study concerns the structure of the measles genome. This virus is particularly prone to invading the CNS and causing a slow subacute sclerosing encephalitis. We prepared cDNA clones of the measles virus as well as the canine distemper virus, a close relative. In the course of the past year, we sequenced much of the measles and CDV genomes and examined them for structural features as well as for homology between the two viruses. Our analyses have revealed that the measles genes have unexpected complex structures. The phosphoprotein gene contains overlapping reading frames, that is, the same stretch of nucleotide sequence codes for two different proteins depending upon the reading frame used. We have demonstrated that both of these proteins are synthesized in the infected cell. These proteins are found in close association with each other in the infected cell and both are believed to be involved in the replication of the measles genome. Similarly, the matrix protein gene of measles is also complex. In this case, there is a second open reading frame that does not overlap the first but follows it in a tandem fashion. This curious feature is also found in canine distemper virus which suggests that this type of structure is important and was conserved during the evolution of these viruses. We do not know, as yet, whether this second open reading frame of the matrix gene is actually expressed in the infected cell.

The final area of negative strand virology receiving our attention is viral assembly: the gathering of all the viral components in the infected cell to form a mature progeny virion. We employed the fast-freeze, deep-etch technique to examine the assembling virions on the inner surface of the cell membrane. These studies revealed that the viral chromosome assumes a tight-coiled configuration just before budding through the surface. Our work suggests that the tight coiling is induced by the binding of the matrix protein to the genome of the virus. We are currently pursuing these studies using mutant viruses that have a thermal labile matrix protein which can be inactivated at 40°C. We anticipate that these studies, together with the positive localization of the matrix protein at the site of tight coiling of the measles chromosome, will establish unequivocally that the binding of the M protein to the viral genome is a prerequisite for tight coiling.

CONTRACT NARRATIVE
Laboratory of Molecular Genetics
Fiscal Year 1984

UNIVERSITY OF VIRGINIA (NO1 NS 12353)

Title: Large Scale Preparation of VSV DI Particles, and E. coli Plasmid DNA Containing VSV Sequences.

Contractor's Project Director: Dr. Jay C. Brown

Current Annual Level: $81,900

Objectives: To establish conditions for the growth and purification of VSV defective particles which will reproducibly yield materials of the requisite purity and activity, to devise procedures for the purification of plasmid DNAs that contain VSV sequences, and to supply such materials to the Laboratory of Molecular Genetics, IRP, NINCDS.

Major Findings:

a) Conditions and procedures have been devised for the purification of the virus particles and plasmids. Materials prepared by this new scheme meet the specifications set forth in the contract.

b) The contractor has delivered to the Laboratory of Molecular Genetics, IRP, NINCDS, the amounts of purified VSV DI particles and plasmid DNA stipulated in the contract.

c) The contractor has established procedures for the preparation of plasmid DNA from E. coli and has supplied the materials designated on the contract.

Significance to the NINCDS Program and Biomedical Research: The procedures and materials developed under this contract are immediately used by the Molecular Genetics Laboratory. This contract, therefore, forms an integral part of the Laboratory's research program, namely, the regulation of viral nucleic acid synthesis in animal cells. This contract has supplied the Program with the raw materials for RNA sequencing of the viral genomes. These studies have characterized sites on the chromosomes that are important for autointerference, DI particle genesis, and the replication of the viral genome.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02528-03 LMG

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation of Myelin Synthesis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. A. Lazzarini	Chief	LMG, IRP, NINCDS
Others:	N. Zeller	Staff Fellow	LMG, IRP, NINCDS
	L. Hudson	Senior Staff Fellow	LMG, IRP, NINCDS
	F. de Ferra	FIC Visiting Fellow	LMG, IRP, NINCDS
	J. Sprague	Chemist	LMG, IRP, NINCDS
	B. Lewis	Biological Lab Technician	LMG, IRP, NINCDS

COOPERATING UNITS *(if any)*

Department of Biology, University of Maryland

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

Recombinant Genetics Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5	3.5	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Four proteins of a peripheral and central nervous system have been targeted for study -- the myelin basic protein, P_2, P_0 and proteolipid. The first phase of the molecular level studies is the cloning of the genes coding for these proteins. To this end, we have obtained the necessary human perinatal brain tissue, prepared cDNA libraries from brain mRNAs, and are presently searching among the five hundred library clones in order to identify those which contain the genes for myelin basic proteins. We have positively identified several such clones and are characterizing them extensively to establish whether they contain the desired genes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02026-12 LMG

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation of Viral Nucleic Acid Synthesis in Animal Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M. Schubert	Research Chemist	LMG, IRP, NINCDS
Others:	E. Meier	Visiting Fellow	LMG, IRP, NINCDS
	G. Harmison, II	Chemist	LMG, IRP, NINCDS
	L. Hudson	Senior Staff Fellow	LMG, IRP, NINCDS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

Molecular Virology Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	3	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Many members of the negative strand RNA viruses cause diseases of the central nervous system (CNS) and are, therefore, important to medical neurology. Infections frequently convert to a persistent state either by the formation of defective interfering (DI) particles, deletion mutants which interfere with the replication of the parental virus or by the accumulation of mutations in the ever changing genome, both affecting the amount of virus released and its cytopathogenicity. Little is known about the regulation of gene expression and replication of these viruses in host cells. All of these processes involve the polymerase and its specific interactions with the nucleocapsid template. The molecular events of transcription and replication of the viral genome are subject of this research project.

Towards these ends, we have cloned and sequenced the VSV L gene (6,400 bases) which codes for the multifunctional RNA dependent RNA polymerase (L). The sequence analysis revealed direct evidence for the high mutability of VSV, suggesting that the polymerase itself has a mutator function which may contribute to the establishment and maintenance of persistent infections.

In order to study the multiple viral essential functions of the polymerase, we have assembled the complete L gene from partial cDNA clones and have expressed this gene in eukaryotic cells. This represents the first successful functional expression of a recombinant polymerase gene of a negative strand virus. This system will now allow for the first time to dissect the functions of the protein as well as their structural organization within this single gene.

We anticipate that the study of these functions will reveal characteristic viral specific mechanisms which may be exploited in the treatment or prevention of viral infections in the CNS.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02600-02 LMG

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Assembly of Enveloped RNA Viruses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	H. Arnheiter	FIC Visiting Associate	LMG, IRP, NINCDS
Others:	M. Dubois-Dalcq	Section Chief	LMG, IRP, NINCDS
	N. Hogan	Senior Staff Fellow	LMG, IRP, NINCDS
	W. Odenwald	Microbiologist	LMG, IRP, NINCDS
	K. Ono	FIC Visiting Fellow	LMG, IRP, NINCDS
	R. Rusten	Biological Lab Technician	LMG, IRP, NINCDS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

Molecular Virology Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	3.5	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The elucidation of mechanisms of synthesis, processing and transport of viral components at the molecular level will help us to understand mechanisms that govern biosynthesis of normal cellular components and to understand problems of defective viral assembly implicated in some neurological diseases. To study the biosynthesis of viral macromolecular components, we have prepared a battery of monoclonal antibodies reacting with different sites of polypeptides of two negative stranded RNA viruses, Vesicular stomatitis virus (VSV) and measles virus, polyclonal antibodies made against synthetic peptides corresponding in sequence to portions of the viral polypeptides, and genes coding for some of the viral polypeptides of the Vesicular stomatitis virus cloned into convenient expression vectors. One part of the project concerns the study of assembly processes in living rather than fixed or fractionated cells. Purified antibodies are microinjected into cultured cells in order to interfere with specific assembly mechanisms. Microinjection of antibodies labeled with a fluorescent tag is used to track the transport of some viral components, and low light intensity video microscopy in combination with differential interference contrast microscopy allows us to document on videotapes the transport of labeled antibodies marking the transport of viral components. Immunocytochemistry at the electron microscopic level is used to determine the ultrastructural localization of injected antibodies. A second part of the project concerns the elucidation of mechanisms which leads to the ultimate assembly of viral components at the sites of viral budding. High resolution stereo views are obtained from platinum-carbon replicas of the outer and the inner side of the plasma membrane of cells infected with either one of the above mentioned viruses. The location of specific viral components at the plasma membrane are marked with antibodies coupled to an electron dense marker, colloidal gold. Temperature-sensitive viral mutants with lesions in polypeptides affecting normal budding are studied to obtain systems in which viral budding can be synchronized.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02580-02 LMG

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Determinants of Virus-Host Cell Tropism

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	W. J. Bellini	Senior Staff Fellow	LMG, IRP, NINCDS
Others:	C. Richardson	Special Expert	LMG, IRP, NINCDS
	S. Rozenblatt	Visiting Associate	LMG, IRP, NINCDS
	N. Hogan	Senior Staff Fellow	LMG, IRP, NINCDS
	G. Englund	Biological Aid	LMG, IRP, NINCDS

COOPERATING UNITS *(if any)*

Neuroimmunology Branch, NINCDS

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

Molecular Virology Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.5	4.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has as a final objective, the elucidation of those viral and host cell components which influence, at the molecular level, the phenomenon known as viral tropism. Currently, the emphasis of this project is focused on those components of measles virus which pertain to the neurotropism of this clinically relevant paramyxovirus.

During the course of natural infection, neurotropic variants of measles virus are generated. Frequently, this gives rise to mild central nervous system involvement and, less frequently, to clinical encephalitis. In rare instances, a delayed encephalitis, subacute sclerosing parencephalitis, is observed. Although the mechanism(s) of this neurotropism is unknown, available evidence suggests that the viral envelope glycoproteins are involved and can be antigenically distinguished from the wild-type or vaccine strains using hybridoma antisera. Therefore, the initial phase of this project is to clone those genes encoding these proteins from a vaccine strain (Edmonston) of measles virus. From the nucleotide sequence, we will deduce the amino acid sequence of the proteins. To positively identify these clones, oligopeptides from the deduced amino acid sequence will be synthesized. Antisera raised against the synthetic peptides will then be used in a variety of immunologic techniques to identify the viral protein recognized and, thus, assign the clones. Once this is established, fragments of these cDNA clones will then be used as probes to identify their counterparts in neurotropic strains of measles virus presently available in our laboratory. The nucleotide and deduced amino acid sequence of the glycoproteins of the neurotropic strains will then be compared with the vaccine and wild-type virus for regions of homology and non-homology. The cloned glycoprotein genes will be placed in appropriate expression vectors to permit the study of their synthesis, regulation of expression, maturation and insertion into the host cell membrane.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02034-12 LMG

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biology of Myelin-Forming Cells In Vitro and In Vivo

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M. Dubois-Dalcq	Section Chief	LMG, IRP, NINCDS
Others:	B. Trapp	Senior Staff Fellow	LMG, IRP, NINCDS
	A. Baron	Ph.D. Student (until 11/83)	LMG, IRP, NINCDS
	T. Behar	Microbiologist	LMG, IRP, NINCDS
	R. Rusten	Biological Lab Technician	LMG, IRP, NINCDS

COOPERATING UNITS *(if any)*

LDBA, NIDR; DMN, IDB, LCNP, LMG, NINCDS; Department of Neuropathology, Albert Einstein College of Medicine; Department of Neurology, Johns Hopkins University School of Medicine

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

Neural and Molecular Ultrastructure Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.7	2.5	1.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Myelin sheath is essential to normal conduction in nerves and is altered in multiple sclerosis and Guillain-Barre diseases. Understanding how myelin is formed and repaired requires basic studies of the differentiation of myelin-forming cells both in vitro and in vivo. To this aim, we are culturing myelin-forming cells in isolation, obtaining enriched populations, and studying the differentiation of these cells. Rat Schwann cells do not synthesize myelin products in the absence of axons, but synthesize components of their basement membrane, such as collagen type IV and laminin. Laminin strongly stimulates Schwann cell adhesion and elongation of their processes. Therefore, laminin may trigger Schwann cell differentiation in vivo during early stages of interaction with the axon. Laminin is also synthesized by astrocytes in the central nervous system and may play a role in modulating the shape of oligodendrocytes. In contrast to Schwann cells, oligodendrocytes, derived from rat brain or optic nerve, synthesize galactocerebroside and basic protein in isolation. Sensitive methods of in situ hybridization also allow us to detect the message for basic protein before the protein, itself. Basic protein may be translated on ribosomes in the oligodendrocyte processes close to the site of insertion of this protein into the myelin membrane. We are presently investigating whether myelin-associated glycoprotein (MAG) and proteolipid of myelin, two transmembrane proteins, are also expressed in isolated oligodendrocytes. Our in vivo studies on MAG show that this protein is consistently associated with the periaxonal space, appears to maintain the axon-Schwann cell contact, and is not found in compact myelin in normal and pathological nerves in vivo, such as in quaking mice. In patients with paraproteinemia and neuropathies, MAG has been shown to be an antigen recognized by monoclonal IgMs. The antigenic site recognized by these antibodies appears to be the carbohydrate moieties of the molecule and the patient's monoclonal IgM also reacts with gangliosides.

TAB 7 -- LABORATORY OF NEURAL CONTROL -- (LNLC)

ANNUAL REPORT

October 1, 1983 through September 30, 1984

Laboratory of Neural Control, Intramural Research Program
National Institute of Neurological and Communicative Disorders and Stroke

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ANNUAL REPORT
October 1, 1983 through September 30, 1984
Laboratory of Neural Control, Intramural Research Program
National Institute of Neurological and Communicative Disorders and Stroke

Robert E. Burke, M.D., Chief

Introduction

Research work in the Laboratory of Neural Control (LNLC) is devoted largely to studies of the central and peripheral neural mechanisms involved in the control of movement in mammals, emphasizing neural organizations at the level of the spinal cord and those regions of the brain stem and cerebral cortex that project directly to the spinal cord.

Present Organization

During FY 1984, the staff of the Laboratory of Neural Control (LNLC) included 9 professional scientists (four permanent senior scientists and five post-doctoral fellows). The permanent staff also includes three senior support personnel (two engineers and one physiologist), a biological technician, and one laboratory secretary. Non-permanent, part-time staff includes two graduate students, one computer programmer, one engineering aide, and one laboratory aide. Because of the close interaction and collaboration among the Laboratory staff, LNLC has not been divided into formal Sections. The research effort can be described under four general headings, divided roughly by methodological approach:

1. Electrophysiological and morphological analysis of the cellular physiology and neuronal circuitry operating in the control of movement at the spinal cord level, largely using acute, reduced preparations (primarily cats).

2. Projects that utilize novel methods for recording the activity of individual neural elements, activity patterns in whole muscles, and kinesiological data in awake, intact animals (both cat and monkey) that are comfortable and performing normal motor behaviors.

3. Theoretical and computer modeling studies of: a.) the electrophysiological properties of identified central nervous system neurons; b.) information processing in neural networks; c.) the mechanical arrangements of bones, joints and muscles in the cat hindlimb with a view to providing a comprehensive description of their dynamic actions; and d.) the properties of complex elements such as muscle spindles.

4. Activities concerned directly with the development of new instruments and techniques, and the further refinement of existing methods, for recording and analyzing neurally-relevant data from intact, freely moving animals and for computer-assisted reconstruction of the anatomy of functionally identified neural elements.

Project Summaries:

Two projects, "Motor Control Systems in the Spinal Cord" and "Intrinsic Properties of Motor Units" can be discussed together. A major effort during FY 84 concerned final data analysis and computer modeling regarding the passive membrane properties of type-identified α-motoneurons, and of the generation of excitatory postsynaptic potentials in these cells by group Ia afferents. The experimental data base for these studies came from a multi-year effort to study the detailed morphology of group Ia muscle spindle afferents and the synaptic connections made by them on type-identified α-motoneurons in the cat spinal cord. Functionally identified afferents and motoneurons were injected intracellularly with horseradish peroxidase (HRP) and reconstructed from serial sections. Detailed description of anatomical results were included in past Annual Reports.

In FY 84, work concentrated on using our anatomical data, together with electrophysiological measurements obtained in motoneurons that were fully reconstructed, to complete computer analyses designed to estimate the passive membrane properties of type-identified alpha-motoneurons, and to compute simulated synaptic potentials in computer models of reconstructed motoneurons, using anatomical and physiological data about group Ia synaptic contacts. This work required the development of specialized application programs that utilize the general purpose circuit analysis program, SPICE, available on the NINCDS VAX 11-750 computer. Another set of analytical programs was developed on the LNLC Hewlett-Packard Model 236 computer system. In combination, these programs have permitted us to model the dynamic electrical behavior of anatomically-correct motoneuron simulations with arbitrary choices for specific membrane resistivity (R_m), capacitance (C_m), and cytoplasmic resistivity (R_i).

The electrical behavior of six model motoneurons was compared with records obtained from the same cells experimentally. A number of assumptions were made about dendritic boundary conditions, and cytoplasmic and extracellular resistivities. This analysis suggests that R_m in alpha-motoneurons is non-uniform, with relatively low values on and near the motoneuron soma and much higher values in the dendritic tree. Given non-uniform R_m, it is possible to match experimental and simulation results with C_m of 1 uF/cm^2, a value which appears to be characteristic of all biological membranes. Unfortunately, the condition of membrane non-uniformity allows matching of experimental results with many forms of R_m non-uniformity. The two models explored systematically were: 1.) a step increase in R_m from low values in the soma to much higher but uniform values in the dendritic tree (step model); and 2.) R_m increasing smoothly from soma to distal dendrites according to a sigmoidal function derived from the cumulative area distribution as a function of distance from the soma (sigmoidal model). The modeling results are consistent with the conclusion of earlier work in LNLC that R_m is systematically higher in type S motoneurons than in cells that innervate fast twitch muscle units. However, absolute values cannot be obtained with currently available techniques. Our work suggests that this will be true for any neuron type when R_m is non-uniform.

Utilizing our reconstructions of 24 group Ia afferent-motoneuron contact systems, we have developed methods for "assigning" Ia synapses to our six model motoneurons according to the observed spatial distributions of Ia-motoneurons contact systems. This has permitted evaluation of synaptic potentials produced in model motoneurons by synapses that, in number and spatial distribution, conform to the best experimental data presently available. In addition, we have used recent results obtained elsewhere to specify the magnitude and duration of conductance transients at group Ia synapses on motoneurons. The results of these synaptic modeling studies show that the EPSPs produced in model motoneurons have amplitudes and shapes that match those observed experimentally in cat motoneurons. Further, the correlations between input resistance and EPSP amplitude match almost exactly the correlation obtained in this laboratory 15 years ago in decerebrate, unanesthetized cats. These studies suggest that the factors that control synaptic potential amplitude in motoneurons, and presumably in neurons generally, involve a complex interaction between the number and spatial distribution of active synapses, in conjunction with postsynaptic characteristics of the recipient neuron. In the case of motoneurons, the available evidence suggests that the key factors controlling Ia EPSP amplitude are synaptic density and the dendritic/somatic conductance ratio, rho.

We have also been studying the organization of excitatory interneuronal pathways to motoneurons in the cat spinal cord, with emphasis on the cutaneous input pathways that project to motoneurons of the flexor digitorum longus (FDL) nucleus. We earlier showed that FDL exhibits a unique pattern of stereotyped activity during locomotion in the intact cat, which persists in fictive locomotion, including that in the low spinal preparation. This implies the existence of a discrete set of excitatory interneurons in the lumbosacral spinal cord that drives this activity and is part of the segmental "locomotor pattern generator". FDL cells also receive what seems to be a special disynaptic excitation from distal skin afferents. Up to now, cutaneous input to hindlimb motoneurons had been thought to be trisynaptic at minimum. Our working hypothesis is that interneurons in this cutaneous pathway may be identical to those postulated to drive the unique flexor burst pattern in FDL during locomotion. We have therefore begun to examine sources of convergent control of the disynaptic cutaneous pathway, with emphasis on supraspinal systems. We have clear evidence that this set of cutaneous excitatory interneurons receives convergent excitation from the rubrospinal and corticospinal tracts, but not from the vestibulospinal or reticulospinal systems. This fits with our previous description of rubro- and corticospinal convergence onto interneurons in the trisynaptic cutaneous pathway to triceps surae motoneurons. We will attempt to further characterize synaptic inputs to the disynaptic excitatory cutaneous pathway to FDL, as a prelude to searching for these interneurons individually, using double microelectrode and spike-triggered averaging methods.

Finally, we have initiated a sub-project to characterize motor units in the cat tenuissimus (TEN) muscle. This is necessary background information for a collaborative project with an investigator in Israel on possible differences in neuromuscular transmission in different types of motor units. However, there is considerable intrinsic interest in the TEN muscle, which has

a unique morphology and probably functions more as a positional transducer than as a generator of output force. The TEN motor pool contains only about 20 α-motoneurons but these innervate muscle units that represent all of the four motor unit types defined in this laboratory in other, larger hindlimb muscles. Of particular interest is the fact that the range of individual unit tensions is much narrower than in the other muscle studied. The TEN motor unit pool also contains a much higher proportion (about 23 percent) of fast twitch, intermediate fatigability units (type FI) than other hindlimb muscles. These findings will be followed up in FY 85 with histochemical analysis of the muscle, including glycogen-depletion of individual, type-identified muscle units.

The project entitled "Neuromuscular Coordination of Movement" includes a variety of studies, using both novel and conventional experimental methods to study motor performance in intact, behaving cats. Most of the new techniques involve the use of chronically-implanted transducer systems developed and perfected in LNLC, as detailed in previous Annual Reports. The motivating philosophy in this work is to obtain information from intact, freely behaving animals in a form that enables interpretation according to the very large data base accumulated about the behavior of neural elements in anesthetized, immobilized, or otherwise reduced preparations.

During FY 84, we have continued a detailed analysis of the cat hindlimb musculature with respect to the anatomical interrelations between muscles and muscle groups in relation to their functional activity. One aspect of this concerns an examination of the functional activity patterns of multiple hindlimb muscles during treadmill locomotion in the intact cat. These normal locomotor patterns are then compared with patterns emitted by the same animal after acute decerebration, and then after cervical spinalization. The temporal pattern of muscle activations in locomotion is very similar in the intact and decerebrate states, but the magnitudes of muscle activation after decerebration shift toward decreased activation of extensors and increased activation of flexors. Paradoxically, the complex patterns of gated flexor reflexes that can be elicited in the intact cat by stimulating cutaneous nerves essentially disappear after decerebration, presumably because transmission through the involved segmental interneurons is blocked.

Earlier work on the organization of muscle spindles and motor pools in the sartorius muscle demonstrated the potential functional complexity of biarticular muscles that can combine flexor and extensor functions in a single muscle, depending on step cycle phase. The sartorius has become a prime test of the "task group" hypothesis formulated in LNLC over the last several years. Work on this problem has continued with studies of the anatomical organization of the sartorius motor pool, using HRP retrograde transport techniques. We have found a rostral-to-caudal correspondence between motoneuron position and the medial-to-anterior location of innervated muscle units in sartorius. However, there is no indication that the three functional task groups of motoneurons present in the cat sartorius are located in different parts of the ventral horn. Rather, the sartorius motor nucleus, like those of other muscles studied in LNLC and elsewhere, forms a continuous column of cells in which α- and γ-motoneurons are admixed. Future work on

this problem will concentrate on intracellular recording methods to examine the functional organization of synaptic inputs to the different task group subsets of sartorius motoneurons.

The issue of complex, bi- and multiarticular muscle organization is also being pursued in studies of the hamstring flexor muscle, semitendinosus, which has a very complex, in-series internal architecture . Similar kinds of questions can be studied in this muscle and in the tenuissimus, which is under study for other reasons (see above). Collaborative work with a group of investigators at Queen's University, Kingston, Canada is directed to the same end, but using the anatomically complex muscles that produce head movement in the cat. The Canadian group has produced extensive anatomical analyses of these neck muscles, which are now being investigated jointly with them in LNLC, using chronic implant techniques available here to examine functional activity patterns in intact, behaving animals.

Work done on the project entitled "Models of Neurophysiological Systems" is closely related to the ongoing studies of locomotor organizations in the cat. Kinesiological data about the length trajectories, EMG patterns, and force production from many hindlimb muscles, which are generated in the various subprojects discussed above, serve as input to refine a general mathematical model of the cat hindlimb. The model is being developed jointly by LNLC and the Department of Electrical Engineering at the University of Maryland, under a research services contract. The overall direction of model development, and the entire data base for it, comes from LNLC, while the software development and mathmatical analysis is being done at the College Park campus. The cat hindlimb is modeled dynamically in two dimensions, using a mechanical plant based on hindlimb anatomy. The output of the system is evaluated in terms of limb trajectories and forces exerted by the foot on the ground. The contributions of individual muscles to this output can be predicted and then compared with the actual EMG patterns and tendon forces measured for the same muscle in situ, leading to refinement of the model. Work has progressed very satisfactorily and there is an excellent working relation between LNLC staff and the contract group. Data is transferred between NIH and University computers by telephone links which were made operational in FY 84.

This project is also importantly involved in the development of computer programs to facilitate the collection and analysis of kinesiological data from chronically implanted devices. The current design philosophy is to develop modular programs that are generally useful and that can be rearranged easily to suit particular applications. This requires considerable effort but should be practical in the long run, since LNLC has now standardized all of its computer installations that are used for on-line data collection.

Finally, work has begun on theoretical models of limb trajectory formation that allow a limb with two joints to "find" locations in a two-dimensional space, given particular algorithms of trajectory prediction and the ability to learn from previous trials. New trajectories are formed by interpolation of past results. Testing of this general hypothesis, which is much simpler than most of the existing concepts of motor program formation that have emerged from robotics engineering, will begin with human subject experiments in FY 85.

Experimental work in the project "Conduction Properties of Peripheral Nerve" was completed during FY 84 and data analysis will be finished in FY 85. The chronic multi-lead nerve cuff technique was used in cats to study the regeneration of nerve fibers with different conduction velocities after nerve crush, and to assess the effects of mechanical constrictions on axonal regeneration. These questions emerge from clinical conditions such as nerve entrapment and carpal tunnel syndromes. Preliminary data analysis indicates that the smaller diameter axons regenerate faster than larger diameter fibers after nerve crush. A mechanical constriction around the nerve, distal to the site of crush injury, delays but does not prevent regeneration through the constricted region. Axons peripheral to the constriction eventually regain a considerable range in conduction velocities but there is permanent slowing at the site of constriction in all axons.

Work on "Cortical Mechanisms of Voluntary Motor Control" has, during FY 1984, continued to examine the organization of motor output regions of the primate motor cortex during the performance of voluntary movement in awake monkeys. The discharge patterns from individual neurons in the arm/hand area of the cerebral cortex that have relatively direct pathways to the spinal cord and brain stem (the sensorimotor cortex and supplementary motor area) are recorded during movement performance in minimally restrained, alert monkeys. Recent results have emphasized the importance of monitoring the electrical (EMG) activity in multiple forelimb muscles during intracortical microstimulation (ICMS). Many other studies have relied on visual inspection or palpation to decide which muscle are activated during ICMS. However, our results show that these simple endpoints may lead to erroneous conclusions, since some intracortical points elicit marked inhibition of muscles with post-inhibitory rebound activation. Only indwelling bipolar EMG electrodes allow accurate assessment of the results of ICMS, including the important category of inhibitions. Unfortunately, many of the existing maps of cortical organization were made using the simpler observations.

We have continued to compare the results of ICMS with the activation patterns of cortical neurons recorded at the same points during voluntary movement, in order to assess the role of particular groups of motor cortical neurons on alternating versus co-contraction patterns of activation of agonist and antagonist muscles. The organization of cortical inhibition, presumably operating through spinal segmental interneuron systems, is of particular interest, since we have found that zones that produce inhibition of target muscles often border, or surround, zones that produce pure excitation. Much of this complexity had been missed in other studies of cortical organization because multi-muscle EMG methods were not utilized.

Work has begun on a subproject to examine the role of certain forms of sensory input to the motor cortex, especially that from proprioceptive afferents travelling in the dorsal columns. After extensive cortical mapping, the animal is subjected to complete section of the dorsal columns at C1, in order to remove ascending proprioceptive input. Postoperative results will be interpretable only after histological confirmation of the extent of the dorsal column lesion. In the only animal done thusfar, the lesion appeared to be less than complete.

Finally, collaborative work in this project has been initiated to assess several forms of multi-lead intracortical recording electrodes, fabricated by outside sources using thin-film technology. Existing evidence suggests that neurons in any given cortical location are probably interconnected in relatively stereotyped ways, producing "modular" neuronal circuits. However, the functional organization within such postulated modules has been difficult to assess because of the technical difficulty with recording reliably from many neurons within a small volume of CNS. Multi-lead electrodes appear to promise a breakthrough in this area but formidable technical problems remain to be worked out.

Work done under the project entitled "Techniques for Making Contact with the Nervous System" largely results from requirements generated by other projects in LNLC, although requests for instance received from outside groups in terms of questions or specific fabrication needs.

During FY 1984, we completed final assembly of a computer - microscope interface system, designed to facilitate collection of quantitative data about neuronal morphology. A relatively simple design strategy, based on the conventional camera lucida method, has been used. The camera lucida superimposes the microscope image with the image of a CRT face displaying the computer file that represents the structure being drawn. Position transducers on the microscope stage permit "movement" of the computer image displayed to match the real microscope image when drawing extensive structures and outlines, and a sensor on the fine focus reads depth information within the individual section. Software development is now complete for reconstruction of neuron positions in spinal cord sections, as needed for studies of motor nucleus anatomy after retrograde HRP labeling. A variation of the this program will allow reconstruction of the dendritic tree of intracellularly labeled neurons in a format compatible with other programs already developed. The hardware and software design should be flexible enough to fit a variety of neuroanatomical problems.

The "map pin" electrode design developed in this project several years ago is currently being evaluated for application by a team of neuroscientists and neurosurgeons at the VA Hospital in Syracuse, New York, in neurophysiological studies in human patients undergoing craniotomy. The interest in the electrode grew out of work with the extramural Neural Prosthesis Contract Program. Results to date indicate that map pin designs using activated iridium insulated with Parylene-C (similar to the electrodes used in animal studies in LNLC) have proved most suitable for the human work.

In this same connection, we have begun to develop a system for moving map pin electrodes after implantation. The main advantage of the map pin electrode - positional stability - is also its main disadvantage, in that the electrodes as currently used cannot be moved at will to other locations. An implantable system that would overcome this limitation would be a major advance for both research and potential clinical use. Several serious technical problems must be overcome in this work but initial evaluations appear promising that, at minimum, a practical system to advance multiple map pin electrodes in their tracks can be developed in an implantable form.

Finally, considerable effort has gone into improving our design for specialized "patch" electrodes for recording EMG potentials in both acute and

chronic implant situations. Several LNLC projects require complex electrode configurations to enable quantitation of EMG signals, and to record potentials from particular regions of muscles. The problem of cross-talk between EMG electrodes in chronic implant situations has also been dealt with. Current designs available in LNLC provide a greater degree of reliability and selectivity than are available elsewhere.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 01686-16 LNLC

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Motor Control Systems in the Spinal Cord

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R.E. Burke	Chief	LNLC NINCDS
Other:	J.W. Fleshman	Staff Fellow	LNLC NINCDS
	Idan Segev	Visiting Fellow	LNLC NINCDS
	Pablo Rudomin	Fogarty Scholar-in-residence	

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Neural Control

SECTION

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.4	1.5	.9

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is designed to provide information on the mechanisms operating within reflex systems in the adult cat spinal cord, which include alpha motoneurons as the output link, as well as on the interconnections and interactions between reflex pathways and control systems descending to the spinal cord from supraspinal centers. Particular consideration is also given to interrelations between synaptic organization, intrinsic neuronal properties, and dynamic behavior of the alpha motoneurons, and the motor unit type, as defined by the physiological characteristics of the innervated muscle fibers. A variety of preparations have been used, including anesthetized, decerebrate animals as well as intact, freely moving cats. Electrophysiological and morphological data are obtained.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 01687-16 LNLC

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Techniques for Making Connections with the Nervous and Musculoskeletal Systems

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M.J. Bak	Electronics Engineer	LNLC NINCDS
Other:	R.E. Burke	Chief	LNLC NINCDS
	G.M. Dold	Engineering Technician	LNLC NINCDS
	G.E. Loeb	Medical Officer (Res.)	LNLC NINCDS
	W.B. Marks	Research Physiologist	LNLC NINCDS
	E.M. Schmidt	Biological Engineer	LNLC NINCDS

COOPERATING UNITS *(if any)*

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INSTITUTE AND LOCATION
NINCDS, NIH, Bethesds, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.7	.2	2.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is intended to develop techniques for the acquisition and processing of neuroelectric signals from the central and peripheral nervous system in acute and chronic neurophysiological preparations. Because of this laboratory's continuing interest in sensorimotor neural activity during unrestrained movements, the project also includes development of chronically implantable mechanical transducers, catheters, and connectors.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 01688-16 LNLC

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Cortical Mechanisms of Voluntary Motor Control

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	E.M. Schmidt	Biological Engineer	LNLC NINCDS
Other:	M.J. Bak	Electronics Engineer	LNLC NINCDS
	G.M. Dold	Engineering Technician	LNLC NINCDS
	Joan S. McIntosh	Physiologist	LNLC NINCDS
	Simon Gil Spottswood	Biological Aid	LNLC NINCDS

COOPERATING UNITS *(if any)*

Fundamental Neurosciences Program, NINCDS (F.T. Hambrecht); Neuroprosthesis Research Program, NINCDS

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Laboratory of Neural Control

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INSTITUTE AND LOCATION

NINCDS, NIH, Bethesds, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	.9	1.6

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is designed to investigate the size and spatial distribution of cortical neuron "colonies" in the primate motor cortex that are associated with individual muscles or closely related groups of muscles, as well as the activity of neurons in such colonies during defined voluntary motor behaviors. Intracortical microstimulation (ICMS) is used to map regions that produce excitation or inhibition of particular muscles or muscle groups, and the resultant cortical maps are compared with those for synergist or antagonist muscle groups. Cortical cell discharge patterns during normal movements are evaluated with respect to the excitation or inhibition of muscle activity that is produced by ICMS. Intracortical capacitor stimulating electrodes are being evaluated for efficacy, stability and safety for chronic implantation. Intracortical multichannel recording electrodes are being evaluated for stability and safety for chronic implantation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02079-11 LNLC

PERIOD COVERED: October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Models of Neurophysiological Systems

PRINCIPAL INVESTIGATOR *(List other professionel personnel below the Principal Investigator.) (Name, title, leboratory, and institute affiliation)*

PI:	W.B. Marks	Research Physiologist	LNLC NINCDS
Other:	G.E. Loeb	Medical Officer (Res.)	LNLC NINCDS
	M.M. Manley	Bio. Lab. Tech.	LNLC NINCDS

COOPERATING UNITS *(if any)*: Dept. of Electrical Engineering, U. MD (W.S. Levine, J.P. Chapelier, He Ji Ping, W.M. Roberts)

LAB/BRANCH: Laboratory of Neural Control

SECTION:

INSTITUTE AND LOCATION: NINCDS, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.3	.7

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

As quantitative data from a wide variety of techniques and levels of investigation become available for a particular nervous system function, it is both possible and advisable to attempt to assimilate such information into a comprehensive model of the underlying mechanisms and their interactions. This project consists of the development of such models and the necessary analytical and mathematical techniques for their implementation and testing in several areas of intensive experimental investigation by LNLC members and the scientific community at large.

The kinematic model of the cat hindlimb initiated last year in collaboration with the University of Maryland has begun to yield experimentally verifiable time courses of muscle lengths and joint angles, and also, recently, of joint torques. Our new system of computer programs for data analysis and display within the UNIX operating system averages EMG signals recorded during a number of steps, compensating for natural variability in the steps. The system superimposes these EMG signals on graphs of lengths of the same muscles from the kinematic model so that muscles active during shortening versus lengthening can be detected and compared.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02080-11 LNLC

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Neuromuscular Coordination of Movement

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	G.E. Loeb	Medical Officer, Res.	LNLC NINCDS
Others:	W.B. Marks	Research Physiologist	LNLC NINCDS
	C.A. Pratt	Staff Fellow	LNLC NINCDS
	S. Duenas	Visiting Fellow	LNLC NINCDS
	C.A. Chanaud	Guest Researcher	LNLC NINCDS
	A.J. Rindos	Guest Researcher	LNLC NINCDS

COOPERATING UNITS *(if any)*
Queen's University Hospital, Dept. of Physiology, Canada (F.J. Richmond)

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INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.8	2.7	1.1

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standerd unreduced type. Do not exceed the space provided.)*

The cat has long been a standard animal for anatomical and acute physiological studies of muscle function and motor control at the spinal cord level. In this project, a wide variety of traditional and novel kinesiological techniques are being used to study motor tasks in unanesthetized, normally behaving cats, including computer-aided reconstruction of skeletal movement from videotape, multiaxis force plates, chronically implanted nerve cuff and EMG electrodes, and strain and length transducers. The major focus has been the study of hindlimb muscles and their afferent and efferent control during walking, which is the subject of a computer modeling project described in Project No. Z01-NS-02079-11 LNLC. Other hindlimb movements studied include jumping, paw shaking, scratching, and reflexes to cutaneous nerve stimulation during normal and decerebrate walking. In a collaborative study, similar data are being collected from a large number of neck muscles.

The major objective is to correlate patterns of usage with the complex mechanics and compartmentalization and proprioceptive specializations of these muscles. A major theme emerging from these experiments is a concept of "Task Groups," which denotes the segregation and specialization of sensorimotor systems to perform kinematically homogeneous tasks in an optimal manner. This is particularly apparent in multiarticular muscles, which in some cases use independent subdivisions of their alpha motoneuron pool to accomplish kinematically diverse tasks. Some of these bifunctional muscles have been found to have a heretofore overlooked internal architecture consisting of short, parallel muscle fibers in series, which poses additional questions regarding their coordination.

Current work asks how well these notions extend to other bifunctional muscles and other programs (such as reflexes) and is examining how much anatomical and physiological independence exists between task groups, in both the spinal cord and in the muscle.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02160-10 LNLC

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Intrinsic Properties of Motor Units

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R.E. Burke	Chief	LNLC NINCDS
Others:	J.W. Fleshman	Staff Fellow	LNLC NINCDS
	C.A. Pratt	Staff Fellow	LNLC NINCDS
	I. Segev	Visiting Fellow	LNLC NINCDS

COOPERATING UNITS *(if any)*
Mathematics Research Branch, NIADDK (W. Rall); Dept. of Anatomy, Hadassah Medical School, Jerusalem, Israel (A. Lev Tov)

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Laboratory of Neural Control

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INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.1	1.6	.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is designed to provide information on the ranges and distributions of the electrophysiological and morphological characteristics of alpha motoneurons and of the interrelated mechanical, histochemical and morphological properties of the muscle fibers innervated by them (i.e., the muscle unit) in various hindlimb muscles in the cat. Methods used include intracellular recording and stimulation, measurement of mechanical properties of muscles and individual muscle units, neuroanatomical techniques of intracellular staining with horseradish peroxidase, along with conventional and computer-aided methods for reconstruction of extensive neuronal structures from serial histological sections, and computer modeling and data processing. In some experiments, motor unit populations in normal animals are compared with those in animals after various conditioning treatments. Studies of alpha motoneuron properties are included in this project when they are related importantly to the type of muscle unit innervated by the studied cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02534-02 LNLC

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Conduction Properties of Peripheral Nerve

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	G.E. Loeb	Medical Officer (Res)	LNLC NINCDS
Other:	A.J. Rindos	Guest Researcher	LNLC NINCDS

COOPERATING UNITS *(if any)*
Neuroimmunology Branch, NINCDS (C. Krarup)

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.1	.9	.2

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is concerned with the conduction of action potentials in peripheral nerve fibers in normal and damaged nerves. One study has been to develop and apply accurate methods for determining conduction velocity in short segments of peripheral nerves and spinal roots. This has resulted in the selection of spike-triggered averaging to obtain incremental latency in adjacent sets of tripolar nerve cuff electrodes, and the finding that there is no significant slowing of myelinated afferents from sciatic nerve to dorsal root in the cat.

A second study has been to apply this technique to the study of electrically evoked nerve potentials in chronically implanted animals during the periods of atrophy and regeneration in compressed peripheral nerves. This has permitted a detailed examination of the effects of and time course of recovery from experimentally induced compression neuropathy. The presence of a chronic constriction slows regeneration distally and that even after the distal segment has reached an almost normal conduction velocity, there may continue to be considerable slowing in the region of the constriction. One unexpected result is that smaller myelinated fibers in a proximal stump of crushed nerve (Group II caliber) appear to regenerate distal projections earlier than the largest (Group I) fibers.

TAB 8 -- LABORATORY OF NEUROBIOLOGY -- (LN)

ANNUAL REPORT

October 1, 1983 through September 30, 1984

Laboratory of Neurobiology
National Institute of Neurological
and Communicative Disorders and Stroke

Table of Contents

ANNUAL REPORT
October 1, 1983 through September 30, 1984
Laboratory of Neurobiology, IRP
National Institute of Neurological and Communicative Disorders and Stroke

Thomas S. Reese, Chief

The new Laboratory of Neurobiology has two Sections, the Section on Structural Cell Biology and the Section on Structural Plasticity. The Section on Structural Cell Biology uses modern cell biological techniques to investigate basic biological problems germane to understanding the function of the central nervous system; the Section on Structural Plasticity applies these and other appropriate approaches directly to problems of both fundamental and clinical importance in the mammalian central nervous system, emphasizing problems related to regeneration and response to injury. In the course of studying release of transmitter at synapses, an important technique for freezing tissue directly was developed in the Section on Structural Cell Biology. The current program of this Section depends on exploiting new avenues of investigation opened by this freezing technique.

In the last year, considerable progress has been made in understanding the basis of the directed organelle movements that carry the materials moved by fast axoplasmic transport. Filaments can be isolated from the axoplasm of the squid giant axon which support directed movements of organelles for many hours, at 1-2 um per sec, provided adenosine triphosphate (ATP) is present. These organelles and filaments are below the resolution limit of the light microscope so fast digital image processing of differential interference contrast images is required to visualize them. Subsequent direct freezing and metal replication of filaments previously observed with the light microscope provide a means to examine these filaments with the resolution of the electron microscope. Their central structure is a single microtubule and the various organelles that move along them are closely attached to this microtubule. Because organelles of all sizes, including mitochondria, move along these filaments at the same rate, it seems likely that all the organelle movements of fast anterograde and retrograde axoplasmic transport are powered by a single "molecular motor". Differences in the rates of transport in intact axoplasm are now thought to be determined by impeding interactions of organelles with other axoplasmic components. Because organelle movements are not blocked by typical inhibitors of either actin-myosin or dynein systems, but are blocked by metabolic inhibitors such as dinitrophenol (DNP), valinomycin, and carbonyl cyanide florophenyl hydrazone (FCCP) (but not oligomycin or azide) even in the presence of ATP, we have proposed that the molecular motor is powered by an electrochemical gradient across the organelle membrane, similar to the way rotation of the bacterial flagellum is powered. This is the first evidence that this motility mechanism, which is common in bacteria, occurs in metazoans.

Recent improvements in the freeze-fracture technique allows the cytoskeleton of axons to be visualized without any of the chemical pretreatments that are typically used. Organelles involved in axoplasmic transport are situated in special "compartments" of the axoplasm, and each type of organelle has characteristic relationships with cytoskeletal elements.

Organelles undergoing directed movements in squid axons occur in longitudinally oriented compartments characterized by their content of microtubules; these compartments also appear to have fewer cross-bridging elements. Further work on intact or partially extracted squid axoplasm is expected to show how structures in the microtubule-associated axoplasmic domains control the rates and, perhaps, direction of organelle movements.

In order to develop further a realistic picture of the detailed organization of cytoplasm, monolayers of cultured myocytes and neurons are directly frozen and examined in a 200 kV electron microscope to determine: the structure of the cytoplasmic "ground substance" lying between the major filamentous elements; how organelles move through the filamentous elements; and the relationships between acetylcholine receptor clustering and the organization of the cytoskeleton. This approach has provided a more detailed understanding of the organization of cytoplasm. A matrix of fine (ca 4 nm) filaments links the major filamentous elements; the soluble proteins and other granular components of cytoplasm are embedded in this fine filament meshwork. Their density and architecture differs in different regions of the cell, and are related to the characteristics of organelle movements in these different regions. Axon terminals on lizard intercostal muscles are unique in lying close enough to the surface of the muscle to be rapid frozen, freeze substituted, and stained with block stains permitting a three-dimensional reconstruction of their cytoplasmic structures. These new freeze-substitution techniques have shown that neurofilament bundles in the axon are continuous, but in the axon terminal they are interrupted by discrete structures (discontinuity plaques) which contain various membrane-limited organelles. These plaques are likely sites for neurofilament degradation since the filaments are thought to be transported down the axon and degraded by proteases in the terminal. How proteases, synaptic activity, and extracellular calcium affect the turnover of neurofilaments in the presynaptic terminal is now under investigation.

Methods have been developed for preparing, from directly frozen tissues, thin cryosections in which dislocations of soluble, diffusible elements are negligible. Compositional analysis, using new, quantitative x-ray microanalysis software, is now routine, but structural analysis is difficult, owing to the inherently low contrast of unstained tissue. Therefore, an element-imaging, computer-driven analytical electron microscope, developed by C.E. Fiori and R.D. Leapman, BEIB, NIH, has been used to obtain simultaneous, quantitative analysis of structure and composition in synapses and other neural tissues. Analysis of the predominantly cholinergic synaptosomes from the optic lobe of the squid has identified a population with internal K concentrations approximating those expected for synaptic elements in vivo. Improved imaging methods presently under development will be used to investigate internal structure in the high-K synaptosomes, and how both structures and elemental compositions change during depolarization and transmitter release. A similar analysis of elemental distributions in cryosections of mouse cerebellum has revealed that there are two- to threefold differences in intracellular K concentration between adjacent processes of axons, dendrites, and glia, and that there are focal areas of elevated Ca (ca 35 mmols/kg) associated with 100-nm structures, which may represent Ca-accumulating organelles in synaptic elements. Improved imaging of these cryopreparations should show which synaptic elements are involved in Ca sequestration, and to which neural and glial elements the different levels of

K belong. Another new approach under development is the application of antimony-based analogs of acetylcholine, which are known to be biochemically similar to acetylcholine and which can be detected in the electron probe to determine the sites of acetylcholine storage and release in synaptosomes.

Direct freezing can also be used to visualize integral membrane proteins in greater detail and closer to their natural state. For this purpose a special apparatus has been developed to freeze-fracture tissue at temperatures near absolute zero (10°K). This approach prevents many of the structural changes which normally occur during fracturing and shadowing. Applications of this technique to open and closed channels called connexons at gap junctions, show changes in the structure of individual channels that depend on their functional state. The substructure of membrane particles at acetylcholine receptor, SR-T tubule junctions in muscle, tight junctions, and in astrocyte membranes involved in the blood-brain barrier are being examined.

The new freeze-fracture technique has been used to show that nonplanar lipids make an important contribution to membrane structure at tight junctions, and the contribution of such nonplanar lipid organization at gap junctions and at sites of membrane fusion is being explored. Assembly of tight junctions is being studied in cultured epithelial cells which form continuous cell monolayers. Ca^{++} removal from the culture medium has been shown to result in rapid disruption of the monolayer and disassembly of the tight junctions, which break down into short cylindrical segments lying in the hydrophobic interiors of the separated epithelial membranes. Cell polarity and cytoskeletal changes accompanying tight junction formation and disassembly are being followed by video-enhanced differential interference microscopy and immunofluorescences. Another approach to the study of lipid polymorphism in biological membranes is to find structures in defined artificial pure lipid membranes similar to the naturally occurring structures. Our recent work has depended on stop-flow mixing of calcium with phosphatidylserine liposomes which produces transient cylindrical micelles similar to the cylindrical structures embedded in bilayers at tight junctions that we postulate are inverted lipid micelles.

Direct freezing and improved freeze-substitution techniques have been applied to growing tips of neuronal processes during development of synaptic connections in the chick optic tectum. Numerous flattened vesicles are found in groups near the growth cone surface and their total area approaches that of the plasmalemma. These membranes would be available to support the rapid expansion of the growth cone surface; experiments are underway to test this idea. Freeze fracture views of adult synapses on lizard and frog muscle showed that structural differences in the membrane organization of neurotransmitter release sites are correlated with physiological differences in the quantal release of transmitter depending on whether the synapse is with a twitch (fast) or tonic (slow) muscle fiber. These structural differences support our earlier hypothesis that the large intramembrane particles found at transmitter release sites are the calcium channels responsible for depolarization-dependent transmitter release because their organization in the presynaptic membrane provides a clear explanation of how levels of quantal transmitter output are determined at different types of synapses. We are currently comparing high with low output synapses in invertebrates to see whether the organization of their transmitter release sites supports this hypothesis.

The Section on Structural Plasticity has recently been concerned with the possibility of reconstructing a neuroendocrine circuit in an accessible portion of the cerebrospinal fluid (CSF) compartment, the IV ventricle. The CSF, which communicates with the extracellular fluid of the brain, may thus mediate interactions between brain and grafts placed within it. Fragments of superior cervical ganglion (SCG), allografted to the IV ventricle, become rapidly vascularized and survive indefinitely. The next step was to co-graft one of the SCG's targets, the pineal gland, to pinealectomized recipients. The goal was to see whether a disrupted neuroendocrine circuit, retina-hypothalamus-spinal cord-SCG-pineal gland, could be reconstructed upon the surface of an otherwise normal brain. An integral part of the attempt was to learn whether the grafts not only survived, but were able to perform their function, the secretion of melatonin. To this end, urinary 6-hydroxymelatonin (6-HO-M) was measured over a 24-hour period. Pineal allografts persisted and retained much of their normal architecture. The identification of their parenchymal cells as pinealocytes was established immunohistochemically and ultrastructurally. However, a single pineal allograft produced no detectable melatonin. It was not until 5 to 8 pineal glands had been transplanted, that appreciable amounts of 6-HO-M were recovered in the urine. The SCG implant sent bundles of unmyelinated axons to pinealocytes and capillaries within the adjacent pineal grafts. Pineal allografts become innervated by SCG co-transplants but a sufficient volume of pineal tissue must be inserted into the IV ventricle in order to yield appreciable amounts of secretory product.

The morphological reactions to focal injury of the brain's surface, a related problem, involves rapidly developing intramembrane changes in two cell types and a slower alteration in the cytoplasm of one of them. The increase in the number of intramembrane particle assemblies in astrocytes, examined after freeze-fracturing, is accompanied by an equally rapid development of tight and gap junctions within the plasma membranes of adjacent arachnoid cells. These events take place from 30 minutes to 3 hours following injury. A slower change, requiring about 24 hours, is the first appearance, detected immunohistochemically, of glial fibrillary acidic protein (GFAP) within astrocyte cytoplasm. Since the increase in the assemblies precedes the appearance of GFAP, it is unlikely that glial intermediate filaments, the source of GFAP antigen, are directly involved in the insertion of new assemblies into the cell membrane. The remarkably extensive development of tight junctions between reactive arachnoid cells indicates that a damaged arachnoid membrane is quickly resealed.

A project which is aimed at determining whether increased transport of horseradish peroxidase into the brain following opening of the blood-brain barrier depends on increasing vesicular transport has led to variable results. Vesicular transfer should be profoundly depressed in hypothermic animals. However, most but not all hibernating squirrels, in which the blood-brain barrier has been opened by the intracarotid infusion of hyperosmotic solutions, had variable numbers of HRP exudates within their brains. Therefore, another means of opening the barrier, hypertension, was tried; the blood pressure was transiently raised pharmacologically. If barrier opening is temperature-dependent, the response could be graded. Accordingly, squirrels were brought to intermediate (about 23°C) body temperatures. The number of HRP exudates was variable in these groups as well. Counts of the number of endothelial vesicles and pits, labeled and unlabeled with HRP, is

expected to provide some idea of whether vesicle formation underlies this blood-brain barrier opening.

It has recently been discovered that the barrier may be circumvented, rather than opened, by insertion into the brain of grafts with permeable blood vessels. Muscle from the neck and diaphragm, pieces of choroid plexus, skin, omentum, and one type of neural tissue, superior cervical ganglion, were grafted upon or into the brains of rats. Superficial muscle from the neck provided the most consistent entry of HRP from blood into brain for some distance. Penetration from the other grafts was not as deep. Localized access to the extracellular compartment of the brain may be provided through such transplants.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 01442-18 LN

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Permeability of cellular layers in the vertebrate nervous system

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

T.S. Reese, Chief, Laboratory of Neurobiology, NINCDS
B. Kachar, Visiting Associate, Laboratory of Neurobiology, NINCDS

COOPERATING UNITS *(if any)*

Marine Biological Laboratory, Woods Hole, MA 02543
R.P. Rand, Brock University, St. Catherine's, Ontario, Canada
J.S. Handler, KE, IR, NHLBI, NIH, Bethesda, MD

LAB/BRANCH

Laboratory of Neurobiology

SECTION

Section on Structural Cell Biology
(Located at the Marine Biological Laboratory, Woods Hole, MA 02543)

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.9	0.8	1.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The substructure of tight junctions is investigated by direct freezing techniques that avoid any chemical fixation and serve to increase the resolution of individual membrane components. The backbone of the tight junction of each of the paired component membranes is a continuous cylinder. This model replaces the previous view that tight junctions are comprised of rows of intramembrane proteins; the rod-shaped structures are now interpreted as inverted cylindrical micelles of membrane lipids. Recent evidence is that a similar model is applicable to tight junctions in invertebrates. Evidence for this model is also being gathered from investigations of pure lipid bilayer systems which are induced to form non-planar micellar phases by addition of calcium ion. Cylindrical micelles identical to those seen at tight junctions are found embedded in these lipid bilayers. Assembly of tight junctions is being studied in cultured anphybian epithelial cells which form continuous cell monolayers. Ca^{++} removal from the culture medium results in rapid disruption of the monolayer structure and disassembly of tight junctions which break down into small single cylindrical segments in the interior of the membrane of each separate cell. Cell polarity and cytoskeletal changes accompanying tight junction formation and disassembly are being followed by video enhanced differential interference microscopy and immunofluorescence. The true inner surfaces of naturally occurring tight junctions are being visualized by deep-etching. A filamentous structure on the surface of this membrane, which is coextensive with the cylindrical micelle, may account for the protein associated with tight junctions, and may explain how cylindrical micelles are stabilized in certain regions of the cell membrane. How tight junctions serve in the blood-brain barrier system to prevent small charged solutes from entering the brain is made clear by this new model of tight junction structure.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 01881-14 LN

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Structural basis of synaptic transmission

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

T.S. Reese, Chief, Laboratory of Neurobiology, NINCDS
J. Walrond, Staff Fellow, LN, NINCDS
T. Cheng, Visiting Fellow, LN, NINCDS

COOPERATING UNITS *(if any)*
Marine Biological Laboratory, Woods Hole, MA 02543
D. Landis, Dept. of Neurology, Massachusetts General Hospital,Boston, MA

LAB/BRANCH
Laboratory of Neurobiology

SECTION
Section on Structural Cell Biology
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INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.2	2.0	1.2

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Three new areas of investigation of synaptic structure are underway. A method of staining freeze-substituted tissue has been developed which requires no further stain after the sections are cut, so the stain extends evenly through the section. Therefore the three dimensional structure of the cytoskeleton and related fine filaments in synapses can be determined in continuous serial sections. The way in which neurofilaments end in synaptic terminals has been determined; this is important because neurofilament lengths are thought to be regulated by Ca-activated proteases at their terminations. Application of the freeze-fracture techniques has shown that the pattern of active zone structure at synapses on fast muscle fibers differs from that on slow muscle fibers; these structural differences provide a basis for understanding why terminals on fast fibers release more transmitter quanta than those on slow fibers. This approach has also shown the cytoplasmic structure of cerebellar spines; these new structural data may provide a basis for rapid changes in spine shape, such as those thought to occur during potentiation. Growing nerve terminals in the brain have been reconstructed from serial sectioned freeze-substituted preparations. These new preparative methods have revealed an internal system of membranes which are thought to be the source of the new membrane added during growth.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02551-03 LN

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Structure of neuronal cytoplasm

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

T.S. Reese, Chief, Laboratory of Neurobiology, NINCDS
B.J. Schnapp, Staff Fellow, Laboratory of Neurobiology, NINCDS
B. Kachar, Visiting Associate, Laboratory of Neurobiology, NINCDS
P. Bridgman, Staff Fellow, Laboratory of Neurobiology, NINCDS
V. Aviv, Guest Worker, Laboratory of Neurobiology, NINCDS

COOPERATING UNITS *(if any)*

Marine Biological Laboratory, Woods Hole, MA 02543
M. Sheetz, Univ. of Connecticut Health Center,Farmington, CT
R. Vale, Stanford Medical School, Stanford, CA

LAB/BRANCH
Laboratory of Neurobiology

SECTION
Section on Structural Cell Biology
(Located at the Marine Biological Laboratory, Woods Hole, MA 02543)

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.5	3.9	1.6

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project determines the structure of neuronal and glial cytoplasm, particularly as it pertains to axoplasmic transport, and the organization of the cytoplasm. Living cells or tissues are directly rapid-frozen and the structure of their cytoplasm is determined by one of two methods, freeze-etching or freeze-substitution. Axons in turtle optic nerves have different cytoplasmic domains, each characterized by specific types of filaments and by their content of organelles. Cultured myocytes, grown on grids, frozen, freeze-substituted, and examined directly at high voltages in an electronmicroscope have a cytoplasmic ground substance consisting of fine filaments instead of a microtubular meshwork, and distinct cytoplasmic domains characterized by different types of organelle movements. Filaments are isolated from the axoplasm of the squid giant axon along which organelles continue to move for many hours, at 1-2 um per sec, provided ATP is present. These organelles and filaments are below the limit of the light microscope so fast digital image resolution processing of differential interference contrast images is required to visualize them. Filaments previously observed with the light microscope and then examined in the electronmicroscope turn out to be single microtubules; organelles move very close to these tubules. Because organelles of all sizes, including mitochondria, move at the same rate, all the organelle movements of fast anterograde and retrograde axoplasmic transport may be powered by a single molecular motor with other cytoplasmic structures determining their final rate. Because organelle movements are blocked by metabolic inhibitors even in the presence of ATP, we now believe that the molecular motor is powered by an electrochemical gradient across the organelle membrane.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02610-01 LN

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Distribution of Mobile Components at Chemical Synapses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

S.B. Andrews, Special Expert,Laboratory of Neurobiology,NINCDS
T.S. Reese, Chief, Laboratory of Neurobiology, NINCDS

COOPERATING UNITS *(if any)*

Marine Biological Laboratory, Woods Hole, MA 02543
Charles E. Fiori, BEIB, DRS, NIH, Bethesda, MD
Richard D. Leapman, BEIB, DRS, NIH, Bethesda, MD

LAB/BRANCH

Laboratory of Neurobiology

SECTION

Section on Structural Cell Biology
(Located at the Marine Biological Laboratory, Woods Hole, MA 02543)

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.4	1.4	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project aims to determine the distribution of diffusible components at chemical synapses. This work is significant because of the relationship between the localization and movement of mobile constituents and their role in synaptic transmission. To attain the necessary spatial and temporal resolution, this study combines three major technological advances: 1) rapid freezing of unfixed tissues in order to achieve a time resolution of 1-2 msec and to limit ice damage; 2) cryosectioning to prepare thin, unstained sections in which diffusion of even very mobile components is controlled; and 3) quantitative, element-specific x-ray imaging in a computerized analytical electron microscope to obtain simultaneous quantitation and localization of tissue components. This approach is being applied to two synaptic preparations from the central nervous system. Experiments on the predominantly cholinergic synaptosomes from the optic lobe of the squid are designed to characterize the biochemically-active synaptosomes which synthesize acetylcholine (ACh) and release this transmitter in response to Ca-dependent depolarization. Morphological and compositional data have identified at least two populations of structures which are candidates for viable synaptosomes. This preparation is also being used, in conjunction with an antimony-labeled ACh analog, to determine where ACh is taken up and stored in cholinergic synaptosomes. Elemental imaging and analysis of the molecular layer of mouse cerebellum indicate that, in certain physiological states, potassium and calcium may be characteristically distributed among different areas that correspond to axons, dendrites, and glia. The detailed relationship between diffusible ions and other synaptic components, as well as how these relationships change during synaptic activity, is now under investigation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 01805-16 LN

PERIOD COVERED: October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Membrane Structure of Astrocytes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

J. Anders, Guest Worker, LN, NINCDS
M. W. Brightman, Head, Section on Structural Plasticity, LN, NINCDS

COOPERATING UNITS *(if any)*: None

LAB/BRANCH: Laboratory of Neurobiology

SECTION: Section on Structural Plasticity

INSTITUTE AND LOCATION: NINCDS, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.6	1.5	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A clue to the function of intramembranous particle assemblies in astrocytes may be gained by correlating their changes with those in the cytoplasm of reactive glial cells. The cytoplasmic change being followed, immunocytochemically, is that in glial fibrillary acidic protein (GFAP). The glia limitans in 2 week old rats provides a good baseline because it has little or no detectable GFAP at that age. Changes in GFAP reactivity were examined from 3 hours to 2 weeks after a localized freezing lesion was made to the cerebral cortex of 2 week old rats. The earliest staining of GFAP appeared by 24 hours, about a day earlier than that reported for a stab wound. The GFAP response occurred in the astrocytes at the periphery of the cold lesion where the assembly numbers increased. However, the increment in assemblies was considerably more rapid: 30 minutes to 4 hours after the lesion was made. Thus, the assemblies which appear to be physically linked with actin filaments and microtubules, do not appear to be directly associated with intermediate filaments; the addition and distribution of new assemblies is unrelated to the presence of GFAP. Two changes took place within the cell membrane of another cell type: arachnoid. At the periphery of the same lesion, the arachnoid response was about as rapid as the assembly increment. Within the first 3 hours after the lesion was made, there was a pronounced increase in the linear extent and number of ridges or strands belonging to tight junctions of subdural arachnoid cells. A greater number of gap junctions formed between tight junction strands between deeply situated, reactive cells than in normal, resting arachnoid cells. In some of the arachnoid cell membranes there were short, discontinuous strands, suggestive of new, forming junctions. In both glial and meningeal reactive cells, the intramembranous responses preceded the cytoplasmic change in the glial cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02086-11 LN

PERIOD COVERED: October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Regeneration in Transplanted Peripheral and Central Neurons

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

M. W. Brightman, Head, Section on Structural Plasticity, LN, NINCDS
S. I. Tsubaki, Visiting Fellow, LCNP, NINCDS
J. Rosenstein, Guest Worker, LCNP, NINCDS
R. Blasberg, Senior Investigator, LCHPH, NCI

COOPERATING UNITS *(if any)*: Laboratory of Chemical Pharmacology, NCI

LAB/BRANCH: Laboratory of Neurobiology

SECTION: Section on Structural Plasticity

INSTITUTE AND LOCATION: NINCDS, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.4	2.3	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Can a disrupted neuroendocrine circuit be reconstructed on an accessible surface of the brain? Having established that superior cervical ganglion (SCG) allografts become vascularized very rapidly and flourish in the IV ventricle, the next step was to see whether one of its major targets, the pineal gland, could become innervated by it and could also function. Pineal allografts have survived for at least 5 months in the IV ventricle. When transplanted to rats with their own SCG left intact, a few myelinated and unmyelinated axons penetrated the pineal graft. Like the sprouting of SCG axons in the iris, damaged during transplantations to the anterior chamber of the eye, the SCG branches to pial and choroidal vessels sent sprouts into the ventricular pineal grafts. In some ganglionectomized hosts that were given both SCG and pineal grafts, many more unmyelinated axons penetrated the grafts. These bundles of axons were ensheathed by Schwann cell processes and lay very close to capillaries and pinealocytes. The pinealocytes were identified, immunohistochemically, by their content of antigen "S" which is probably rhodopsin kinase, and electron-microscopically, by the presence of synaptic ribbons in some of the cells. The function of the allografted pineals was considerably depressed. Urinary 6-hydroxymelatonin (6-HO-M) was undetectable by a sensitive gas chromatographic and mass spectrophotometric method in hosts that had been given a single pineal graft. It was not until 5 to 8 pineal grafts were inserted that the urinary 6-HO-M became detectable over a 24 hour collection period. In most of the recipients only about one tenth of the amount secreted by a single, intact, pineal was recovered from the urine. In 2 host rats, however, the amount--102 and 174 ng/24 hr--was within normal limits. Thus, pineal allografts survive in the IV ventricle and become innervated by co-grafted SCG, but they function at low levels. The depressed function of the grafts may be due, in part, to the death of some pinealocytes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02144-10 LN

PERIOD COVERED: October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 charecters or less. Title must fit on one line between the borders.)*: The Blood Brain Barrier

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigetor.) (Name, title, laboratory, and institute affiliation)*

M. W. Brightman, Head, Section on Structural Plasticity, LN, NINCDS
S. I. Tsubaki, Visiting Fellow, LCNP, NINCDS

COOPERATING UNITS *(if any)*: None

LAB/BRANCH: Laboratory of Neurobiology

SECTION: Section on Structural Plasticity

INSTITUTE AND LOCATION: NINCDS, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.3	0.9	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

It was predicted that, in hibernating ground squirrels, the blood-brain barrier (BBB) to blood-borne horseradish peroxidase (HRP) could be opened with hyperosmotic solutions even though active, vesicular transport would be suppressed during hypothermia; the opening was expected to be by way of intracellular clefts. In 7 of 10 squirrels infused with the solution at a high flow rate, 2.9 ml over a 30 second period, some exudates formed. Could the high flow rate and pressure of injection have ruptured capillaries? When a slower rate, 90 seconds, was used, 5 of 7 animals had exudates. Moreover, there were no exudates in the brains of squirrels infused at the higher rate with isosmotic solutions. Therefore, the escape of HRP after hyperosmotic exposure of cerebral vessels was not due to endothelial damage. Although most of the animals had some exudates after the hyperosmotic treatment, the number was highly variable, so the barrier was opened by a second method: hypertension. Aramine or nor-epinephrine was given intravenously to 8 hypothermic squirrels and the arterial blood pressure elevated from 40 to 120 mm Hg. There were some exudates in 7 of these animals. Five additional squirrels were warmed to a body temperature of about 23°C. Of these, 3 had exudates. However, the variability in the number of exudates was sufficiently great to prohibit designating the number as "intermediate" between hypo- and normo-thermic brains. Instead of opening the BBB in rats, we bypassed it with a series of grafts into the cerebral cortex. Transplants of skeletal and cardiac muscle, skin, choroid plexus, omentum and superior cervical ganglion were compared. The greatest leak of blood-borne HRP into the surrounding brain with an intact BBB consistently occurred via the isografts of superficial neck muscle. The insertion of gel foam in the same area and to the same cortical depth did not lead to any discernible entry of protein.

TAB 9 -- LABORATORY OF NEUROCHEMISTRY -- (LNC)

ANNUAL REPORT

October 1, 1983 through September 30, 1984

Laboratory of Neurochemistry
National Institute of Neurological and
Communicative Disorders and Stroke

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ANNUAL REPORT

October 1, 1983 through September 30, 1984

Laboratory of Neurochemistry
National Institute of Neurological and
Communicative Disorders and Stroke

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ANNUAL REPORT
October 1, 1983 through September 30, 1984
Laboratory of Neurochemistry, Intramural Research
National Institute of Neurological and Communicative Disorders and Stroke
Janet V. Passonneau, Chief

The Laboratory of Neurochemistry currently is composed of three section: Enzyme Chemistry, Cellular Neurochemistry, and Neuronal Development and Regeneration.* A fourth section on neurochemical pharmacology is being phased out because of the resignation of its section chief.

Section on Enzyme Chemistry

Research in the Section on Enzyme Chemistry is centered on the roles of ion transport and intracellular regulation as related to neural functions. The mechanism and regulation of the Na,K-ATPase is the major project. Current studies include the determination of the rates of conformational transitions of the phosphorylated ATPase which establishes that this stage of the reaction is fast relative to the transition of the non-phosphorylated enzyme. Steady-state kinetic studies of conditions which favor the formation of the phosphorylated enzyme from ortho-phosphate indicates that the accessibility of water to the catalytic site is an important factor in the energy state of the enzyme acylphosphate bond. A series of monoclonal antibodies are being developed as structural and functional probes of the Na,K-ATPase. Together with new approaches to the solubilization and purification of the brain Na,K-ATPase, these antibodies are being employed in the characterization of the transport system in different brain cell types. Preliminary work suggests that Electrophorus electric organ may constitute a useful source of mRNA for structural studies of the Na,K-ATPase.

A new project involves studies of the control of Ca^{2+} distribution in mast cells. Of particular interest is the high calcium content of mast cell secretory granules. These studies may provide useful insights into the control of secretion as it relates to neurotransmitter release.

Section on Cellular Neurochemistry

Metabolic sequelae to transient brain ischemia have been the subject of studies in both the Section on Cellular Neurochemistry and the Section on Neuropharmacology. These investigations have established temporal profiles of major metabolites and metabolic pathways subsequent to ischemia in standardized animal models. An important finding is that the profound and rapid depletion of high-energy phosphate compounds is not as rapid as the efflux of potassium ions from brain cells. Thus, the cause of K^+ loss must be sought elsewhere and will be the subject of future studies. Ca^{2+} influx has also been shown to parallel K^+ loss after the initiation of ischemia and further examination of physiological and pharmacological regulation of intracellular Ca^{2+} is in progress.

* Late in Fy '84 this section was transferred to the office of the Associate Director for Laboratories.

Most acute alterations in metabolite levels are reversed in minutes after cerebral reperfusion. Exceptions are glycogen and protein synthesis. Because transient ischemia has long-term effects leading to selective neuronal death, these may be important clues to their proximate cause. Discrete steps of both processes will be examined in future work. In particular, studies are directed toward evaluating the possible role of the phosphorylation state of eIF-2, a protein synthesis initiation factor.

The layered histological structure of the retina and its experimental accessibility afford advantages for the study of neural metabolism. Two projects in the Section on Cellular Neurochemistry concern the retina. The output of photoreceptor neurons are subject to extensive processing by two other general classes of retinal neurons: ganglion cells and amacrine cells. Both of these are found to be composed of several sub-classes in terms of functions and connectivities. Elucidation of the characteristics of these sub-groups is being pursued by means of combined neurochemical, microelectrode, and anatomical techniques. Recent work has correlated the distribution of dopamine-containing amacrine cells with that of retinal rod photoreceptors. The other retina project concerns the specialized metabolism of retinal neurons and, in particular, guanine nucleotide metabolism. The detection of a unique cGMP diesterase in the extracellular matrix of photoreceptors is being extended by purification and characterization of this enzyme. In particular, the possible relationship to the intracellular cGMP diesterase is under study. Quantitative ultramicro technique is being applied to retinal cell layers to measure the temporal responses of retinal metabolites to light stimulation. The same technique is applied to determine metabolite alterations in canine retinal dystrophy.

Section on Neuronal Development and Regeneration

Current research in the Section on Neuronal Development and Regeneration is determining factors which control the ability of neurons to regenerate axonal connections. The two major parameters under investigation are (1) histocompatibility antigens and (2) the influence of macromolecular components of basement membranes in muscle and Schwann cells.

The immunosuppressive agent cyclosporin-A has been shown to be effective in permitting nerve allografts to guide reinnervation peripherally and also to permit their survival in the CNS environment. Future plans include the identification of the major antigen-bearing cells in peripheral nerve and to determine whether antigenicity persists in long-term grafts.

Peripheral nerve basement membrane is found to be insufficient to support axon regeneration. However, certain basement membrane components may be important in the activation of myosatellite cells. Additional studies are planned to determine which components of a nerve graft are necessary for its function as a support for regeneration and to assess the functional competence of the regenerated axons.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-NS-02256-08 LNC

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Metabolic Profiles in Normal and Diseased Retina

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I. : Janet V. Passonneau, Head Sec. on Cellular Neurochem. LNC, NINCDS

Other : Elizabeth K. Barbehenn Expert LNC NINCDS

COOPERATING UNITS *(if any)*

Laboratory of Vision Research, NEI
University of Pennsylvania School of Veterinary Medicine

LAB/BRANCH

Laboratory of Neurochemistry, IRP, NINCDS

SECTION

Section on Cellular Neurochemistry

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.4	1.4	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Three studies are in progress. The purification and characterization of the phosphodiesterase from the interphotoreceptor matrix continues. It has been found that fast, gentle washes of fresh retinas at 0^{o} provide the best starting material with fewest contaminating species. A concanavalin A affinity column has been used as a second step to remove the major contaminant, a glycoprotein. Chromatography on HPLC sizing columns has yielded a final preparation which is approximately 40% pure. Two affinity chromatography steps (protamine agarose and cGMP-sepharose) are being tested to provide the additional purification needed to obtain a homogenous enzyme. An antibody highly specific for the rod outer segment phosphodiesterase cross reacts with the interphotoreceptor matrix phosphodiesterase. The inhibitors bound to each of these two enzymes are interchangeable and inhibit up to 98% of the activity.

The enzyme, guanylate cyclase, in the retina is activated by light. The rate and extent of the activation is under study. A microassay has been set up in the "oil well" to measure femtomoles of product. Our initial findings indicate that the enzyme is unstable at room temperature and humidity in freeze-dried sections with a t 1/2 of about 12 hours. Fresh sections, properly cared for, will be required for future studies.

High energy phosphate compounds are being measured in retinas of dogs bred to develop a retinal dystrophy. Eyes from controls, carriers, and diseased animals of varying chronological age are sectioned, freeze-dried, and the retinas dissected into 8 layers plus tapetum. They are analyzed by micro methods ("oil well" technique). ATP levels drop 5-fold in the tapetum after 5 weeks of age which correlates with the developmental process.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-NS-02455-04 LNC

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Metabolic Correlates of Neuronal Transmission in the Hippocampal Slice

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P. I. : Janet V. Passonneau, Head, Sec. on Cellular Neurochem. LNC NINCDS

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Neurochemistry, IRP, NINCDS

SECTION

Section on Cellular Neurochemistry

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-NS-02429-05 LNC

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Coordinate Changes in Brain Energy Metabolism and Protein Synthesis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P. I. Thaddeus S. Nowak, Jr., Senior Staff Fellow LNC NINCDS

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Neurochemistry, IRP, NINCDS

SECTION

Section on Cellular Neurochemistry

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	1.0	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Coordinate changes in brain energy metabolism and protein synthesis have been investigated using several models of altered brain metabolism in order to elucidate physiologically relevant mechanisms for the control of protein synthesis. Experimental systems have included transient ischemia in gerbils, amphetamine-induced hyperthermia in mice and electroconvulsive shock in rabbits.

During transient ischemia in gerbils brain metabolism is drastically altered. Within 30 minutes of reperfusion, most measures of energy metabolism have returned to control levels, while brain protein synthesis recovers over several hours. Of the several metabolites measured, we have found that only glycogen shows a delayed recovery comparable to that observed for protein synthesis, suggesting the involvement of a common regulatory mechanism. Current efforts focus on determining the phosphorylation state of protein synthesis initiation faction eIF-2 during ischemia and recirculation, to evaluate the role of protein phosphorylation/dephosphorylation in the regulation of protein synthesis.

Previous studies have demonstrated the direct role of hyperthermia in the reduction of brain protein synthesis activity in mice following amphetamine administration. We have now employed two-dimensional gel electrophoresis to demonstrate the synthesis of heat shock proteins during recovery from amphetamine-induced hyperthermia.

We have determined that the unique sensitivity of the rabbit to the effects of electroconvulsive shock on protein synthesis in brain and other tissues arises from the hyperthermia produced by electroconvulsive shock in this species.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-NS-02142-10 LNC

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Cerebral Metabolism in Altered Metabolic States of the CNS

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I. :	W. David Lust	Section Head	LNC	NINCDS
:	Bogomir Mrsulja	Visiting Associate	LNC	NINCDS
:	Yukimasa Yasumoto	Visiting Fellow	LNC	NINCDS
:	Dan Heffez	Visiting Fellow	LNC	NINCDS
:	Thaddeus S. Nowak, Jr.	Sr. Staff Fellow	LNC	NINCDS

COOPERATING UNITS *(if any)*
Laboratory of Neurophysiology, NINCDS
Laboratory of Cerebrovascular Neuropathology, NINCDS

LAB/BRANCH
Laboratory of Neurochemistry, IRP, NINCDS

SECTION
Section on Cellular Neurochemistry

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.75	1.25	1.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Changes in brain metabolism which occur during ischemia and recirculation have continued to be investigated in the gerbil bilateral ischemia model. While previous studies have focussed on regional differences in metabolism related to the selective vulnerability of hippocampal CA 1 neurons, major emphasis in current work has been on the temporal relationships between metabolic events and their experimental manipulation.

Microwave fixation has allowed the detailed analysis of metabolic changes which occur during the first minute of ischemia, with the demonstration that pentobarbital pretreatment does not prevent, but rather delays the rapid fall in high energy phosphate equivalents during ischemia.

Alterations in levels of brain $[K^+]_e$ and $[Ca^{++}]_e$ have been correlated with metabolite changes during ischemia and recirculation in anesthetized gerbils. Anoxic depolarization occurs at approximately 1.5 min ischemia, with a rapid increase in $[K^+]e$ and a decrease in $[Ca^{++}]e$ which gradually recover during recirculation. While glucose and phosphocreatine levels are depleted by the time of anoxic depolarization, ATP levels have not fallen below 50% of control. Attempts to manipulate the timing of extracellular ion changes during ischemia using hyperglycemia produced by intraperitoneal glucose administration have led to a delay in the onset of anoxic depolarization, and a more rapid recovery.

Elevated intracellular Ca^{++} has been implicated in the cellular damage produced by various insults, including ischemia, in brain and other tissues. Preliminary studies with nimodipine, a selective Ca^{++} channel blocker, have suggested that pretreatment with this drug may delay the fall in ATP and rise in cAMP during the early minutes of ischemia in some brain regions.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z02-NS-02257-08 LNC

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Neuropharmacology of Cerebral Metabolism

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P. I. : W. David Lust Head, Sec. on Neurochemical Pharmacology LNC NINCDS

COOPERATING UNITS *(if any)*

Pharmacology Laboratory, Epilepsy Branch, CDNDP, NINCDS

LAB/BRANCH

Laboratory of Neurochemistry, IRP, NINCDS

SECTION

Section on Neurochemical Pharmacology

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-NS-01586-17 LNC

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Trophic Interactions of Neuronal and Target Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P. I. :	A. A. Zalewski	Section Head	LNC	NINCDS
:	A. K. Gulati	Visiting Associate	LNC	NINCDS
:	J. D. Ziemnowicz	Bio. Lab. Tech. (Micro.)	LNC	NINCDS

COOPERATING UNITS *(if any)*

Mineralized Tissue Research Branch, NIDR, NIH (A. H. Reddi)

LAB/BRANCH

Laboratory of Neurochemistry, IRP, NINCDS

SECTION

Section on Neuronal Development and Regeneration

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In earlier studies, we described immunocytochemical changes in basement membrane (BM) components of myofibers and Schwann cells after transplantation injury in rats. We speculated that the loss of BM components might be important in permitting myosatellite and Schwann cells to detach from their BM and to proliferate and migrate. In order to determine whether degradation of Schwann cell BM influenced axonal regeneration, we froze normal or predegenerated (8-weeks post axotomy) nerve autografts (4 cm long) prior to transplantation. Freezing was intended to kill Schwann, vascular and perineurial cells in the graft leaving behind unchanged (normal nerve) or degradated (predegenerated nerve) BM tubes. After 3 months, we found no significant axonal regeneration through either type of frozen nerve graft. This finding supports our contention that viable cells, and not BM alone, are required for axonal growth through long nerve grafts.

To further examine the role of extracellular matrix in regeneration, we applied fluorescein-conjugated lectins, to tissue sections of autografts of regenerating skeletal muscle. We found an intense binding of wheat germ agglutinin (WGA) to the myogenic zone of regenerating muscle. Since WGA binds specifically to N-acetylglucosamine, this binding may mean than an N-acetylglucasamine-rich environment is favorable for myosatellite activation, proliferation and fusion to form myotubes. We are currently in the process of using labelled lectins to study nerve and taste bud regeneration.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-NS-02254-08 LNC

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Repair of Injured Nerve with a Nerve Allograft

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P. I.	: A. A. Zalewski	Section Chief	LNC	NINCDS
	: A. K. Gulati	Visiting Associate	LNC	NINCDS
	: B. J. Mrsulja	Visiting Associate	LNC	NINCDS
	: J. D. Ziemnowicz	Bio. Lab. Techn. (Micro)	LNC	NINCDS

COOPERATING UNITS *(if any)*
Department of Anatomy, Wayne State University, School of Medicine
(H. G. Goshgarian)

LAB/BRANCH
Laboratory of Neurochemistry, IRP, NINCDS

SECTION
Section on Neuronal Development and Regeneration

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Nerve allograft (a graft between genetically different members of the same species) rejection can be prevented by treating the host with the immunosuppressive drug cyclosporin-A (Cy-A). We have used Cy-A to demonstrate that during immunosuppression host axons will regenerate through a long (4 cm or more) nerve allograft and reinnervate denervated tissue. Further studies were carried out to determine (A) the response of long-term surviving nerve allografts to injury and (B) whether viable cells were needed in the nerve allograft.

(A). Our results demonstrated that after injury (crush or cut) of 3-month old allografts (which contained regenerated host axons) in Cy-A treated rats, the allografts underwent Wallerian degeneration which was followed by the regrowth of host axons. This finding indicated that nerve allografts behave like normal nerves after injury in that they permit repeated axonal regeneration through them.

(B) To determine the role of cell viability in nerve allografts, the grafts were frozen prior to their insertion into Cy-A treated rats. We found that, after 3 months, host axonal growth into frozen nerve allografts was restricted to the initial cm of a 4-cm graft. This observation demonstrated that nerve graft matrix alone was not sufficient to permit host nerve fiber regeneration over a long distance.

Other data revealed that Cy-A prevented neuronal allograft rejection in the central nervous system (spinal cord) of sensitized rats. In addition, hamster neurons survived in Cy-A treated rats whereas guinea pig neurons were rejected. Finally, histochemical studies of normally myelinated and remyelinated axons revealed a high activity of gamma-glutamyl transpeptidase and Na-ATPase at the paranode. The physiological meaning of this paranodal enzyme localization remains to be determined.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-NS-00813-23 LNC

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Enzymological Aspects of Neural Functions

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P. I.	:	R. W. Albers	Actg. Chief, Sec. on Enzyme Chemistry	LNC NINCDS
	:			
Others	:	S. P. Chock	Expert Consultant	LNC NINCDS
	:	A. K. Hazra	Visiting Associate	LNC NINCDS
	:	A. S. Hobbs	Research Associate	LNC NINCDS

COOPERATING UNITS *(if any)*

J. P. Froehlich, National Institute on Aging, NIH
R. H. Huang, Dept. of Biochemistry, Univ. of South Alabama

LAB/BRANCH

Laboratory of Neurochemistry, IRP, NINCDS

SECTION

Section on Enzyme Chemistry

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.5	3.0	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project consists of five parts, all of which center on the structure and functioning of the Na,K-ATPase. (1) Transient kinetic studies have established rate constants for the conformational transition, E1-P== E1-P, and have also demonstrated an ADP-stimulated hydrolysis of E1-P. (2) Conditions which promote the formation of phosphoenzyme from ortho-phosphate are under study. Results support the hypothesis that the energy state of the enzyme acylphosphate is determined by factors which control access of water to the catalytic site. (3) Quantitative solubilization and partial purification of the Na,K-ATPase activity from rodent brain has been achieved utilizing a new detergent and affinity columns. The objective of this work is to examine the question of the occurrence of isozymes of the ATPase in brain. (4) The relation between structure and function in the Na,K-ATPase is being studied through the application of a battery of monoclonal antibodies which are being screened for structural specificity and functional interactions. Monoclonal antibodies are also being applied to the investigation of ATPase isozymes. (5) The Electrophorus electric organ is being investigated as a source of mRNA for the Na,K-ATPase. Preliminary studies have shown that proteins in the correct molecular weight range are coded for by the partially purified RNA preparation. The identity of the proteins will be tested immunologically. Depending on the results, further work may include the preparation of cDNA for sequencing and hybridization studies.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-NS-02631-01 LNC

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Structure and Function in Retinal Neurons

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P. I. :	Ralph Nelson	Physiologist	LNC	NINCDS

COOPERATING UNITS *(if any)*
Department of Physiology, University of Utah, Salt Lake City (H. Kolb); Max-Planck-Institut fur Physiologishe and Klinishe Forchung Bad Nauheim FRG (E. Zrenner); Laboratory of Vision Research, NEI, NIH (A. Mariani)

LAB/BRANCH
Laboratory of Neurochemistry, IRP, NINCDS

SECTION
Section on Cellular Neurochemistry

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.8	.8	none

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The goal of this research is to improve understanding of the inner workings of mammalian retinas using combined electrophysiological, anatomical, and neurochemical approaches.

A. Dopaminergic amacrine cells of monkey retina parallel rods in spatial distribution. Visualized in whole, flat mounted retinas using an aqueous, formaldehyde-induced fluorescence method, dopaminergic amacrines can be observed everywhere in monkey retina outside the foveal pit. Their density is non-uniform, however, being minimal in foveal and peripheral regions and maximal (30-40 mm^{-2}) at 3 mm eccentricity, the region of peak rod density. There are about 7500 such cells per retina. Dopamine may thus be associated with rod-system function.

B. A17 amacrine cells of cat retina depolarize in sustained fashion and have the spectral sensitivity of the rods. Revealed by intracellular recording, HPR injection, light and electron microscopy, A17 cells receive input only from rod bipolars and other amarine cells, among them the dopamine containing amacrine cell of cat retina. A17 is about 800 um in both dendritic and receptive field and broadly stratified in the cat inner plexiform layer.

C. A19 amacrine cells of cat retina are rare types with transient, on-off depolarizations. Wide in dendritic field and narrowly stratified in s2 these receive input from cone biplars and (primarily) other amacrines, but not dopaminergic amacrines.

D. Biplexiform cells in monkey retinas are unique ganglion cells that send dendritic processes to contact rods directly. Intracellular recording and staining of such a cell in Macaca fascicularis has revealed a depolarizing on-off waveform, a broad receptive field, and activation by both rod and cone mechanisms. The axon traveled through the inner plexiform layer for 0.3 mm before descending to the optic nerve fiber layer and changing course to proceed to the optic disk.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-NS-02605-01 LNC

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Mechanism of Mast Cell Secretion

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P. I.	:	S. P. Chock	Expert	LNC	NINCDS
Other	:	R. W. Albers	Head, Sec. on Enzyme Chemistry	LNC	NINCDS

COOPERATING UNITS *(if any)*

E. W. Chock, Department of Biochemistry, Armed Forces Radiobiology Research Institute (AFRRI)

LAB/BRANCH
Laboratory of Neurochemistry, IRP, NINCDS

SECTION
Section on Enzyme Chemistry

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.25	.25	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Mast cells are secretory cells associated with the connective tissue. They secrete over twenty preformed mediators, including histamine, in response to the binding of their surface sensitized IgE by ligand such as allergen. The mechanism of its secretion is not yet understood. The study of mast cell release may lead to a better understanding of the mechanism of neuronal secretion.

Calcium is known to play a crucial role in the mechanism of secretion in general. There is strong evidence that calcium is also intimately involved in the mechanism of mast cell degranulation. We have localized a high calcium store in the granule using elemental X-ray microanalysis. Since intracellular free calcium is normally kept to a very low level ($<10^{-6}$M), we have looked for the presence of calcium binding proteins. By using the coupled enzyme assay method of Chock and Huang (Anal. Bioch. 138: 34 (1984)), we have elucidated a calmodulin-like activity associated with the mast cell granules. Experiments are now being conducted to localize this calmodulin using an immunocolloidal gold ultrastructural technique.

The possibility of the existence of different calmodulin binding proteins in mast cell is also of interest to us. Since we are already able to prepare calmodulin affinity column, we will employ this technique to identify these calmodulin binding proteins.

We believe that the packaging of the granule content plays a crucial role in the regulation of the intragranular osmotic pressure in accordance to the chemo-osmotic model for secretion. We have been able to observe the ultrastructural organization of the granule by detergent extraction. A more systematic study to correlate the ultrastructural with biochemical changes will be undertaken.

TAB 10 -- LABORATORY OF NEURO-OTOLARYNGOLOGY -- (LNO)

ANNUAL REPORT

October 1, 1983 through September 30, 1984

Laboratory of Neuro-otolaryngology
National Institute of Neurological and Communicative Disorders and Stroke

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ANNUAL REPORT
October 1, 1983 through September 30, 1984
Laboratory of Neuro-otolaryngology, IRP
National Institute of Neurological and
Communicative Disorders and Stroke

Jorgen Fex, M.D., Ph.D., Chief

The Laboratory has continued to provide new knowledge within the framework of its two Projects: Project Number Z01NS02216-09 LNO, Inner Ear Neuronal Mechanisms: A Multidisciplinary Analysis, Project number Z01NS02217-09 LNO and Synaptic Transmission and Neuronal Connections of the Mammalian Cochlear Nucleus. Through these Projects we aim at a better understanding of how the inner ear can make us hear and how the cochlear nucleus processes the auditory information that it receives from the inner ear.

There is a consensus among scientists concerned with how the organ of hearing works that its outer hair cells partially control the micromechanics of the inner hair cells, while the inner hair cells in their turn very much control auditory nerve activity, i.e. what we hear. We share with many the hypothesis that the neurons from the brainstem that synapse with outer hair cells modulate this control. We have continued to study these particular efferent neurons, by general agreement classified as the medial system of olivocochlear neurons. The other efferent neurons in the organ of hearing, classified as belonging to the lateral system of olivocochlear efferents modulate information that the auditory nerve carries from the sensory organ to the central nervous system; these efferents do so primarily through synapses on auditory nerve endings (dendrites) in the organ of hearing. We study also these efferents.

We have submitted for publication a study using several different antisera to choline acetyltransferase (ChAT), showing ChAT-like immunoreactivity in the different types of efferents in the organ of hearing. This adds strong evidence to previous evidence that both the medial and the lateral system of olivocochlear efferents are cholinergic.

We made the discovery a few years ago (published 1981, described in a previous Annual Report), that the mammalian organ of hearing, specifically the olivocochlear fibers in this organ, contains enkephalin. Our discovery has been confirmed in other laboratories. Later we have shown, using both immunohistochemistry on the one hand and high performance liquid chromatography (HPLC) together with radio immunoassays (RIA) on the other hand, that there are several different opioid peptides in the organ of hearing. These peptides include methionine enkephalin and leucine enkephalin. The evidence strongly indicates that the lateral system of efferents contains methionine enkephalin and that the medial system does not contain methionine enkephalin but other opioid peptide(s). We describe these findings in a biochemical study that is in press (Brain Research) and in a light and electron microscopy study of enkephalin-like immunoreactivity that is in press (Hearing Research).

Our studies show there is likely co-containment of acetylcholine and opioid peptides in efferents in the organ of hearing. We have not yet

determined that this is so. We have, however, published a study showing enkephalin-like immunoreactivity and acetylcholinesterase in cells, which are cells of origin of efferents to the organ of hearing. Also, we have in press a study showing for the first time co-containment of ChAT-like and enkephalin-like immunoreactivity in neurons, again in cells of origin of efferents to the organ of hearing.

We have extended our peptide studies to retina and hippocampus for technical reasons, as mentioned in the Annual Report of last year. Discussed in that Report and now published are: i) a study of hippocampus in which was shown through biochemical means the presence of three different enkephalin-like peptides and through histochemical means their distribution; ii) a biochemical study of retina with chromatographic identification of enkephalins; and a brain slice study of the pharmacology of hippocampus showing that Naloxone blocks long term potentiation of certain field potentials.

We have an immunocytochemical study in press on the distribution of glutamic acid decarboxylase (GAD)-like immunoreactivity in efferent nerve fibers and endings in the organ of hearing. The findings are of general interest in that we now have shown multiple immunoreativities in a system of nerve fibers, the cochlear efferents. GAD is considered to be the marker of choice of neurons that use the inhibitory neurotransmitter gamma-aminobutyric acid (GABA). Whether in this case the marked efferents actually use GABA as neurotransmitter remains to be seen. On the other hand, we found that efferents of both systems were marked, but far from all efferents of either of the systems. In other words, our findings strongly indicate that there is a small subsystem of efferent that is chemically different from the other two systems. We now are complementing this study by determining the distribution in the organ of hearing of GABA-like immunoreactivity.

We have another first finding in showing neuron-specific enolase-like immunoreactivity in major sensory cells, in this case in the inner hair cells of the organ of hearing of guinea pig. An extra twist to the finding is that the outer hair cells do not show this immunoreactivity. The immunoreactivity was also seen in efferent nerve endings in the organ of hearing. The study has been submitted for publication.

The enzymes aspartate aminotransferase (AATase) and glutaminase (GLNase) have remained of interest to us. The enzymes are closely associated with the metabolism of the ubiquitous amino acids glutamate and aspartate that are major candidates for excitatory neurotransmitters in the mammalian central nervous system. In previous Annual Reports, we have described our studies with biochemical, pharmacological and immunohistochemical evidence that these amino acids are candidates for excitatory neurotransmitters of the auditory nerve. The immunohistochemical evidence was obtained through studying the distribution of the enzymes AATase and GLNase, using antisera to them and immunohistochemistry and light and electron microscopy. We extended those studies to studies testing our featured hypothesis that AATase and GLNase may serve as markers of glutamergic and aspartergic neurons. We have reported that Type I spiral ganglion cells contain both enzymes, Type II cells may contain neither. Our study on immunohistochemical localization of GLNase-like immunoreactivity in the auditory nerve is now published (Brain Research). A study of AATase-like and GLNase-like immunoreactivities in hippocampus is in

press (Brain Research). A study on such immunoreactivities in neurons of the cerebral neocortex is submitted for publication.

GLNase-like immunoreactivity has been found by us in fibers and endings of efferents of the medial system in the organ of Corti (manuscript in preparation), similar to what we found for AATase-like immunoreactivity (previously published). For the GLNase study we have used a bright field light microscope equipped for video enhanced Asymmetric Illumination contrast. This has given an excellent resolution of surface and single cell preparations of the organ of hearing in vitro. This again is a technique that we, in collaboration with Dr. Bechara Kachar, have introduced in the field of auditory research.

Except for the presence of AATase-like and GLNase-like immunoreactivities, there is no evidence that these immuno-stained efferents would be neurons that use aspartate and glutamate as neurotransmitters. This has bearing on our hypothesis that the enzymes AATase and GLNase may be good markers of such aspartergic and glutamatergic neurons. We therefore intend to try to determine by biochemical means if cochlear efferents contain activities of these two enzymes. As part of such an effort we are trying to improve our surgical techniques for de-efferenting the organ of Corti.

The use of brainstem slices for the physiological and pharmacological study of auditory nerve synapses has continued with drugs applied to the bath of the slice chamber and through microiontophoresis. Slice preparations of the mouse and of the chicken have been used. A study of excitatory amino acid pharmacology of the auditory nerve and nucleus magnocellularis of the chicken has been submitted for publication. The results of the study suggest that, as in mammals, an excitatory amino acid is released from the chicken auditory nerve and that its action is terminated by an uptake process. However, the results also suggest that a kainate-type receptor is activated post-synaptically at the chicken auditory nerve synapse, while the corresponding mammalian receptor is of the NMDA-type.

The pharmacologist/neurophysiologist, who has been with the LNO since 1977, was recently given a permanent position at the LNO. A biochemist, who previously had been with the LNO for 6 years, joined the LNO on July 1, 1984 to take up a permanent position at the Laboratory. If we are granted the permanent position that has been requested for the neuro-anatomist that has been with the LNO for 6 years, then the LNO will finally have an adequate, permanent core of experienced but still very flexible scientists for the multidisciplinary research that needs to be carried out.

We intend to turn an appreciable part of our activities to studies of cells, in isolation or in interaction with other cells, of the organ of hearing and spiral ganglion of small mammals. We expect to begin such studies during the fall of 1984; preliminary studies are under way.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02216-09 LNO

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Inner Ear Neuronal Mechanisms: A Multidisciplinary Analysis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.)* *(Name, title, laboratory, and institute affiliation)*

PI:	Jorgen Fex	Chief	LNO, NINCDS
Others:	R. A. Altschuler	Senior Staff Fellow	LNO, NINCDS
	D. W. Hoffman	Staff Fellow	LNO, NINCDS
	J. A. Rubio	Visiting Fellow	LNO, NINCDS
	M. H. Parakkal	Histopathology Technician	LNO, NINCDS
	K. A. Reeks	Histopathology Technician	LNO, NINCDS

COOPERATING UNITS *(if any)* Laboratory of Neurobiology, NINCDS (B. Kachar); Laboratory of Clinical Science, NIMH (P. J. Marangos and N. Zamir); Max Planck Institute für Psychiatrie, Abteilung Neurochemie, Am Klopferspitz, D-8033, Martinsried, Germany (F. Eckenstein); Univ. Florida, Gainesville, FL (W. Brownell)

LAB/BRANCH
Laboratory of Neuro-otolaryngology

SECTION

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.5	2.7	2.8

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this project is to provide new knowledge of the auditory mechanisms of the inner ear.

We have continued to study the distribution in the organ of hearing of neurotransmitter candidates and associated enzymes, using small mammals (guinea pigs, rats and mice). We have used polyclonal antisera and monoclonal antibodies, studying the distribution of immunoreactivity through light and electron microscopy in immunohistochemical studies. High performance liquid chromatography (HPLC), radio immunoassays (RIA), and receptor binding experiments were used in biochemical studies of opioid peptides in the organ of hearing. Both the normal and the de-efferented organ of hearing were studied.

Several opioid peptides were found in the organ of hearing of the guinea pig. Its lateral system of efferent neurons specifically contains methionine enkephalin, while the medial system contains other peptide(s). In cells of origin of the lateral system of efferents enkephalin-like and choline acetyltransferase-like immunoreactivities are co-contained; such co-containment has previously not been demonstrated in nerve cells.

Glutamic acid decarboxylase (GAD)-like immunoreactivity is present in a subpopulation of efferent neurons in the organ of hearing, in both the lateral and the medial system of efferents. This indicates that the present dichotomy of these neurons may need to be modified.

We have also used antiserum against neuron-specific enolase (NSE) in a light microscopy study of the hearing organ. We found NSE-like immunoreactivity in inner hair cells but not in outer hair cells, indicating that there may be a major difference in demands on metabolism between the two types of hair cells. The likely presence of NSE in a major type sensory cell has previously not been demonstrated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02217-09 LNO

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Synaptic Transmission and Neuronal Connections of the Mammalian Cochlear Nucleus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Jörgen Fex	Chief	LNO, NINCDS
Others:	R. A. Altschuler	Senior Staff Fellow	LNO, NINCDS
	M. R. Martin	Senior Staff Fellow	LNO, NINCDS
	M. J. Frye	Electronics Technician	LNO, NINCDS
	M. H. Parakkal	Histopathology Technician	LNO, NINCDS
	K. A. Reeks	Histopathology Technician	LNO, NINCDS

COOPERATING UNITS *(if any)* Laboratory of Neurophysiology, NIMH (J.P. Donoghue); Dept. Neurophysiol., Univ. Wisconsin, Madison, WI (R.J. Wenthold); Dept. Psychobiol., Univ. CA, Irvine, CA (C.W. Cotman and D.T.T. Monaghan); Dept. Biochem., Univ. PA, Pittsburgh, PA (N.P. Curthoys and W.G. Haser) SUNY, Stony Brook, NY (J.L. Mosinger)

LAB/BRANCH

Laboratory of Neuro-otolaryngology

SECTION

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.0	1.8	2.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is to provide new knowledge of how the cochlear nucleus processes information coming from the organ of hearing through the auditory nerve.

We described in previous Reports our evidence that the excitatory amino acids glutamate and aspartate are neurotransmitter candidates for the auditory nerve synapses in the cochlear nucleus. We also reported on our hypothesis that the enzymes glutaminase (GLNase) and aspartate aminotransferase (AATase) may serve as markers for glutamergic and aspartergic neurons. We have continued our immunocytochemical studies on the distribution of these two enzymes in the central nervous system of guinea pigs and rats using mainly immunoperoxidase techniques, with light and electron microscopy for visualization of the immunoreactivities. We have now added studies of the distribution of AATase-like and GLNase-like immunoreactivities in cerebellum, neocortex and hippocampus and of AATase-like immunoreactivity in retina. Findings from these studies provide evidence for the hypothesis that GLNase may serve as an immunocytochemical marker for excitatory amino acid neurons and that AATase-like immunoreactivity may define sub-populations of excitatory amino acid neurons or, perhaps, GABAergic neurons. Further studies are needed to confirm, or refute, the hypothesis.

Our in vitro studies of auditory nerve synapses in the brain stem have been continued. Chamber mounted slices of the brainstem of chickens were prepared, and drugs were applied to synapses. Antidromic and orthodromic responses were evoked; field potentials were recorded using a glass electrode filled with artificial cerebro-spinal fluid. The results indicate that, as in mammals, an excitatory amino acid is released from the chicken auditory nerve and has its action terminated by an uptake process, but that this amino acid activates a kainate-type receptor on nucleus magnocellularis neurons. In the mammal, the corresponding receptor is of the NMDA-type.

TAB 11 -- LABORATORY OF NEUROPATHOLOGY AND NEUROANATOMICAL SCIENCES -- (LNNS)

ANNUAL REPORT

October 1, 1983 through September 30, 1984

Laboratory of Neuropathology and Neuroanatomical Sciences
National Institute of Neurological and Communicative Disorders and Stroke

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ANNUAL REPORT
October 1, 1983 through September 30, 1984
Laboratory of Neuropathology and Neuroanatomical Sciences, IRP
National Institute of Neurological and Communicative
Disorders and Stroke

Igor Klatzo, Chief

The Laboratory of Neuropathology and Neuroanatomical Sciences (LNNS) is devoted to experimental research concerning the nature, pathophysiology and therapy of cerebrovascular disorders. The laboratory consists of three sections: Section on Cerebrovascular Pathology, Section on Cerebrovascular Physiology and Section on Neurocytobiology, each focussed on different aspects and using different approaches in elucidation of pathophysiology of cerebrovascular disorders.

The Section of Cerebrovascular Pathology has continued to investigate major factors in dynamics of the vasogenic brain edema, which constitutes a serious complication of ischemic brain lesions. Using the specially designed model, in which an opening of the blood-brain barrier (BBB) is not associated with any evidence of injury to cellular elements, our previous studies have been extended to the use of different BBB tracers and to quantitative determinations of extravasated protein and water content. Our new observations demonstrated, that after closure of the barrier to proteins at 9 hours, there was a progressive clearance of edema, although the barrier to micromolecular substances, such as sodium fluorescein, remained open for 24 hours. The quantitative studies using ^{125}I labeled bovine serum albumin (BSA) revealed a direct relationship between amounts of BSA and water, measured in the same tissue samples. Thus, these studies provided an overwhelming evidence of the direct relationship between the extravasation of serum proteins and retention of water in the brain tissue, which constitutes the main feature of the vasogenic edema.

The relationship between edema and extravasation of serum proteins was further demonstrated in the studies on prevention of the BBB opening to proteins in cats which were subjected to one-hour occlusion of the middle cerebral artery (MCA). In these experiments, the regional cerebral blood flow (rCBF) was below 12 ml/100g/min in the ischemic territory. When the reactive hyperemia was prevented by withdrawing blood at the time of release of the MCA occlusion, the animals, sacrificed at 3 hours following release of occlusion, showed no evidence of previous barrier opening. The ischemic regions revealed significantly lesser edema. The corresponding areas in control animals, subjected to an ischemia of similar intensity, but without hypovolemia and showing the opening of the barrier to Evans Blue (EB) tracer. Cats sacrificed after 3 days revealed in the hypovolemic group much less severe tissue damage in the ischemic areas than in the control group. Our studies thus demonstrated that extravasation of proteins which follows release of occlusion of major cerebral artery significantly aggravates the intensity of ischemic brain edema and it contributes to severity of ischemic brain tissue damage.

In the course of our investigations on effects of 5-minute ischemia in gerbils, our studies were able to bring together two major concepts concerning thresholds and selective vulnerability. Our studies demonstrated that during and

shortly after 5-minute carotid occlusion, various brain regions, although showing similar intensity rCBF reduction, similar profiles of ionic disturbances and similar changes in main energy metabolites, reveal during post-ischemic periods very different sensitivity to ischemic injury and this provides a basis for linking together the concepts of thresholds and of selective vulnerability, the latter being related to intrinsic properties of neuronal structures. Furthermore, our new project on elucidation of differences between the young and adult brains with regard to reactivity to ischemic injury revealed that the thresholds to ischemic damage are also age-dependent. Five minute bilateral carotid occlusion resulted both in 3 week old and in adult (12-14 week old) animals in similar severe reduction of rCBF (below 10 ml/100g/min) in most of the hemispheres. However, the rate of depletion of main energy metabolites was considerably slower in young gerbils indicating slower brain metabolism in the young animals. After 2 weeks the brain of the young gerbils revealed no evident morphological damage, whereas the brains of adult gerbils showed characteristic severe destruction of CA1 sector of the hippocampus. These studies introduce several new questions concerning which factors play most important role in the mechanisms of post-ischemic injury and they will be considerably expanded.

The Section on Cerebrovascular Physiology has been involved in study of the effects of focal ischemic brain edema upon cerebral extracellular space determined by impedance measurements. Cats were subjected to left MCA occlusion for 1 hr. Immediately after recirculation, 2% EB tracer was injected for blood-brain barrier (BBB) evaluation. The cats were sacrificed between 6 and 42 hrs later. Cerebral electrical impedance (CEI) and rCBF were measured using a platinum microelectrode array inserted into the ipsilateral caudate. During ischemia (rCBF= 11ml/100g/min), impedance rose to $\bar{x}$=211%. Immediately after release, CEI decreased but it was followed by a second rise to $\bar{x}$=176% within 15 hrs. of recirculation and this late rise was not accompanied by ischemia. A secondary rise was also observed in cats in which the MCA was permanently occluded. All these cats revealed extravasation of EB in ischemic areas. The secondary rise in CEI appeared to be related to increased intracranial pressure (ICP) induced by ischemic brain edema. To test this hypothesis, brain compression was produced by epidural balloon inflation. When the epidural pressure rose from a baseline value of 5 mmHg, to 26 mmHg, the CEI increased to 216% and rCBF dropped from a baseline value of 48ml to 25ml/100g/min. This study suggests that an increase in ICP itself can produce a reduction in extracellular spaces without lowering rCBF to critical ischemic values and that secondary rise of CEI in cerebral ischemia might be therefore related to compression of extracellular spaces due to increased tissue pressure induced by the development of edema. Further study is planned.

The study of extracellular ionic concentrations were carried out in gerbils subjected to 5-minutes of cerebral ischemia due to bilateral occlusion of the carotids. Ion-selective electrodes were used, which permit the continuous and simultaneous measurement of concentrations of certain selected ions such as K^+ and Ca^{++}. Changes in concentration of these ions reflect movements of these ions in or out of the extracellular spaces where the electrode tips are located. Measurements were made in the hippocampus and in the cortex. While exact values of ion concentrations and phase durations were slightly different, the ionic concentration changes showed basically similar profiles. When the carotids were occluded, marked changes in the concentrations of K^+ and Ca^{++} were observed which we believe reflect major movements of these ions. With respect to time, these changes could be easily separated into phases which demonstrated the complex

character of the ischemic event and offer opportunities to study separately the processes involved. Some of these changes could be associated with depolarization of the neural membranes. Changes in [Ca^{++}] are of considerable interest because of the possible role of calcium in cell damage. Futher study is planned.

In collaboration with Professor Thomas Devlin of Hahnemann Medical College and Hospital in Philadelphia, PA, an effort has been made to evaluate a prostaglandin derivative called PGBx for its protective action against ischemic brain damage. This compound, isolated in the course of studies on stress, was observed to protect in vitro mitochondrial metabolism from hypoxia. Our interest rested on the opportunity to test the efficacy of PGBx in a model of cerebral ischemia which seemed definitive and relatively easy to assess. This model is the adult mongolian gerbil subjected to 15 minutes of bilateral carotid occlusion. Extensive tests have shown that the 7-day survivability of the animals is close to 30%. While untreated controls showed the expected 30% survivability at 7 days, over 92% of the treated gerbils survived. This beneficial result was present only if the PGBx was given 30 minutes after occlusion release followed by repeat doses at 1, 2 and 3 hrs. If given before or during occlusion or more than 1hr after release from occlusion, the drug was essentially ineffective. As there are few drugs that offer benefit when administered after the ischemic injury, this drug appears to deserve further study.

The continuous goals of the Section on Neurocytobiology have been to develop and utilize new model systems for the investigation of basic mechanisms operative on the level of normal and pathologically altered blood-brain barrier (BBB) and cerebral blood flow (CBF) to study the metabolic processes occurring in cerebral ischemia and ischemic edema, especially their prevention and therapy. During the last year, both the newly established pure muscle cell culture (Spatz et al. Brain Res.) 280: 387-391, 1983, and the previously developed endothelial culture derived from dissociated cerebral microvessels (Spatz et al. Brain Res.) 191: 577, 1980, have been very useful models for the continuous studies of cerebrovascular function related to the BBB.

Previous studies, concerned with characterizations of adrenergic receptors linked to adenylate cyclase (AC) in cerebrovascular smooth muscle cultures, demonstrated the presence of β_2 - and α_1- but an absence of α_2 - type adrenergic receptors coupled to AC. However, both α_1 and α_2 - type receptors were shown to mediate central and peripheral vascular contraction. Therefore, a possibility of the presence of α_2 - adrenergic receptors was investigated by binding studies using radiolabeled clonidine as a ligand and various (cold) adrenergic agonists and antagonists as displacers of the radiolabeled clonidine. With the utilization of this technique, the adrenergic receptors were detected in smooth muscle cells showing characteristics of multiple population of the α_2 - adrenergic binding sites. Hence, the existence of these receptors not linked to AC activity observed in the cerebrovascular smooth muscle cells strongly suggest that their reactivity which is mediated by α_2 - adrenergic receptors might be associated with Ca^{++} fluxes.

Three different aspects relative to the cerebral capillary function in vivo have been investigated in the in vitro model using the pure cerebrovascular endothelial culture: a) the synthesis of prostaglandins and its stimulation by various hormones implicated in the regulations of events occurring on the level of BBB, b) the characterizations of glucose 6-phosphatase activity which has been postulated to participate in the transport of glucose across the BBB and c) the

examination of intrinsic cellular regulatory mechanisms of the endothelium by its exposure to hypotonic environment. Prostacyclin measured as 6-keto - $PGF_{1\alpha}$ was the main prostaglandin (PG) formed from endogenous arachidonic acid by the cultured endothelial cells. Noradrenalin, isoproterenol, serotonin, histamine but not angiotensin II increased the synthesis of PG. However, the greatest stimulation of PG snythesis was seen with additions of calcium ionophore A-23187 to the intact cells. Thus, these findings support the contention of the endogenously synthezised prostaglandin's interaction with various hormones in the cerebral capillaries. Moreover, the observed reactivity of the prostaglandin synthesis to various vasoactive substances is in agreement with their implicated participation in the regulation of CBF, BBB permeability and/or BP.

Glucose 6-P (G 6-P) was the best substrate among the tested sugar phosphates (glucose 1-P, erythrose 4-P and 4-P glycerate) in both cell types. Ribose 5-P gave the same response as glucose 6-P while fructose 6-P was a good substrate for the cultured cerebrovascular endothelium but not for the isolated microvessels. The cerebrovascular endothelial G 6-Pase, in contrast to that of the liver, failed to phosphorylate glucose using carbamyl phosphate as donor. Kinetically a marked activation of G 6-Pase occurred at high concentration of G 6-P (over 2 mmoles/l up to 25 mmoles/l). A biphasic response curve of the G 6-Pase activity was seen in the presence of either increased relative concentration of substrate or the amount of tissue enzyme. ATP as well as the nonhydrolyzable analogue adenyl (β,γ-methylene) diphosphonate stimulated also the activity of endothelial G 6-Pase. The gel electrophoresis showed a single site of enzymatic activity corresponding to a single protein band irrespective of the tissue source.

The high concentration of G 6-Pase in the cerebrovascular endothelium, its kinetic activation pattern [(allo-) steric] distinctly different from other tissues are indicative of a specific role of this enzyme in the cerebral microvasculature compatible with the proposed participation of G 6-Pase in the glucose transport across the BBB.

The exposure of viable cerebrovascular endothelium to a medium of half normal osmolality resulted in immediate cellular swelling, reduction of transmembraneous potential and intracellular pH but without evidence of permeability changes to trypan blue bound proteins. A rapid recovery of cell volume and membrane potential but with limited restoration of intracellular pH took place within 30-60 minutes although the osmolality remained low. At the same time, the intracellular Na^+- and K^+- concentrations were markedly reduced in the endothelial cells as compared to controls. The decrease of intracellular Na^+- and K^+- levels accounted for a fall in cellular osmolality of 63 mOsm which were discharged from the intra- to the extracellular compartment during the adjustment of cell volume to the hypotonic solution. The results of these studies demonstrate that the cerebrovascular endothelium, the active constituent of the BBB, has a built-in capacity for selfregulation which is undoubtedly important for the normal function of the BBB interface. Therefore, this system provides a suitable model for the investigation of various mechanisms participating in normal and altered processes occurring on the BBB level.

The studies on cerebral ischemia, its pathophysiology, prevention and therapy in gerbils have been concerned with continuous evaluation of the effects of naturally occurring central nervous system depressant [γ-butyrolactone (GBL) and γ-hydroxybutyrate (GHB)] on cerebral ischemia focusing on the elucidation of the possible mechanisms responsible for the observed beneficial effect of GBL and

GHB on ischemic brain edema. These investigations showed that the postischemic GHB treatment 3 hours after release of bilateral carotid occlusion markedly reduced the ischemically accumulated tryptophan (the precursor of 5HT), raised significantly the level of 5HT and normalized 5-HIAA concentration in the cortex, hippocampus and striatum as compared to the respective values found at 4 hours of reflow in untreated gerbils. These findings indicate that the postischemic GHB treatment stabilizes the ischemically disturbed serotonin metabolism. Therefore, the observed short term improvement in one of monoamines metabolism following a relatively late postischemic treatment warrants further studies of GHB as a potential therapeutic agent in cerebral ischemia.

The development and progression of ischemic cerebral edema have been associated with changes in serotonin (5-HT) metabolism. To shed some more light on the pathomechanism of edema formation, we investigated the kinetic properties of cellular 5-HT (S_2-postsynaptic) receptors and their relationship to the 5-HT and water content of the brain in gerbils subjected to 15 min bilateral carotid artery occlusion with and without 1 hour release. The S_2-receptors in the brain homogenate were detected using ^3H ketanserin, the potent 5-HT antagonist which labels specifically S_2-receptors sites. Bilateral cerebral ischemia of 15 min duration did not alter the kinetics of ^3H ketanserin cerebral bindings sites although it reduced significantly 5-HT and specific gravity levels as compared to the brain values of sham-operated gerbils. The S_2-receptors displayed the normal characteristics of high affinity bindings sites with ^3H ketanserin, K_D=1nM. At 1 hour reflow, two types of S_2-receptors were detected and a high and low affinity bindings sites (K_D=1nM and K_D=10nM, respectively) with normal 5-HT levels and lowest specific gravity of the brain. The appearance of additional bindings sites for ^3H ketanserin is indicative of supersensitivity of S_2-receptors in the presence of unchanged 5-HT content in the brain. Therefore the mechanisms responsible for the supersensitization of postsynaptic 5-HT receptors might be the result of either direct effect of the presynaptically released 5-HT on the membrane (without interacting with S_2-receptors) or due to reduced availability of 5-HT. Nevertheless, the altered properties of S_2-receptors suggest serotoninergic involvement in the pathogenesis of ischemic brain edema.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02275-08 LNNS

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Cerebral capillary endothelial cultures: Prostaglandin Synthesis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

M. Spatz, Head, Section on Neurocytobiology, LNNS, NINCDS

COOPERATING UNITS *(if any)*
L.S. Wolfe, Montreal Neurological Inst. McGill Univ. Montreal CA.

LAB/BRANCH
Laboratory of Neuropathology and Neuroanatomical Sciences

SECTION
Section on Neurocytobiology

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	.2	.8

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Cultured and propagated cerebrovascular endothelial cells form prostacyclin from endogenous arachidonic acid. Noradrenalin, isoproterenol serotonin but not histamine-increased prostacyclin (6-keto-$PGF_{1\alpha}$) synthesis from 100 to 200 percent. However the greatest increase of the synthesis (4.5-fold) is seen with the addition of calcium ionophore. The pattern of the lipoxygenase products obtained after the calcium ionophore stimulation of intact pieces of cerebral cortex was different from this seen in the endothelium. 12-L-hydroxyheptadecatrienoic acid (HHT) was found in significant amounts of brain tissue but not by endothelial cells indicating absence of thromboxane A_2 formation in the latter.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02324-07 LNNS

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Studies on the blood-brain barrier (BBB) to 5-HT and NE metabolites

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, end institute affiliation)*

M. Spatz, Head, Section on Neurocytobiology, LNNS, NINCDS

COOPERATING UNITS *(if any)*

None

LAB/BRANCH
Laboratory of Neuropathology and Neuroanatomical Sciences

SECTION
Section on Neurocytobiology

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.6	1.6	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standerd unreduced type. Do not exceed the space provided.)*

Previously we showed that the 5-HT present in the microvessels is not only derived from serontonergic nerve endings but it is also formed in the endothelium where its level is most probably controlled by prostaglandin. (J. Cerebral Blow Flow and Metabolism 3 Suppl 1 S311, 1983) The continuous investigation comprised daily monitoring of 5-HT and 5-HIAA content in the feeding solution incubated with the cerebrovascular endothelium (experimental) and without these cells (control) , permitting a direct determination of 5-HT metabolism and indirect assessment of endothelial 5-HT synthesis. The daily concentrations of each substance was assayed by HPLC. The findings of double or tripled 5-HIAA level with an absent or significantly lower content of 5-HT in the experimental than control medium could not be entirely derived from the available 5-HT in the feeding solution since the 5-HIAA concentration exceeded even the amount of 5-HT apparently taken up and metabolized by the cells (after 72-96 hrs of incubation). Thus the accumulated 5-HIAA in the experimental medium most likely was derived from newly snythetized 5-HT in these cultures.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02357-06 LNNS

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The therapeutic GHB effect on experimental cerebral ischemia in Mongolian gerbils

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Y. Ueki, Visiting Fellow, LNNS, NINCDS
B. M. Djuricic, Visiting Fellow, LNNS, NINCDS
M. Spatz, Head, Section on Neurocytobiology, LNNS, NINCDS

COOPERATING UNITS *(if any)*

None

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SECTION
Section Neurocytobiology

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.8	.1	.7

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The studies on cerebral ischemia, its pathophysiology, prevention and therapy in gerbils have been concerned with continuous evaluation of the effects of naturally occurring central nervous system depressant [γ-butyrolactone (GBL) and γ-hydroxybutyrate (GHB)] on cerebral ischemia focusing on the elucidation of the possible mechanisms responsible for the observed beneficial effect of GBL and GBH on ischemic brain edema. These investigations showed that the postischemic GHB treatment 3 hours after release of bilateral carotid occlusion markedly reduced the ischemically accumulated tryptophan (the precursor of 5HT), raised significantly the level of 5HT and normalised 5-HIAA concentration in the cortex, hippocampus and striatum as compared to the respective values found at 4 hours of reflow in untreated gerbils. These findings indicate that the postischemic GHB treatment stabilizes the ischemically disturbed serotonin metabolism. Therefore, the observed short term improvement in the metabolism of one of the monoamines following a relatively late postischemic treatment warrants further studies of GHB as a potential therapeutic agent in cerebral ischemia.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02361-07 LNNS

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Investigations on blood-brain barrier (BBB) permeability

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

M. Spatz, Head, Section on Neurocytobiology, LNNS, NINCDS

COOPERATING UNITS *(if any)*

Prof. K.G. Go, and Dr. H.J. Hauthoff, Department of Neurosurgery and Pathology, University of Groningen, The Netherlands

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INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.4	.4	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the spece provided.)*

This project has been temporarily discontinued.

Publication:

K.G. Go, H.J. Hauthoff, S. Huitema and M. Spatz
Protein Tracer Permeability of the Blood-Brain Barrier
After Transient Cerebral Ischemia In Gerbils.
Recent Progress In The Study and Therapy Of Brain Edema
Edited by K.G. Go and A. Baethmann

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02362-06 LNNS

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)* Effect of DMSO on the histochemical demonstration of glycogen in the perfused-fixed brain

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

J. Cammermeyer, Guest Worker, LNNS, NINCDS

COOPERATING UNITS *(if any)*

None

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Laboratory of Neuropathology and Neuroanatomical Sciences

SECTION
Section on Cerebrovascular Pathology

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

EFFECT OF DIMETHYL SULFOXIDE ON THE HISTOCHEMICAL DEMONSTRATION OF GLYCOGEN IN THE PERFUSED-FIXED BRAIN

This project has been completed and the resulting manuscript has been published.

Cammermeyer, J., and Fenton, I.M.: Factors restricting maximal preservation of neuronal glycogen after perfusion fixation with dimethyl sulfoxide and iodoacetic acid in Bouin's solution. Histochemistry 76: 439-456, 1982.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02548-03 LNNS

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Evaluation of electrical impedance in cerebral ischemia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

H.G. Wagner, Chief, Section on Cerebrovascular Physiology, LNNS, NINCDS
P. Ting, Special Expert, LNNS, NINCDS
K. Kito, Visiting Fellow, LNNS, NINCDS
I. Klatzo, Chief, LNNS, NINCDS

COOPERATING UNITS *(if any)*

None

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Laboratory of Neuropathology and Neuroanatomical Sciences

SECTION

Section on Cerebrovascular Physiology

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	0.9	.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

EVALUATION OF CEREBRAL ELECTRICAL IMPEDANCE (CEI) IN FOCAL CEREBRAL ISCHEMIA PRODUCED BY OCCLUSION OF THE MIDDLE CEREBRAL ARTERY (MCAO) FOR ONE HOUR

A series of cats were subjected to left MCAO for 1 hour. During ischemia rCBF fell to less than 11 ml/100g/min. CEI rose about 211%. After release of occlusion, CEI decreased to pre-ischemic levels but a second rise of about 176% within 15 hrs of release from MCAO followed. During this late rise the rCBF was 54±5ml/100gm/min. The second rise was also observed in cats in which the MCAO was permanently occluded (rCBF= less than 1 ml/100gm/min. The secondary rise is thought to be related to brain compression induced by brain edema.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02552-03 LNNS

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Investigation of extraneuronal catechol-synthesizing enzymes in the CNS

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

M. Spatz, Head, Section on Neurocytobiology, LNNS, NINCDS

COOPERATING UNITS *(if any)*

Dr. Ikuko Nagatsu, Fujita-Gakuen Univ. School of Med., Toyoake, Aiche, Japan
Dr. Toshiharu Nagatsu, Tokyo Institutes of Technology, Yokohama, Japan

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Laboratory of Neuropathology and Neuroanatomical Sciences

SECTION

Section on Neurocytobiology

INSTITUTE AND LOCATION

NINCDS, NIH, Bethedsa, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.8	.1	.7

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the spece provided.)*

Our previous immunohistochemical and biochemical studies of cerebral microvessels and cerebrovascular endothelial cultures showed the presence of phenyl-ethanol-amine-N-methyltransferase (PNMT) activity in both tissues. Since these extra-neuronal tissues contain a catecholamine-synthesizing enzyme which is responsible for conversion of norepinephrine to epinephrine, we extended these studies to determine whether vascular PNMT is indeed capable of producing epinephrine from norepinephrine. For this purpose a direct assay of endothelial epinephrine formed from norepinephrine was determined by using high pressure liquid chromatography. These studies, which are still in progress, have shown that the cultured cerebro-vascular endothelium (2nd-4th generation) derived from dissociated cerebral micro-vascular fractions (obtained from rats) are capable of converting norepinephrine to epinephrine.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02571-02 LNNS

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Blood-brain barrier breakdown to proteins and water content of brain tissue

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

T. Kuroiwa, Visiting Fellow, LNNS, NINCDS
R. Cahn, Visiting Fellow, LNNS, NINCDS
I. Klatzo, Chief, LNNS, NINCDS

COOPERATING UNITS *(if any)*
None

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SECTION
Section on Cerebrovascular Pathology

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.1	.6	.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The effect of blood-brain barrier (BBB) breakdown to proteins on the water content of brain tissue was studied in rabbits subjected to unilateral bolus injection of the animal's own blood into the internal carotid artery under pressure. The BBB was assessed with Evans Blue (EB) and with sodium fluorescein (NaFl) tracers. Also, the penetration of horseradish peroxidase (HRP) tracer, as well as the morphology of the brain tissue, was studied on the electron microscopic level. Water content of the brain tissue was evaluated with a modified specific gravity (SG) method. Quantitative evaluation of protein penetration into brain tissue was carried out using ^{125}I labeled bovine serum albumin (BSA). Following closure of the barrier to proteins there was a progressive resolution of edema, in spite of the fact that the barrier remained open much longer for micromolecular substances, such as NaFl. Quantitative assayes revealed a direct relationship between amounts of extravasated BSA and water increments in the brain tissue. The results of this study indicate that breakdown of the BBB, allowing entry of serum proteins into extracellular spaces of brain parenchyma, free of any evidence of injury, is associated with significant increment in water content of this tissue, signifying development of vasogenic brain edema. This project is completed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02572-02 LNNS

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Effect of abolition of BBB opening on water content of ischemic brain tissue

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P. Ting, Special Expert, LNNS, NINCDS
T. Kuroiwa, Visiting Fellow, LNNS, NINCDS
I. Klatzo, Chief, LNNS, NINCDS

COOPERATING UNITS *(if any)*

None

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Laboratory of Neuropathology and Neuroanatomical Sciences

SECTION
Section on Cerebrovascular Pathology

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.1	.6	.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The effect of prevention of reactive hyperemia, which invariably follows release of arterial occlusion in areas of the brain subjected to ischemia of intensity below threshold levels, was evaluated with regard to opening of the blood-brain barrier (BBB) associated with extravasation of serum proteins, and to development of ischemic brain edema. The reactive hyperemia was abolished by hypovolemic withdrawal of the blood at the time of recirculation. Such animals showed no opening of the BBB to proteins and significantly reduced edema, when tested at 3 hours following recirculation, in comparison to edema in normovolemic animals subjected to similar intensity of one hour ischemia. Brain injury determined at 3 days after recirculation varied from none to moderate in cats with severe ischemia (below 12 mg/100g/min) in which reactive hyperemia and opening of the BBB was prevented by hypovolemia, whereas the cats with ischemia of similar severity, accompanied by reactive hyperemia and extravasation of Evans Blue, revealed a frank cerebral infarction. These studies demonstrate further the significance of serum protein extravasation in the development of brain edema and with regard to severity of ischemic injury.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02573-02 LNNS

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Changes in water content of brain and BBB in convulsive seizures

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

R. Cahn, Visiting Fellow, LNNS, NINCDS
T. Kuroiwa, Visiting Fellow, LNNS, NINCDS
I. Klatzo, Chief, LNNS, NINCDS

COOPERATING UNITS *(if any)*

None

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SECTION
Section of Cerebrovascular Pathology

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.3	.8	.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This study demonstrates that epileptic seizures in unrestrained, spontaneously breathing animals produce very high elevations of osmolarity in blood plasma, for which release of lactic acid produced by muscular contractions appears to be mainly responsible. The high plasma osmolarity induces osmotic dehydration of the brain which lasts for several hours. Elevation of plasma osmolarity and dehydration of the brain are absent in animals in which muscular contractions were pharmacologically abolished and which were artificially ventilated. In both groups of animals, it was evident that the opening of the blood-brain barrier (BBB) to protein tracers induces an increase in water content, which eventually, may lead to development of edema. These studies indicate that epileptic seizures associated with violent muscular contractions result in dehydration of the brain which may last for several hours. Also, the studies indicate that opening of the blood-brain barrier to proteins during the epileptic seizures leads to edema in the affected brain regions.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02574-02 LNNS

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
A new histochemical method for the detection of adenylate cyclase with forskolin

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

G. Szumanska, Guest Worker, LNNS, NINCDS
M. Spatz, Head, Section on Neurocytobiology, LNNS, NINCDS

COOPERATING UNITS *(if any)*

None

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SECTION
Section on Neurocytobiology

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.3	.4	.9

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A new histochemical method was developed for the detection of adenylate cyclase (AC) by stimulation of the enzyme activity with forskolin. This method was compared with the technique in which isoproterenol and 5-guanylylimidodiphosphate (GppNp) were used as activators of AC.

The studies revealed that forskolin is not only a suitable activator of AC but is more effective than isoproterenol and GppNp for the demonstration of this enzyme histochemically.

The availability of the method for the detection of AC activity without the necessity of using a hormonal stimulator has a great potential for the evaluation of this enzyme in normal and pathological tissues especially in those cases showing an absence or desensitization of the specific hormonal receptor linkage to AC.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02575-02 LNNS

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The establishment of cerebrovascular smooth muscle culture

PRINCIPAL INVESTIGATOR *(List other professionel personnel below the Principel Investigator.) (Name, title, laboretory, and institute affiliation)*

M. Spatz, Head, Section on Neurocytobiology, LNNS, NINCDS

COOPERATING UNITS *(if any)*

Dr. Ronald F. Dodson, Division of Experimental Pathology, East Tyler Chest Hospital, Tyler, Texas

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SECTION
Section on Neurocytobiology

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	.1	1.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This study was concerned with the development and establishment of pure cerebrovascular smooth muscle culture. The established cell line originates from dissociated cells of microvessels obtained from brains of rats by mechanical dispersion and filtration technique.

The cultured cells display histochemical and ultrastructural features characteristic of smooth muscle cells. They are as follows: an ovoid nucleus with one to four nucleoli and a granular, slightly basophilic perinuclear cytoplasm arranged in bundles throughout the cytoplasm, particularly adjacent to the opposing cellular membranes of the cells.

In view of these observations, the cultured cerebrovascular smooth muscle cells provide a new model system for studying their function, especially related to the function of cerebral blood flow, blood pressure and overall to the blood-brain barrier function.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02576-02 LNNS

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Cerebrovascular smooth muscle cultures: Binding studies of α_2-adrenergic receptors

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

B. Wroblewska, Visiting Fellow, LNNS, NINCDS
M. Spatz, Head, Section on Neurocytobiology, LNNS, NINCDS

COOPERATING UNITS *(if any)*

None

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SECTION
Section on Neurocytobiology

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	.8	1.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The presence of α_2-adrenergic receptors was investigated in smooth muscle cell cultures using ^{3}H clonidine (α_2-adrenergic agonist) as a ligand.

Specific binding sites of ^{3}H clonidine were defined as the excess over "blanks" taken in the presence of 1μm "cold" clonidine. For the competitive studies different concentration of various adrenergic agonists and antagonists were used to displace binding with 4nM ^{3}H-clonidine.

The rank order of potency for α-adrenergic agonists and antagonists was clonidine > phentolamine = yohimbine >> prazosin. The IC_{50} for the investigated displacers were: 25nM, 300 nM, 1μM and 9mM respectively. Competition curve produced by competing "cold" for ^{3}H-clonidine (4nM) showed a biphasic pattern indicative of multiple binding sites. Besides, the Scatchard analysis of saturation curve (concentration of ^{3}H-clonidine ranged from 6μM to .3 nM) and dissociation rate were characteristic of multiple population of the α_2-adrenergic binding sites in cultured smooth muscle cells. Thus, the existence of α_2-type adrenergic receptors not linked to AC activity observed in the cerebrovascular smooth muscle cells strongly suggest that their reactivity which is mediated by these receptors might be associated with Ca++ fluxes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02620-01 LNNS

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Reactivity of young gerbil brain to cerebral ischemia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

H. Martinez, Visiting Associate, LNNS, NINCDS
R. Cahn, Visiting Fellow, LNNS, NINCDS
B. B. Mrsulja, Visiting Scientists, LNC, NINCDS
I. Klatzo, Chief, LNNS, NINCDS

COOPERATING UNITS *(if any)*

None

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SECTION

Section on Cerebrovascular Pathology

INSTITUTE AND LOCATION

NINCDS,NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	2.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Effects of 5 minute cerebral ischemia due to bilateral occlusion of the common carotid arteries were studied in 3-week old and adult gerbils. The evaluation of cerebral blood flow with ^{14}C iodoantipyrine radioautography revealed severe, uniform (below 10 ml/100g/min) ischemia in most of the both hemispheres, similar in intensity in both young and adult gerbils. However, biochemical assays revealed a considerable difference concerning the rate of depletion of the main energy metabolites indicating a slower energy metabolism in the young gerbils. Morphological studies carried out after 2 weeks revealed no evident ischemic injury in the young animals, whereas the brain of adult gerbils showed characteristic severe destruction of the CA1 sector of the hippocampus. These studies indicate that the thresholds for ischemic injury are age-dependent and that the young animals show a lesser sensitivity than adult ones to ischemic brain damage.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02621-01 LNNS

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 charecters or less. Title must fit on one line between the borders.)*
Properties of Glucose 6-Phosphatase in Cerebrovascular Endothelium

PRINCIPAL INVESTIGATOR *(List other professionel personnel below the Principel Investigetor.) (Name, title, leboratory, end institute affiliation)*

B.M. Djuricic, International Research Fellow, LNNS, NINCDS
M. Spatz, Head, Section on Neurocytobiology, LNNS, NINCDS

COOPERATING UNITS *(if eny)*

None

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Laboratory of Neuropathology and Neuroanatomical Sciences

SECTION
Section on Neurocytobiology

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.8	1.0	.8

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use stendard unreduced type. Do not exceed the spece provided.)*

The biochemical characteristics were investigated of G 6-Pase in cerebrovascular endothelium using two models: isolated microvessels and pure cultures of cerebrovascular endothelial cells (4th - 6th generation) derived from the microvascular fraction of rat brain.

Glucose 6-P (G 6-P) was the best substrate among the tested sugar phosphates (glucose 1-P, erythrose 4-P and 4-P glycerate) in both cell types. Ribose 5-P gave the same response as glucose 6-P while fructose 6-P was a good substrate for the cultured cerebrovascular endothelium but not for the isolated microvessels.

The cerebrovascular endothelial G 6-Pase in contrast to that of the liver failed to phosphorylate glucose using carbamyl phosphate as donor. Kinetically a marked activation of G 6-Pase occurred at high concentration of G 6-P (over 2 mmoles/l up to 25 mmoles/l). A biphasic response curve of the G 6-Pase activity was seen in the presence of either increased relative concentration of substrate or the amount of tissue enzyme. ATP as well as the non-hydrolyzable analogue adenyl (β,γ-methylene) diphosphonate stimulated also the activity of endothelial G 6-Pase. The gel electrophoresis showed a single site of enzymatic activity corresponding to a single protein band irrespective of the tissue source.

The high concentration of G 6-Pase in the cerebrovascular endothelium, kinetic activation pattern [(allo-) steric] distinctly different from other tissues are indicative of a specific role of this enzyme in the cerebral microvasculature compatible with the proposed participation of G 6-Pase in the glucose transport across BBB.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02622-01 LNNS

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The effects of hypoosmotic solutions on cultured cerebrovascular endothelium

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigetor.) (Name, title, leboretory, and institute affiliation)*

O. Kempski, Visiting Fellow, LNNS, NINCDS
M. Spatz, Head, Section on Neurocytobiology, LNNS, NINCDS

COOPERATING UNITS *(if any)*
G. Valet, Max-Planck Inst. Biochem. Martinsried FRG
A. Baethmann, Inst. Surg. Res. Univ. Munich, FRG

LAB/BRANCH
Laboratory of Neuropathology and Neuroanatomical Sciences

SECTION
Section on Neurocytobiology

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL.	OTHER:
1.5	.8	.7

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Since the capillary endothelial ability to regulate its volume should be prerequisite for maintaining the function of the barrier, we investigated the response of intrinsic endothelial mechanisms to hypotonicity.

The exposure of viable endothelium to a medium of half normal osmolality resulted in immediate cellular swelling, reduction in transmembraneous potential and intracellular pH but without evidence of permeability changes to trypan blue bound proteins. A rapid recovery with complete normalization of cell volume and membrane potential but with limited restoration of intracellular pH took place within 30-60 minutes although the osmolality of the medium remained low. These results strongly suggest that the cerebrovascular endothelium has a build-in high capacity for self-regulation which undoubtfully is important for normal function of BBB.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02623-01 LNNS

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Serotonin(S_2)-Receptors in ischemic brain edema

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

B. Wroblewska, Visiting Fellow, LNNS, NINCDS
Y. Ueki, Visiting Fellow, LNNS, NINCDS
B. M. Djuricic, Visiting Fellow, LNNS, NINCDS
M. Spatz, Head, Section on Neurocytobiology, LNNS, NINCDS

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Laboratory of Neuropathology and Neuroanatomical Sciences

SECTION
Section on Neurocytobiology

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	2.0	.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The development and progression of ischemic cerebral edema have been associated with changes in serotonin (5-HT) metabolism. To shed some more light on the pathomechanism of edema formation, we investigated the kinetic properties of cellular 5-HT (S_2-postsynaptic) receptors and their relationship to the 5-HT and water content of the brain in gerbils subjected to 15 min. bilateral carotid artery occlusion with and without 1 hour release. The S_2-receptors in the brain homogenate were detected using ^3H Ketanserin, the potent 5-HT antagonist which labels specifically S_2-receptors sites.

Bilateral cerebral ischemia of 15 min. duration did not alter the kinetics of ^3H Ketanserin cerebral binding sites although it reduced significantly 5-HT and specific gravity levels as compared to the brain values of sham-operated gerbils. The S_2-receptors displayed the normal characteristics of high affinity binding sites with ^3H Ketanserin, K_D=1nM. At 1 hour reflow, two types of S_2-receptors were detected a high and low affinity binding sites (K_D=1nM and K_D=10nM, respectively) with normal 5-HT levels and lowest specific gravity of the brain. The appearance of additional binding sites for ^3H Ketanserin is indicative of supersensitivity of S_2-receptors in the presence of unchanged 5-HT content in the brain. Therefore the mechanisms responsible for the supersensitization of postsynaptic 5-HT receptors might be the result of either direct effect of the presynaptically released 5-HT on the membrane (without interacting with S_2-receptors) or due to reduced availability of 5-HT. Nevertheless, the altered properties of S_2-receptors substantiate the serotoninergic involvement in the pathogenesis of ischemic brain edema.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02625-01 LNNS

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Efficacy of PGBx to protect against cerebral ischemia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
H. Martinez, Visiting Associate, LNNS, NINCDS
B.B. Mrsulja, Visiting Scientist, LNC, NINCDS
H. Masaoka, Visiting Fellow, LNNS, NINCDS
R. Cahn, Visiting Fellow, LNNS, NINCDS
J. Dambrosia, Biostatistician, OBFS
H.G. Wagner, Chief, Section on Cerebrovascular Physiology, LNNS, NINCDS
I. Klatzo, Chief, LNNS, NINCDS

COOPERATING UNITS *(if any)*
Laboratory of Neurochemistry, IRP, NINCDS
Hahnemann University, Philadelphia, PA

LAB/BRANCH
Laboratory of Neuropathology and Neuroanatomical Sciences

SECTION
Section on Cerebrovascular Pathology

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 202050

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.5	0.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In collaboration with Professor Thomas Devlin of Hahnemann University in Philadelphia, PA an effort has been made to evaluate a prostaglandin derivative called PGBx for its protective action against ischemic brain damage. This compound, isolated in the course of studies on stress, was observed to protect vitro mitochondrial metabolism from hypoxia. Our interest rested on the opportunity to test the efficacy of PGBx in a model of cerebral ischemia which seemed definitive and relatively easy to assess. This model is the adult mongolian gerbil subjected to 15 minutes of bilateral carotid occlusion. While untreated controls showed the expected 30% survivability at 7 days, over 92% of the treated gerbils survived. This beneficial result was present only if the PGBx was given 30 minutes after occlusion release followed by repeat doses at 1, 2 and 3 hrs. If given before or during occlusion or more than 1 hr after release from occlusion, the drug was essentially ineffective. As there are few drugs that offer benefit when administered after the ischemic injury, this drug appears to be deserving further study.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02627-01 LNNS

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Relationship between electrical impedance and intracranial pressure

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

H. G. Wagner, Chief, Section on Cerebrovascular Physiology, LNNS, NINCDS
P. Ting, Special Expert, LNNS, NINCDS
K. Kito, Visiting Fellow, LNNS, NINCDS
I. Klatzo, Chief, LNNS, NINCDS

COOPERATING UNITS *(if any)*

None

LAB/BRANCH
Laboratory of Neuropathology and Neuroanatomical Sciences

SECTION
Section on Cerebrovascular Physiology

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.6	1.3	.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

AN EVALUATION OF THE ROLE OF INCREASED INTRACRANIAL PRESSURE (ICP) IN PRODUCING BRAIN COMPRESSION AND CHANGES IN CEREBRAL ELECTRICAL IMPEDANCE (CEI) HAS BEEN STARTED

Earlier studies showed that focal brain ischemia produced by one hour occlusion of the middle cerebral artery in cats produces a rise in the cerebral electrical impedance of the affected grey matter which returns approximately to pre-ischemic levels when the occlusion was released. In many of these animals a second-later rise in CEI was observed to occur which appeared to be related to an increase in intracranial pressure. To test this hypothesis, brain compression was produced by epidural balloon inflation. When the epidural pressure was increased the CEI increased as much as 216%. The regional blood flow (rCBF) was lowered but not to ischemic levels. This study suggests that brain compression produced by edema can itself produce a reduction in extracellular space without necessarily lowering rCBF to critical ischemic levels.

TAB 12 -- LABORATORY OF NEUROPHYSIOLOGY -- (LNP)

ANNUAL REPORT
October 1, 1983 through September 30, 1984
Laboratory of Neurophysiology

National Institute of Neurological and Communicative Disorders and Stroke

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ANNUAL REPORT

October 1, 1983 through September 30, 1984

Laboratory of Neurophysiology
National Institute of Neurological and
Communicative Disorders and Stroke

Jeffery L. Barker, Chief

During the past year work has continued in several well-established lines of investigation and considerable effort has been expended developing innovative strategies for examining cell biological properties of central neurons maintained in vitro. The Laboratory's current research programs have also been subjected to trienniel review by the Board of Scientific Counselors.

Most of the projects involve characterization of the cellular properties and functions of elements that comprise the vertebrate CNS, using a number of the techniques current in cellular neurobiology. Several projects are focussed on clonal pituitary cells, which, like central neurons, are excitable and exhibit a variety of commonly expressed transmitter recepter proteins and ion channels. These latter cells serve not only as useful models for elucidating hormonal-type transmitter regulation of membrane excitability but also as immunogenic material for generating monoclonal immunoreagents specific for surface determinants associated with receptors and ion channels.

Our immediate goal is to develop strategies for isolating transmitter-type-specific CNS neurons for maintenance in monolayer culture. Our long-term objective involves characterization of cellular properties expressed in phenotypically distinct populations of cells. Ideally, we hope to determine whether transmitter-type-specific cells exhibit distinctive morphologies and membrane specializations, especially with regard to transmitter receptors and ion conductances, and, in addition, what roles these latter properties play in transmitter synthesis and secretion. By culturing these cells in isolation (following fluorescence-activated cell sorting) or in a community of cells growing in vitro under ideal conditions of contemporary cell cultivation with appropriate target elements we hope to elucidate details regarding chemical signals and their physiological roles in regulating cellular excitability. Are the signals mediated by the same transmitter relatively constant in different regions of the CNS or do these signals vary in a systematic way? Do different transmitters mediate functionally unique forms of intercellular communication and, when compared in a quantitative manner, how do they vary? What roles do they play in regulating synthesis and secretion of other signals?

Results obtained this past year may be briefly summarized as follows. Spinal and supraspinal regions of the embryonic CNS have been dissociated into cellular suspensions using mechanical and/or enzymatic methods and then analyzed with the fluorescence-activated cell sorter (FACS). Although there is little, if any ambient fluorescence signal associated with the material, several distinct peaks in the histogram of forward angle light-scatter can be

identified and these depend primarily on the digestion protocol. As might be expected cellular debris and sub-cellular organelles scatter the least amount of light, while non-viable elements, which have lost their shape and shrunk, scatter significantly less light than vital cells, which are variable in volume but bounded by relatively tight, polarized membranes. Enzymatic, as opposed to mechanical digestions of cellular tissue consistently yield the optimum number of viable elements that can be maintained in culture. These results indicate that application of flow cytometric techniques to embryonic CNS tissues yields data that can be quantitatively analyzed at the brain region level and is also compatible with survival in dissociated cell culture.

Two strategies have been developed for labelling specific sub-populations of embryonic cells prior to flow cytometric analysis. One involves retrograde transport of a plant lectin coupled to a fluorescent marker by cells projecting from the CNS to peripheral tissues. Accumulation of the fluorescent marker in spinal cord cells and sensory ganglia permits sorting on the FACS. Using such a strategy, cells have been isolated from the spinal cord by flow cytometry and maintained in vitro for several weeks. Preliminary analysis indicates that a proportion of the cultured cells differentiate into large, multipolar elements and exhibit a spectrum of chemically and electrically excitable membrane properties. Further characterization should reveal whether these cells are phenotypically motoneurons. If so, we should be able to isolate enough to use as immunogenic substrate for generating immunoreagents specific for motoneurons and we might be able to develop some monoclonals that mark only cells of the cholinergic phenotype.

The other strategy involves the use of surface-reactive monoclonal immunoreagents complexing with specific sub-populations of embryonic CNS cells. Cells isolated with this strategy and maintained in vitro have begun to be characterized. The majority of these cells stain for the presence of GAD, the rate-limiting enzyme in the synthesis of GABA. Some of these also synthesize immunohistochemically detectable levels of one or another peptide. It should be obvious that if the yield of these cells can be improved, then this immunoreagent in particular, and this strategy in general have both immediate promise and increasing potential for isolating and studying in vitro cells whose transmitter phenotypes are expressed in vivo.

Although considerable effort has gone into perfecting methods for culturing embryonic central neurons in this and other laboratories, difficulties persist and developing a consistent, reliable protocol remains a continuing priority. At present the quality of the preparation and its utility as a monolayer system to simplify the complexity of the CNS and to gain access to problems involving neuronal functions at cellular and sub-cellular levels are both limited. Therefore, systematic study of the culture conditions that are required for optimizing the survival of embryonic CNS cells, including cellular supporting elements, connective tissue matrices and diffusable trophic factors, is a pre-requisite for the success of many of the lines of investigation constituting this long-term program. This is especially true now that we have begun to culture cells following isolation by flow cytometry.

Those cells that do survive in culture have been characterized with a variety of assays important in quantitating cellular functions. These include 1) electrical measurements of excitable membrane properties in both micron-sized patches and in whole cells; 2) intact-cell binding studies of putative transmitters, clinically important drugs and cell-type-specific or receptor-subunit-specific immunoreagents; and 3) immunohistochemistry at light- and electron-microscopic levels. The results clearly indicate that a variety of cytoplasmic and membrane functions are variously expressed in vitro. All of these can be recognized in normally organized tissues and several are being compared with data derived from the intact CNS. Efforts this past year have been directed at the following problems: 1) quantitative resolution of the principal electrically and chemically gated ion conductances resident both in neurons cultured from different regions of the embryonic CNS and in cells cloned from a rat tumor that secretes prolactin and growth hormone; 2) biochemical characterization of the surface antigens on embryonic CNS neurons and cloned cells interacting with monoclonal immunoreagents; 3) the development and biochemical characterization of GABA and GABA-related drug receptors in intact cells under physiological conditions and comparison with data obtained in binding experiments on membrane fractions derived from cultured and normally developed cells; 4) multi-disciplinary analysis of immunoreaction signals generated in embryonic cells with anti-glycine-receptor-subunit-specific monoclonal antibodies; and 5) immunohistochemical studies of cultured cells doubly-stained for surface marker antigen and transmitter phenotype expressions.

The results indicate that monolayers of CNS neurons are indeed a valuable preparation to study the distribution of neuronal functions at the cellular level. However, it is evident that the temporal resolution of excitability afforded by all of the conventional electrophysiological techniques is unavoidably compromised both by the difficulties inherent in conducting such assays, by the single-cell rate at which they can be carried out and analyzed, and by the unidentified nature of the cell type recorded electrically. Conversely, ligand-binding strategies suffer from the facile generation of signals without statistical resolution of their meaning in terms of cellular distribution. Thus, we plan to develop new methods, specifically those involving a variety of optical recording techniques, for studying the cellular biology of central neurons at a more expeditious rate of insight.

I anticipate that all of the on-going projects, as well as those currently in development will benefit significantly from the arrival of Drs. Craig Venter and Claire Fraser, their associates and their expertise in the biochemistry and immunology of transmitter receptors and ion channels. They plan to focus on specific aspects of the structure and chemistry of receptors and ion channels. Many of their experiments will quite naturally lead to collaborative efforts with other members of the Laboratory on problems of mutual interest.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02019-12-LNP

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Electrophysiological Studies on Neuronal Excitability

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

J. L. Barker	Chief	LNP, IRP, NINCDS
T.G. Smith	Section Chief	LNP, IRP,NINCDS
G.D. Lange	Senior Scientist	LNP, IRP, NINCDS
A.B. MacDermott	Staff Fellow	LNP, IRP, NINCDS
A.E. Cole	PRAT Fellow	NIGMS, NIH
M.A. Rogawski	PRAT Fellow	NIGMS, NIH
G-G Chen Fogarty Fellow	LNP, IRP, NINCDS;	J. Mazzetta, Tech., LNP, IRP, NINCDS
D. Owen Fogarty Fellow	LNP, IRP, NINCDS;	V. Smallwood, Tech., LNP, IRP, NINCDS

COOPERATING UNITS *(if any)*

M. Segal (Weizmann Institute, Rehovot, Israel); B. Dufy (Laboratoire de Neurophysiologie, Bordeaux); H. Betz (Univ. of Heidelberg, West Germany); G. Redmann and H. Lecar, LB, IRP, NINCDS

LAB/BRANCH

Laboratory of Neurophysiology, IRP, NINCDS

SECTION

Office of the Chief and Section on Sensory Physiology

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
8	7	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The aim of this research program involves elucidation of the ion conductances expressed by cells cultured from the embryonic CNS and from primary and clonal endocrine cells. Specific lines of investigation include projects on the embryonic development, cellular distribution and functional roles of the conductances in membrane excitability. Electrical measurements of ion conductance activities either at the microscopic level in membrane patches or at the whole-cell level with the patch-clamp technique and with single- and double-microelectrode penetrations are strongly influenced by the assay method itself. Thus, although the different assay techniques are all quite useful and provide complementary data for characterizing the membrane mechanisms underlying ion conductances in these cells, there are advantages and disadvantages to the application of each. Principal observations to date include the following: 1) electrically excitable membrane properties develop early, well before birth as soon as cells can be studied; 2) although a number of cation and anion conductances have been characterized in a relatively quantitative manner, none appear unique to the vertebrate or to the cell studied; and 3) the conductances underlie specific patterns of excitability, which serve to transform, in a still ill-defined way, synthetic events in the cytoplasm into defined secretory activities. Particular attention has been given to those mechanisms most commonly expressed and to identifying those exhibited by specific cell types (e.g., motoneurons, GABAergic cells). It appears that certain, relatively ubiquitous mechanisms have similar electrical properties in phenotypically distinct types of cultured neurons. Pharmacological experiments involving anti-glycine-receptor antibodies have revealed that certain immunoreagents can mimic agonist actions. Such a line of investigation will allow us to examine the roles played by different subunits in the generation of an electrical response on the post-synaptic membrane by glycine. This strategy is undoubtedly generalizable to immunoreagents specific for other transmitter receptor/ion channel complexes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02330-07 LNP

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Cellular Biological Studies of CNS Neurons

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigetor.) (Name, title, leboratory, and institute affiliation)*

J.L. Barker	Chief	LNP, IRP, NINCDS
G.D. Lange	Senior Scientist	LNP, IRP, NINCDS
M.T. Caserta	Staff Fellow	LNP, IRP, NINCDS
G. Kapatos	Staff Fellow	LNP, IRP, NINCDS
P.A. St.John	Staff Fellow	LNP, IRP, NINCDS
A.E. Schaffner	Staff Fellow	LNP, IRP, NINCDS
W. Kell	Technician	LNP, IRP, NINCDS

J. Mazzetta Tech. LNP, IRP, NINCDS; V. Smallwood, Tech. LNP, IRP, NINCDS

COOPERATING UNITS *(if any)*
H. Betz (Univ. of Heidelberg, West Germany); R.E. Siegel (Lab. Cell Biology, NIMH); J. Moskal (Lab. Cell Biology, NIMH)

LAB/BRANCH
Laboratory of Neurophysiology, IRP, NINCDS

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NINCDS, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
9	7	2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The immediate aim of this research is the development of complementary cell biological strategies for cellular and sub-cellular fractionation of embryonic nervous tissue for the purpose of investigating the process of phenotypic expression and the cellular distribution of neuronal functions. During the past year embryonic elements dissected from the mammalian central nervous system have been studied using a variety of techniques, including dissociated primary and clonal cell culture, flow cytometry and fluorescence-activated cell and organelle sorting, immunohistochemistry with surface-reactive and transmitter-related reagents, light- and electron-microscopy, and intact-cell ligand binding. Preliminary protocols have been developed for analyzing in a quantitative manner subpopulations of cells by flow cytometry and for isolating specific cell types both for biochemical characterization and for potential use as immunogenic material, as well as for maintenance in culture. Principal findings include: 1) enzymatic digestions of embryonic CNS tissue yield maximal number of viable elements; 2) putative motoneurons and a subset of sensory cells can be back-filled and isolated; and 3) a subpopulation of embryonic spinal cord cells that synthesize GABA-related marker enzyme can be stained with a surface-reactive immunoreagent and isolated in cell culture. Distinguishing cellular properties have been revealed in these isolated sub-populations, indicating that studying the processes underlying phenotypic differentiation may be feasible with this combined flow-cytometric/cell-culture strategy.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 01659-16-LNP

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Synaptic Contacts of Retinal Neurons

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

A. Lasansky	Chief, Section on Cell Biology	LNP, IRP, NINCDS
A. Mariani	Laboratory of Vision Research	NEI, NIH
Julie Lohr	Technician	LNP, IRP, NINCDS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Neurophysiology, IRP, NINCDS

SECTION
Section on Cell Biology

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	1	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Rod telodendria in turtle retina end as postsynaptic elements at ribbon synapses of cones and other rods. This finding represents the first clear evidence of chemical synapses between vertebrate photoreceptors.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02339-07-LNP

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Neural Coding and Processing of Information in the Visual System

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

H.G. Wagner. Chief, Section on Neuronal Interactions, LNP, IRP, NINCDS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Neurophysiology

SECTION

Section on Neuronal Interactions

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.2	0.1	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been discontinued since Dr. Wagner, the principal investigator, has transferred to another laboratory.

TAB 13 -- BIOMETRY AND FIELD STUDIES BRANCH -- (BFSB)
(Formerly Office of Biometry and Field Studies - OBFS/OD)

ANNUAL REPORT
October 1, 1983 through September 30, 1984

Office of Biometry and Field Studies

Intramural Research Program
National Institute of Neurological and Communicative Disorders and Stroke

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Annual Report
for period October 1, 1983 through September 30, 1984

Office of Biometry and Field Studies

Intramural Research Program*
National Institute of Neurological and Communicative Disorders and Stroke

Jonas H. Ellenberg, Ph.D., Acting Chief

The Office of Biometry and Field Studies (OBFS) supports a program in biostatistics and computer science to advance the mission of NINCDS in the areas of neurology and communicative disorders. The scientists in OBFS offer expertise in biostatistics and computer science, as well as demography and survey design. The Office participates in a wide range of extramural and intramural collaborative projects, including large and small scale observational clinical studies, studies of incidence and prevalence of disease, clinical trials, and laboratory studies. The research program of OBFS is conducted through both direct staff research and through research and development contracts.

I. COLLABORATION WITH THE INTRAMURAL AND EXTRAMUAL RESEARCH PROGRAMS, NINCDS

OBFS continues an active collaborative role with both the Intramural and Extramural Research Programs at NINCDS. The typical collaboration gives OBFS responsibility for the statistical design, data management and statistical analysis aspects of the project with the Program providing the project initiatives, subject matter expertise and overall project leadership.

Several projects with the Developmental Neurology Branch, CDNDP are ongoing. The clinical trial of cognitive and developmental effects of phenobarbital on children with febrile seizures is in its second year of patient accrual and will continue through 1985, with each patient to be followed for a minimum of 2.5 years. The survey of physicians regarding the treatment and management of children who have had febrile seizures has been completed and the statistical analysis of the survey response data will be completed in FY '85. A large prospective population-based study of the prognostic value of the EEG for predicting subsequent febrile seizures for children who have had a febrile seizure is being carried out in Yugoslavia, and will continue patient accrual through the end of calendar year 1984; the total number of patients registered into the study will be 500, with a minimum three-year follow-up for each patient.

OBFS is participating with the Communicative Disorders Program on a study of factors that are predictive of reading and writing skills in the congenitally deaf. Sixty-five congenitally deaf adolescents from each of three language training groups (aural-oral, total communications, and American Sign Language) will be recruited. The study which is in the planning and design stage will last for approximately three years.

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*As of September 12, 1984, OBFS was transferred to the Intramural Research Program, NINCDS, and has been retitled the Biometry and Field Studies Branch (BFSB).

OBFS is working with the Stroke and Trauma Program on three studies of interventional stroke therapies conducted under the aegis of the Cerebrovascular Clinical Research Master Agreement. These studies provide information necessary for investigators to develop randomized, controlled clinical trials for the treatment of stroke with new or untested treatments and management modalities. A Phase I dose response study of Naloxone for the treatment of acute cerebral infarction is nearing completion, and will be followed by a Phase II study. A Phase II study of the benefits of hypervolemic hemodilution (Dextran-40) for the treatment of stroke-in-evolution has begun and will continue to accrue patients through 1985. A third Master Agreement study of a calcium channel blocker for the treatment of hemorrhagic strokes is in the planning and design stages.

OBFS has continued its involvement in the Copiah County Study, a prevalence survey of major neurologic disorders in a biracial population. In collaboration with the Intramural Research Program and the University of Mississippi Medical Center, the Office has published research reports on essential tremor and cerebral palsy. Manuscripts on Parkinson's Disease and dementia have been submitted for publication. Work is continuing on the analysis of data on epilepsy, psychomotor delay, and stroke.

A significant effort continues on the analysis of data from the Collaborative Perinatal Project in collaboration with the Developmental Neurology Branch, CDNDP and the Infectious Diseases Branch, IRP. Intensive studies are proceeding in the areas of epilepsy, cerebral palsy, and maternal infection during pregnancy. Papers on the incidence of clinical infections in pregnant women, obstetric conditions as risk factors for cerebral palsy and convulsive disorders in children, the age of onset of seizures in young children and the risk of recurrence of nonfebrile seizures in children have been published in this fiscal year. A study of migraine in pregnant women, also using the Collaborative Perintal Project data, is continuing. Statistical investigations are focused on the association of migraine with other diseases, the familial relationship of migraine in mothers and seizures in their children, and the relationship between migraine in the mothers and cerebral dominance in the child.

OBFS also collaborates with IRP on a number of clinical and laboratory studies. These studies include the development of screening techniques to detect viral infections, space-time clustering of disease, comparisons of viral antibody titer levels in MS patients and controls, determination of transmission rates of slow viruses, sample size determination for animal model studies, drug efficacy studies in animal models, and the development of new bioassay methods.

II. CLINICAL DATA BANKS

OBFS is responsible for the management of two prospective data collection projects, the Stroke and the Traumatic Coma Data Banks. These data banks provide a framework in which to address research questions regarding the characteristics and clinical course of hospitalized stroke and coma patients. The approach, which involves the collection of clinical and laboratory data at multiple clinical centers uses a common set of data collection forms so as to provide data which may be pooled across centers. Each of the Data Banks is a collaborative effort between OBFS, acting as the coordinating center, a computer data base maintenance center, and four hospital centers.

Stroke Data Bank

The pilot phase of the Stroke Data Bank ended in 1981. Data collection for the Main Phase Stroke Data Bank began in the middle of FY '83 and by the end of FY '84, approximately 1,000 patients will have been enrolled. The protocol calls for a two-year patient follow-up from initial hospitalization.

Studies to measure the reliability and validity of portions of the Stroke Data Bank data were undertaken during this year. In one study inter-observer agreement of a group of neurologists' responses using the data bank forms for a common group of patients was measured. The results indicated a high level of consistency. A study of agreement in diagnosis followed, where each of the neurologists received several completed data bank forms, CT and angiograph slides on the same group of patients, and were then asked to complete the data bank summary diagnosis and CT scan forms. This study is currently being analyzed. The coding of the CT scans used for the latter study will itself be evaluated for inter-observer agreement.

The University of Maryland, a Stroke Data Bank Center, is collecting data for a validity study of the Center for Epidemiologic Studies Depression Symptoms Scale (CES-D). This coincides with a non data bank University of Maryland research study on depression and stroke which utilized a lengthy diagnostic battery for depression. By coordinating the timing of our CES-D with the administration of their battery, we will be able to assess the validity of the CES-D scale as it relates to diagnosis of depression in stroke patients.

The data collected during the pilot phase of the Stroke Data Bank have been used for several scientific studies in stroke: a report on hemiparesis in acute stroke has been accepted for presentation; papers on mechanism of stroke and outcome and on gaze palsy in hemispheral stroke were presented; and data analysis is also proceeding on studies of lacunar infarction syndromes, hyperglycemia and its impact on stroke severity, and on factors impacting stroke survival.

Coma Data Bank

The pilot phase of the Traumatic Coma Data Bank ended in 1982, and data collection for the main phase Coma Data Bank began in January, 1984. Contracts were awarded six months prior to the January start up, and during these six months the data collection forms, administrative manual and protocol from the pilot study were revised and augmented. The need to study intracranial pressure (ICP) data obtained directly from the intensive care unit has led to the design and testing of a system for converting continuous pressure readings into summary elements for entry into the Data Bank. Results from CT scans taken during follow-up visits will become part of the information collected in the data bank as well. The Outcome Measures Battery has been expanded from that of the pilot phase and now includes such items as quality of life interviews with patients and families and neuropsychological tests. By the end of FY '84 approximately 200 severe head injury patients will have been enrolled.

A number of studies have used data from the Pilot Traumatic Coma Data Bank: a study of brain stem cisterns as a prognostic factor in severe head injury; a

manuscript which examines the prediction of delayed intracranial hypertension and patient course using the patients' status and pressure values during their first two days in intensive care and a paper describing the pilot patients with epidural hematomas have been accepted for publication. A manuscript relating duration of coma to duration of post-traumatic amnesia, a paper on sex differences and other characteristics of youthful traumatic coma patients 15-24 years of age and a report on the outcome of children with traumatic coma have also been completed.

The preliminary scientific results and the structural aspects of the data banks have been presented at several workshops, symposia and scientific meetings this year. At a workshop on the Stroke Data Bank which was held at the American Heart Association Stroke Council Meeting, the research foci of the Stroke Data Bank were described; data banks were discussed at the 14th Princeton-Williamsburg Conference on Cerebrovascular Diseases, where a paper on approaches to the pathophysiology of stroke through the NINCDS Data Bank was presented; a seminar on neurological data banks was given at the 1984 American Academy of Neurology Meetings; nurse coordinators from the Stroke Data Bank and OBFS staff members presented a symposium at the American Association of Neuroscience Nurses Meeting in April, 1984, which gave an overview of the Stroke Data Bank and focused on the role of the research nurse in the implementation of large, collaborative clinical studies. OBFS staff also presented at the Ninth International Health Records Congress on the design of the Stroke Data Bank and its use in medical research; at the Seventh Annual Head Trauma Rehabilitation Conference on the Traumatic Coma Data Bank and its approach to evaluation of patient outcome; and at the Eighth Annual Conference on the Rehabilitation of the Brain-Injured Adult and Child, on the epidemiology of childhood head injury.

III. SURVEYS AND DEMOGRAPHIC STUDIES

OBFS continues to serve as a resource for the analysis and interpretation of data on the morbidity and mortality of the neurologic disorders. Over the past few years the Office has been developing alternatives to single-disease surveys funded by the Institute. First, greater use is being made of data gathered by other agencies. These efforts include expanding our work in the field of morbidity and mortality of the neurologic disorders using Vital Statistics data and data from the National Ambulatory Care Survey. We are also exploring the feasibility of using data from the Health Care Financing Administration Long Term Care Survey. The second approach is cooperating with other agencies undertaking major survey initiatives. By cooperating with these groups prior to survey design and implementation, questions of concern to the Institute can be included in a national survey with the Institute sharing only a minimal part of the cost. We have included questions on stroke in the NIA, National Health and Nutrition Examination Survey Follow-Up and questions on stroke and TIA's on NCHS's Mortality Follow-Back Survey.

Research on the trends and implications of stroke mortality is continuing, using Vital Statistics data. A paper on stroke as an associated and underlying cause of death was published. The major finding was that, in addition to the 180,000 deaths per year during the period 1975-77, with stroke as the underlying cause, there were an additional 100,000 deaths with stroke coded as the associated cause. Currently we are investigating which diseases appear in

conjunction with stroke on the death certificate. This analysis will consider both underlying and associated causes of death.

To satisfy a need for current information on the morbidity and mortality of neurologic disorders, two disease reports were commissioned. The first, "Huntington's Disease: Genetics and Epidemiology", has been published and the second, "Parkinson's Disease: A Review of the Epidemiology and Pathogenesis", has been submitted for publication.

OBFS participated in several studies of multiple sclerosis. Data from the National Multiple Sclerosis Survey were used to examine the pattern of remission/exacerbations for selected symptoms. A paper on mobility and multiple sclerosis has been published, a paper examining the factors affecting employment among people with multiple sclerosis has been submitted for publication, and a paper describing the symptomatology of multiple sclerosis is being prepared in conjunction with the staff of the Demyelinating, Atrophic and Dementing Disorders Program. A study determining the prevalence of multiple sclerosis in Colorado is completed, and a series of articles is being prepared. A paper on the methodology and preliminary results of this study has been presented.

OBFS is engaged in the study of severe and debilitating headache. The planning and design for an area survey of the prevalence of severe headache in the general population has been completed. The initial findings of a feasibility study have been presented, and further analysis of the data from the feasibility study is now proceeding. These analyses are focusing on the interrelationship between headache symptoms and features and their association with the four major types of headache, and the potential for development of a new classification system of headache based on objective criteria.

A paper in press, reporting findings from the National Survey of Intracranial Neoplasms, gives the incidence of primary and secondary intracranial neoplasms by age and sex in the United States and also presents clinical findings related to histological type and location of tumors. A brochure on the findings is now in preparation.

OBFS addressed the problem of how to obtain morbidity statistics on the rare neurologic disorders. A review of existing survey strategies for studying rare characteristics in populations was completed, as well as a design for a survey of rare neurologic disorders based on visits to neurologists. The methodological issues involved in surveys of dementia were examined based on experience with the Baltimore Dementia Study, an NINCDS initiated project carried out jointly with the NIMH and Johns Hopkins University, and a paper has been prepared.

IV. OTHER RESEARCH PROJECTS IN NEUROLOGY

The collaborative investigation of head injury, designed by the Departments of Neurosurgery at the University of Virginia and the All-India Institute (AIIMS), New Delhi, is proceeding. Data from about half of the 650 head injury cases from AIIMS have been edited and entered into the computer, and parallel analyses of this data set and data from a case series of over 1,000 patients from the University of Virginia are planned for Fall of 1984. Current plans are limited to a descriptive study contrasting the two case series.

Papers describing various aspects of hearing loss in the Framingham Heart Study cohort were presented at several meetings. A paper summarizing the major findings, that the pattern of hearing loss is different by sex and that the most important risk factor for hearing loss is age, has been submitted for publication.

V. METHODOLOGICAL RESEARCH IN STATISTICS

OBFS statisticians continue to develop new statistical methodology and derive innovative modifications of statistical techniques to meet the needs of the Institute for design of experiments and field studies, analysis of data, and statistical modeling of biological processes and phenomena. Most of the statistical methodology problems arise in collaborative studies with the Intramural and the Extramural Programs. In general there are two objectives associated with these various statistical activities of OBFS. The primary objective is the development and improvement of statistical methodology to meet the needs of the Institute. The secondary objective is to make contributions to the development of statistical methodology which may be more generally useful in neurological as well as other medical research.

A partial listing of the statistical applications developed by OBFS includes: modified metrics for space-time clustering of rare diseases applied to a population in a defined geographic area; application of a selection procedure based on a signal-to-noise ratio for determining the "best" ELISA assay plate preparation; an autoregressive model of patient response for a k-period-crossover drug trial that accounts for both treatment residual effects and random effect for the individual patient; a method of monitoring patient recruitment relative to a target sample size; methods to determine suitable sample sizes developed in the contexts of detection of disease transmission rates; unequal allocation of subjects to treatment for clinical trials with binomial outcomes; and in the area of pain research, a report on the utility of Sensory-Decision-Theory measurements of experimental pain.

Theoretical statistical work included: regression in Poisson data; modeling Markov transition probabilities for a two-state chain with the incorporation of covariate information; new hypothesis testing procedures in the presence of inequality constraints; development of a new family of sequential tests for binomial distributions with early stopping potential; a demonstration of the adequacy of the diffusion process as an approximation of a binomial random walk for estimating absorption probabilities; and the development of a quantitative measure of bias of the Kaplan-Meier statistic as a function of the dependence of the censoring process.

In summary, OBFS has a vigorous program of collaborative research. Its collaboration extends to many intramural and extramural research groups throughout the Institute, as well as to centers outside NINCDS. The scope of its research activities is also quite broad, and ranges from small, one-on-one collaboration with intramural scientists, to the conduct of large-scale, multicenter clinical data banks. OBFS continues to serve as a resource for NINCDS for data on the morbidity and mortality of neurologic disorders and makes an important and continuing contribution to statistical methodology development applicable to neurological research.

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CONTRACT NARRATIVE
Office of Biometry and Field Studies, IRP, NINCDS
Fiscal Year 1984

1. UNIV. OF MARYLAND (NO1-NS-2-2302)

2. UNIV. OF S. ALA. (NO1-NS-2-2397)

3. BOSTON UNIV. (NO1-NS-2-2398)

4. MICHAEL REESE HOSPITAL & MEDICAL CENTER (NO1-NS-2-2399)

Title: Full Phase Stroke Data Bank

Date Contracts Initiated: July 1, 1982

Contractors' Principal Investigators:
1. Dr. Thomas Price
2. Dr. Jay Mohr
3. Dr. Philip Wolf
4. Dr. Louis Caplan

Current Annual Levels FY'84:
1. $222,319
2. $248,793
3. $223,320
4. $194,602

Objectives: The primary objective of this project is to implement a full phase computerized interactive data bank which will collect uniform longitudinal data on stroke patients to aid clinical research. This is a collaborative project involving a data base maintenance center to store and manipulate the data, clinical centers for the collection of data, and staff at OBFS who have the responsibility for data analysis.

Methods Employed: The Steering Committee, composed of the Principal Investigators and OBFS personnel, met during the first year of this project and outlined research objectives, developed forms and data collection protocols. Initial studies in the main phase, during the current year, have focused on research methodology and have included assessment of similarities and differences in administering and recording neurological examinations among the centers.

Significance to the NINCDS Programs and Biomedical Research: The Full Phase Stroke Data Bank Network will provide a resource of high quality data on the clinical course of stroke. The project serves as a prototype for national data bank networks for other neurological disorders.

Proposed Course of the Project: This is the beginning of the third year of a five-year project. The initial course has included determination of research questions to be investigated and design of forms to collect the data. Data collection began in July, 1983, and, as of June, 1984, information on over 700 patients had been collected. Data exploration and analysis on approximately 500 patients in the computer system (DBMC) began in July, 1984, with initial findings to be reported next year. In addition, the Stroke Data Bank has been invited to publish a Supplement to STROKE describing the data bank and

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(NO1-NS-2-2399)

including the forms that have been developed for data collection. Work on this is proceeding. The following paper has been accepted for publication:

Shinar, David, et. al. Interobserver Variability in the Assessment of Neurologic History and Examination in the Stroke Data Bank. Archives of Neurology. (In press)

CONTRACT NARRATIVE
Office of Biometry and Field Studies, IRP, NINCDS
Fiscal Year 1984

1. UNIV. OF TEXAS-GALVESTON AND BAYLOR UNIV. MEDICAL COLLEGE (NO1-NS-9-2308)

2. UNIV. OF CAL. IN SAN DIEGO (NO1-NS-9-2309)

3. MEDICAL COLLEGE OF VIRGINIA (NO1-NS-9-2307)

4. UNIV. OF VIRGINIA (NO1-NS-9-2306)

Title: Pilot Data Bank Network in Traumatic Coma

Contractors' Principal Investigators

1. Dr. Howard Eisenberg
2. Dr. Lawrence Marshall
3. Dr. Donald Becker
4. Dr. John Jane
5. Dr. Robert Grossman
6. Dr. Kamran Tabaddor

Current Annual Level FY'84

1. -0-
2. -0-
3. -0-
4. -0-

Objectives: The primary objectives of this project were to develop a computerized interactive data bank network for traumatic coma patients, to provide a structure within which to test the feasibility of data bank methodology for the study of coma and to provide a data resource for clinical research.

Major Findings: This data bank project developed and used a uniform vocabulary to collect patient data including the details of the accidents, clinical and laboratory test results, therapies and outcomes. Data from 581 severely head-injured patients were collected from January 1980 to February 1982 and are continuing to be analyzed. Several papers have been accepted in the Journal of Neurosurgery based on this project. One describes the Traumatic Coma Data Bank's design, purpose, goals, and results. One focuses on implications for treatment of patients who talk and deteriorate. Two others deal with absent cisterns as ominous predictors of outcome, and with delayed intracranial hypertension in severe head injuries.

Significance to the NINCDS Program and Biomedical Research: Longitudinal data on severely head-injured traumatic coma victims were collected at six centers using uniform definition and procedures. This information has provided a large

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(NO1-NS-9-2309)
(NO1-NS-9-2307)
(NO1-NS-9-2306)

body of high quality data for clinical research on the factors influencing survival and quality of life following severe head injury. The number of therapies and monitoring devices commonly utilized during the acute phase of managing traumatic coma necessitates a highly organized data handling capacity, and the data bank has served as an efficient mechanism for collecting, storing and retrieving both acute and followup information collected on a single patient and groups of patients. Analysis of data from the pilot phase is continuing at OBFS (see Z01-NS-02516-03). The following papers have been produced:

1. Marshall, L.F. et al. The National Traumatic Coma Data Bank. Part I: Design, purpose, goals, and results. J. Neurosurg. 59: 276-284, 1983.

2. Marshall, L.F. et al. The National Traumatic Coma Data Bank. Part 2: Patients who talk and deteriorate: implications for treatment. J. Neurosurgery. 59: 285-288, 1983.

3. Marshall, L.F. et al. Absent cisterns are an ominous predictor of outcome in severe head injury. J. Neurosurgery. (In press)

4. Marshall, L.F. et al. A predictive graph for delayed intracranial hypertension for severe head injury. J. Neurosurgery. (In press)

5. Marshall, L.F. et al. Traumatic acute epidural hematoma: unrecognized lethality in comatose patients. Neurosurgery. (In press)

Proposed Course of the Project: These contracts have been terminated, and the full phase Traumatic Coma Data Bank contracts are now operational (NO1-NS-3-2339, NO1-NS-3-2340, NO1-NS-3-2341, NO1-NS-3-3242).

CONTRACT NARRATIVE
Office of Biometry and Field Studies, IRP, NINCDS
Fiscal Year 1984

1. UNIV. OF TEXAS-GALVESTON AND BAYLOR UNIV. MEDICAL COLLEGE (NO1-NS-3-2339)

2. UNIV. OF CAL. IN SAN DIEGO (NO1-NS-3-2340)

3. MEDICAL COLLEGE OF VIRGINIA (NO1-NS-3-2341)

4. UNIV. OF VIRGINIA (NO1-NS-3-2342)

Title: Full Phase Traumatic Coma Data Bank

Contractors' Principal Investigators
1. Dr. Howard Eisenberg
2. Dr. Lawrence Marshall
3. Dr. Donald Becker
4. Dr. John Jane

Current Annual Level FY'84
1. $193,429
2. $219,562
3. $167,205
4. $175,124

Objectives: The primary objective of this project is to implement a full phase computerized interactive data bank which will provide a research resource for numerous ongoing clinical investigations of patients with head injury. This is a collaborative project, involving a data base maintenance center to store and manipulate the data, clinical centers for the collection of data, and staff at OBFS who have the responsibility for data analysis.

Methods Employed: The Steering Committee, composed of the Principal Investigators and OBFS personnel, have met during the initial year of this project and have outlined the research objectives, developed forms and a new data collection protocol based on the findings of the pilot Traumatic Coma Data Bank. A major subproject has been initiated. This is a study of how to optimally monitor, record, sample, synthesize, and report intracranial pressure (ICP) data. Data collection began in January, 1984.

Significance to the NINCDS Program and Biomedical Research: Longitudinal data on head-injured victims will be collected at four centers, using uniform definitions and procedures. This information will provide a large body of comparable data for clinical research on the factors influencing survival and quality of life following severe head injury. The number of therapies and monitoring devices commonly utilized during the acute phase of managing traumatic coma necessitates a highly organized data handling capacity, and the data bank will serve as an efficient mechanism for collecting, storing and retrieving this information as well as follow-up data collected on a single patient or groups of patients.

Proposed Course of the Project: This is the second year of a five-year project. Data collection will continue, and analysis will begin as soon as sufficient data becomes available for specific research questions.

CONTRACT NARRATIVE
Office of Biometry & Field Studies, IRP, NINCDS
Fiscal Year 1984

BETH ISRAEL HOSPITAL (N01-NS-2-2308)
BOSTON, MASSACHUSETTS

Title: Data Bank Maintenance Center for Data Bank Network Projects in Stroke and Traumatic Coma

Date Contract Initiated: September 30, 1982

Contractor's Project Director: Dr. Howard Bleich

Current Annual Level FY'84: $235,066

Objectives: Beth Israel Hospital is the Data Bank Maintenance Center (DBMC) for the Stroke and Traumatic Coma Data Banks. The DBMC is the computer system for these projects and is contracted to provide data editing to safeguard against transmission errors, storage, subsetting and retrieval, as part of the MISAR Medical Information Retrieval System. The DBMC, as part of the scope of work, is to provide a method for rapidly accessing subgroups of patient data collected at physically discrete hospital centers. Methods for retrieving data from the central repository should allow access to all or parts of the data by the Stroke and Coma Data Banks and the OBFS.

Methods Employed: The DBMC created the computer data dictionary for the Stroke project's data elements and developed methods for entering these data, transmitted from the microprocessors located in each hospital, into the central data bank. Data entry personnel at the clinical centers were trained. A pretest was performed to test out retrieval capabilities. Required enhancements should be implemented to meet the needs of the data bank projects.

Significance to the NINCDS Program and Biomedical Research: The functions of the Data Bank Maintenance Center are central to the success of the eight centers which comprise the Data Bank Networks for Stroke and Traumatic Coma. It serves as the central data repository, maintains data integrity and provides a system for efficient data retrieval.

Proposed Course of Contract: The Maintenance Center is now focusing on storage and retrieval for the Stroke and Traumatic Coma projects, as well as providing the Users Manual for the system. The contractors are expected to provide designated staff to satisfy the requirements of their workscope. The departure of key personnel from the DBMC has hampered its progress. The Project Officer has submitted a series of specific tasks to be completed as per contract requirements, and requested staffing levels for designated programmers and systems analysts commensurate with the funded levels. The Contracts Officer of NINCDS has been kept informed of the DBMC's record of contract compliance and has made plans to terminate the contract if the workscope is not adequately fulfilled.

CONTRACT NARRATIVE
Office of Biometry and Field Studies, IRP, NINCDS
Fiscal Year 1984

RLR & ASSOCIATES, INC., Fairfax, Virginia (N01-NS-2-2315)

Title: Front-end Microprocessor Support for Data Bank Projects

Contractor's Project Director: Robert L. Rush

Current Annual Level: $76,249

Objectives: To provide the medical data bank projects with a software package for cost-efficient data entry, updating, editing and nighttime transmission to the host computer to implement patient management tools to aid the clinical centers with patient care.

Major Findings: The pilot studies yielded over 1700 patients in the two Data Banks. New procedures are being developed and will be implemented to enhance the front-end capabilities.

Significance to Biomedical Research and the Program of the Institute: The front-end is an integral part of the National Data Bank Projects, which were established to collect and maintain medical data for both patient management and clinical research.

Proposed Course of the Project: This project will continue throughout the Main Phase Stroke and Traumatic Coma Projects.

CONTRACT NARRATIVE
Office of Biometry and Field Studies, IRP, NINCDS
Fiscal Year 1984

UNITED STATES BUREAU OF THE CENSUS (YO1-NS-7-0031)
UNIVERSITY OF MISSISSIPPI (NO1-NS-7-2357)

Title: Survey of Major Neurological Disorders in Copiah County, Mississippi (Copiah County Study)

Contractor's Project Director: Mr. Robert W. Mangold (Bureau of the Census); Dr. Armin F. Haerer (University of Mississippi)

Current Annual Level: $0 (Bureau of the Census); $0 (University of Mississippi)

Objectives: The primary objective of the project is to establish the prevalence of major neurological and developmental disorders (cerebrovascular disease, convulsive disorders, cerebral palsy, psychomotor delay, Parkinson's disease, essential tremor, and dementia) in a well-defined population of southern blacks and whites. A secondary objective is to evaluate certain screening questions for possible use in other morbidity surveys.

Major Findings: The background information and methods employed in the study have been published. Prevalence of essential tremor and cerebral palsy, noting racial differences, have been published also. Manuscripts on dementia and Parkinson's disease have been submitted for publication. Work is in progress on the following: cerebrovascular disease, convulsive disorders, and psychomotor delay.

Significance to the NINCDS Program and Biomedical Research: The Copiah County Study, a survey of major neurological disorders, was prompted by a need for prevalence data that emphasized comparisons between blacks and whites. The study population was geographically defined. It consisted of all residents of Copiah County (as of January 1, 1978), including individuals living in institutions--viz., nursing homes and state hospitals. There are four characteristics that combine to make the data extraordinarily valuable. First, the same methods were used for blacks and whites in the study, thereby eliminating a major source of confounding. Second, in identifying potential cases for enumeration, there was no requirement that individuals must have previously entered the health-care system for the conditions of interest. The racial comparisons, therefore, were not obscured by the question of racial differences in access to health care. Third, senior, board-certified neurologists evaluated each individual suspected of having a condition of interest. In establishing diagnoses, the neurologists performed examinations and made use of other pertinent records and information. Fourth, participation in the study was unusually high for an American survey of this type: over 97% of households responded to a preliminary screening, and of individuals thought to have a condition of interest, 85% were examined during the study. Because of other sources of information, only a small portion of the remaining 15% had insufficient information to fulfill the diagnostic criteria used in the investigation.

Proposed Course of the Project: The field operations have been completed. The data were processed by the Bureau of the Census. Staff of NINCDS, with the assistance of the Project Director from the University of Mississippi, are now analyzing the data and preparing scientific reports. The contract portion of this project has been completed. Research studies investigating disease prevalence will continue and be reported as an individual research project. To date, the following reports have appeared:

1. Anderson, D.W., Schoenberg, B.S., and Haerer, A.F.: Racial differentials in the prevalence of major neurological disorders: Background and methods of the Copiah County Study. Neuroepidemiology 1: 17-30, Jan. 1982.

2. Haerer, A.F., Anderson, D.W., and Schoenberg, B.S.: Prevalence of essential tremor: Results from the Copiah County Study. Arch. Neurol. 39: 750-751, Dec. 1982.

3. Haerer, A.F., Anderson, D.W., and Schoenberg, B.S.: Prevalence of cerebral palsy in the biracial population of Copiah County, Mississippi. Dev. Med. Child Neurol. 26: 195-199, April 1984.

CONTRACT NARRATIVE
Office of Biometry and Field Studies, IRP, NINCDS
Fiscal Year 1984

NATIONAL INSTITUTE OF MENTAL HEALTH (1Y01-0-0004-00)

Title: ECA Dementia Supplement

Contractor's Project Director: William Eton, Ph.D.

Current Annual Level: $ 0

Objectives: The study will identify a group of demented individuals who are non-institutionalized and the type of dementia will be ascertained by means of a medical examination. An estimate of the social and economic costs will also be generated.

Major Findings: None. The data are still being collected.

Significance to the NINCDS Program: As the population of this nation ages, the dementias will become an increasing medical problem. There are currently no reliable data on the cost of these disorders and this information is needed to assist in future health planning efforts.

Proposed Course of the Project: This project is an add-on to an existing NIMH program of mental health surveys. After an initial screening for cognitive disability, the subjects who are disabled will be given a medical examination. A close relative or friend of those with verified dementias will be used to help establish the history of the disease and estimate the social and economic costs to the affected individual and their friends or relatives. This contract has been terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02114-11 OBFS

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Etiology and Natural History of Convulsive Disorders and Cerebral Palsy*

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Jonas H. Ellenberg	Deputy Chief	OBFS, OD, NINCDS
Others:	Karin B. Nelson	Chief, Cerebral Palsy and Other Disorders Section	DNB, CDNDP, NINCDS
	Deborah Hirtz	Pediatric Neurologist	DNB, CDNDP, NINCDS

COOPERATING UNITS *(if any)*
Cerebral Palsy and Other Motor Disorders Section, DNB, NDP, NINCDS

LAB/BRANCH
Office of Biometry and Field Studies

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.6	0.4

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This study examines the relationship between perinatal and early postnatal factors and the occurrence of seizure disorders and cerebral palsy in childhood. The project derives from the data of the Collaborative Perinatal Project, a large prospectively-followed population (approximately 60,000 mothers, with their children followed to seven years of age). The univariate screen of maternal, obstetric and pediatric risk factors, and demographic analysis have been completed. Multivariate assessment of the data bank has been substantially completed, including correlation and regression analyses. Final manuscripts in each area are in progress, including pre and postnatal predictors of both disorders.

*[This study is the OBFS/NINCDS portion of a larger study entitled: Convulsive Disorders Data Analysis Group, and Cerebral Palsy Data Analysis Group. The Principal Investigator for these studies is Dr. Karin B. Nelson, Chief, Cerebral Palsy and Other Motor Disorders Section, DNB, CDNDP, NINCDS.]

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02312-08 OBFS

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Maternal Infection Study*

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Jonas H. Ellenberg	Deputy Chief	OBFS, OD, NINCDS
Other:	John L. Sever	Chief	IDB, IR, NINCDS
	Alan Talbert	Mathematical Statistician	OBFS, OD, NINCDS
	Martha Griswold	Statistician	OBFS, OD, NINCDS
	Anita Ley	Microbiologist	IDB, IR, NINCDS
	Dorothy Edmonds	Clinical Nurse	IDB, IR, NINCDS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Office of Biometry and Field Studies

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.10	0.10	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Analysis of the Collaborative Perinatal Project (CPP) data continues in the area of maternal infection. (The CPP is a prospective study of approximately 60,000 gravidae and the follow-up of their children through the seventh year of life.) The relationship of maternal infection during pregnancy with the later status of the child is being examined using both clinical and serologically-confirmed infections in the mother.

*[This study is the OBFS/NINCDS portion of a larger study entitled: Perinatal Infections Causing Damage to the Child - Collaborative Perinatal Project, Z01 NS 00402-28 ID. The principal investigator on the overall study is Dr. John L. Sever, Chief, IDB, IR, NINCDS.]

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02497-04 OBFS

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 charecters or less. Title must fit on one line between the borders.)*

Indo-U.S. Study of Head Injury

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	William Weiss	Chief	OBFS, OD, NINCDS
Other:	Cynthia R. Gross	Biostatistician	OBFS, OD, NINCDS

COOPERATING UNITS *(if any)*

University of VA Dept. of Neurosurgery, Charlottesville, VA
All-India Institute of Medical Science, New Delhi, India

LAB/BRANCH

Office of Biometry and Field Studies

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Office of the Chief

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.15	0.10	0.05

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the spece provided.)*

Information on head-injured persons has been collected in independent research efforts in Charlottesville, Virginia, and in New Delhi, India. A preliminary review of these data collection efforts has indicated significant overlap in the type of information collected. A preliminary analysis of the collected data is proposed to identify differences and similarities between these head-injured populations, and to determine the feasibility of prospective cooperative association for the study of head injuries.

The Government of India has approved the research proposal and has allocated 767,000 rupees for the three-year Indian portion of the collaborative study. The proposal has been peer-reviewed by NIH, and approval has been given to proceed with the pilot phase of the study.

A staff professional from OBFS has travelled to New Delhi to examine the quality of the data, and has returned with the Indian data to be processed and analyzed together with the UVA data.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02504-04 OBFS

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Epidemiological Study of Pain

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: Ta-Chuan Chen Mathematical Statistician OBFS, OD, NINCDS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Office of Biometry and Field Studies

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this project is to evaluate the overall and age-specific incidence rates of various chronic pain syndromes, and to investigate the relationship between occurrences of these pain conditions with various epidemiological factors. The incidence rates of disabling and/or severe headache were evaluated with data obtained from a Mid-West non-clinical population survey. The use of incidence and prevalence rates in the estimation of length of illness due to headache has been examined. A report of the results of this study has been prepared.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02505-04 OBFS

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Headache in Pregnant Women

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Ta-Chuan Chen	Mathematical Statistician	OBFS, OD, NINCDS
Other:	Karin Nelson	Chief, Cerebral Palsy and Other Motor Disorders Section	DNB, CDNDP, NINCDS
	Sylvia Edelstein	Systems Analyst	OBFS, OD, NINCDS

COOPERATING UNITS *(if any)*

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Office of Biometry and Field Studies

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.4	0.2	0.2

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project investigates the relationship between migraine headache and pregnancy based on the data collected from a large group of women in pregnancy - the Collaborative Perinatal Project gravidae. Subgroups of pregnant women characterized by the absence and presence of migraine and other recurrent headaches prior to or during pregnancy, are identified. Characteristics of these subgroups are investigated on a variety of demographic, sociological, medical and obstetric factors, and the association of headache with other disorders. Seven data files were created bearing information of migraine history, use of headache medications, and frequencies of headache during pregnancy. Preliminary results showed pregnant women with a migraine history had more other symptoms and illnesses than women without a migraine history. Children of mothers with a history of migraines appear to have higher incidence of seizures than children born to mothers in the nonmigraine group. More intensive statistical analyses will be carried out to examine the apparent associations.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02506-04 OBFS

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Antibody Titers in Macacas on Cayo Santiago

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	William Weiss	Chief	OBFS, OD, NINCDS
Others:	William T. London	Chief, Experimental Pathology Section	IDB, IR, NINCDS

COOPERATING UNITS *(if any)*
Infectious Diseases Branch, IR, NINCDS

LAB/BRANCH
Office of Biometry and Field Studies

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland, 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.05	0.05	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project will provide a test of four antigens on adult and juvenile Macacas on Cayo Santiago, Puerto Rico. The initial statistical problem was to determine the percent of postive antibody titers that could be determined from the adult sample for whom blood sera are presently available, and the number of juveniles that should be sampled. The entire monkey colony on Cayo Santiago will have been trapped and bled by January of 1985, and the serological analysis for the four antigens completed by late 1985.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: ZO1 NS 02517-03 OBFS

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Statistical Methodology for the Measurement of Pain

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: Ta-Chuan Chen Mathematical Statistician OBFS, OD, NINCDS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Office of Biometry and Field Studies

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project investigates the statistical problems involved in the measurement of experimental and clinical pain. (1) A study has been conducted to investigate the statistical technique used in deriving psychophysical measurements of pain. A report has been prepared for this work dealing with the interrelationship of sensory-decision-theory measures such as d' and β and nonparametrical measurement indices, such as p(A), Hodo's percent bias and MacNicol's index of response bias, β. The investigation of this part of the work is completed. The report of this study is going to be presented at the 4th World Congress on Pain in 1984. (2) A study of statistical quantification of the temporal characteristics of persistent, episodic pain such as migraine headache is currently being developed. A group of measurements for this type of pain has been selected for investigation.

An external committee has reviewed the current state-of-the-art of the methodology for the measurement of pain. This meeting recommended that a full-scale symposium be supported to discuss various aspects of pain measurement problems.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02408-06 OBFS

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Epidemiologic Research with Clinical Data Banks*

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Cynthia R. Gross	Biostatistician	OBFS, OD, NINCDS
Others:	Selma C. Kunitz	Chief, CAS	OBFS, OD, NINCDS
	Irene G. Fishman	Statistician	OBFS, OD, NINCDS
	Christine L. Wolf	Programmer	OBFS, OD, NINCDS
	Margaret Meadows	Statistical Assistant	OBFS, OD, NINCDS
	David Shinar	Psychologist	OBFS, OD, NINCDS

COOPERATING UNITS *(if any)*

Depts. of Neurology: B.U. School of Medicine, Michael Reese Hospital, New York Neurological Institute and U.Md. Depts. of Neurosurgery: U.Va, M.C.V., U. Texas at Galveston and U.C.S.D.

LAB/BRANCH

Office of Biometry and Field Studies

SECTION

Computer Applications Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.3	0.2

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Work on determining which epidemiologic approaches are most appropriate for use with clinical data banks was begun in conjunction with the Pilot Stroke and Traumatic Coma Data Bank Networks (NO1-NS-2-2302, 97, 98, 99 OBFS; NO1-NS-3-2339, 40, 41, 42 OBFS;) and is continuing with the full phases of these projects. This project has focused upon quality assurance methods and epidemiological considerations in the data collection, analysis and interpretation of data bank results. Aspects under study include 1) methods for detecting inter-center variation, 2) determining conditions when pooling of results is appropriate, and 3) use of the data bank as a source of cases for case-control studies.

Work initiated in FY 84 included quality assurance studies on the validity of a depression symptoms scale which was developed for epidemiologic surveys, (CES-D) for use with the Stroke Data Bank on the incidence and severity of depression in Stroke Data Bank patients, and a review of coding accuracy in the use of the Abbreviated Injury Scale (A.I.S.) to record multiple trauma to traumatic coma patients. Some work begun under project Z01-NS 02597-02 for the Stroke Data Bank is continuing under this project. Data collection for the validity study of the CES-D depression scale for stroke patients is currently underway. Analysis is planned for FY 85.

*[Formerly "Clinical Data Banks as a Resource for Epidemiologic Research"]

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02443-05 OBFS

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 charecters or less. Title must fit on one line between the borders.)*

Development of Offline Data Entry System for Stroke and Coma Projects

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Barbara Nichols	Computer Specialist	OBFS, OD, NINCDS
Others:	Christine Wolf	Programmer	OBFS, OD, NINCDS

COOPERATING UNITS *(if any)*

RLR & Associates, Inc., Fairfax, VA

LAB/BRANCH

Office of Biometry and Field Studies

SECTION

Computer Applications Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.7	0.5	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A "front-end" general purpose software package was developed for the Datapoint terminals in the Data Bank Clinical Centers, which allows data to be entered, edited and stored locally by time and date. The software operates with menu processing, in which a nonprogrammer can choose the options for data entry from a list. It produces screen images which replicate the order of data on the data collection record. During data entry, data are edited for valid numeric ranges, alpha-numeric checks, code lists, requested items and special formats such as dates. Prior to data transmission the package provides relational checks for data inconsistencies and produces error messages for the clinical centers to facilitate correction. A cost-efficient communication discipline has been added to insure the accuracy of data transmission. Patient management reports were designed and are now being implemented to serve as tools for patient care at the Data Bank Centers.

The front-end support team provides all user documentation and is available on a daily basis for any assistance needed by the clinical centers. Since the Data Banks are dynamic, changes in data collection procedures require concomitant modification in the front-end software, and this work is proceeding.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02493-04 OBFS

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 charecters or less. Title must fit on one line between the borders.)*

Stroke Diagnosis: The Pilot NINCDS Data Bank Algorithm

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Selma Kunitz	Chief, Computer Applications Section	OBFS, OD, NINCDS
Others:	Cynthia R. Gross	Biostatistician	OBFS, OD, NINCDS
	James M. Dambrosia	Chief, Mathematical Statistics Section	OBFS, OD, NINCDS

COOPERATING UNITS *(if any)*

Departments of Neurology: Boston University, University of South Alabama, University of Maryland and Duke University

LAB/BRANCH

Office of Biometry and Field Studies

SECTION

Computer Applications Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.15	0.15	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In conjunction with the NINCDS Pilot Stroke Data Bank Network (NO1-NS-3-2306, 7, 8, 9, -OBFS) a diagnostic classification schema for strokes was devised which consisted of cerebral pathology, vascular pathology, location, diagnostic source and diagnostic role. Approximately 1,100 stroke patients have been classified by this algorithm and the results have been analyzed. A manuscript describing the pilot Data Bank and explaining the algorithm was published in Stroke in 1984. This pilot project has been completed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02498-04 OBFS

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Observer Agreement Studies*

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Cynthia Gross	Biostatistician	OBFS, OD, NINCDS
Others:	Selma C. Kunitz	Chief, CAS	OBFS, OD, NINCDS
	Irene G. Fishman	Statistician	OBFS, OD, NINCDS
	Karlin I. Richardson	Programmer	OBFS, OD, NINCDS
	Christine L. Wolf	Programmer	OBFS, OD, NINCDS
	Margaret A. Meadows	Statistical Assistant	OBFS, OD, NINCDS
	David Shinar	Psychologist	OBFS, OD, NINCDS

COOPERATING UNITS *(if any)*

Depts. of Neurology: B.U. School of Medicine, Michael Reese Hospital, N.Y. Neurological Institute, U.MD School of Medicine. Depts. of Neurosurgery: U.Va., M.C.V., U.T. at Galveston and U.C.S.D.

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Office of Biometry and Field Studies

SECTION

Computer Applications Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.4	0.1

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

To demonstrate that data from the Stroke and Traumatic Coma Data Banks are reliable, studies of inter-observer agreement have been implemented. These studies include a pilot study of agreement among CT Scan readers in the pilot Traumatic Coma Data Bank (NO1-NS-3-2306,7,8,9,OBFS), and studies of variations in neurological examination, diagnosis and CT scan reading for the Stroke Data Bank (NO1-NS-2-2302,97,98,99). Two studies have been analyzed to date using Kappa statistics.

Kappa statistics are often applied to the analysis of this type of study, yet the restrictive assumptions of this method are rarely met. An extension of the usual methods, to allow for a fixed, not random, set of raters is being developed, based upon the work of Davies and Fleiss (Biometrics, 1982), and guidelines for sample size (number of raters, number of subjects to be rated) are being sought for polychotomous, categorical data.

Work begun under project Z01-NS-02597-02 on interobserver agreement studies is being continued under this project.

*[Formerly "CT Scan Observer Variability Study"]

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02500-04 OBFS

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Polymyositis/Dermatomyositis Study

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Irene G. Fishman	Statistician	OBFS, OD, NINCDS

COOPERATING UNITS *(if any)*
Neurological Center of the Pennsylvania Hospital (Christopher Clark)

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Office of Biometry and Field Studies

SECTION
Computer Applications Section

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☒ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The low incidence of myositis and its chronic course necessitate collaboration of a number of investigators. The project involves consultation by OBFS staff to a group of neurologists who are discussing the collection of clinical information on myositis patients. An initial set of data items for collection has been proposed, and forms were designed to enter data on demographic information, initial evaluation, and subsequent follow-up. These forms were distributed to interested researchers, and refinements were made incorporating experience with their use. OBFS staff is acting in a consultative role to this extramural group of investigators.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02502-04 OBFS

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Medical Studies Database System

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Karlin Richardson	Systems Programmer	OBFS, OD, NINCDS
Others:	Sylvia Edelstein	Systems Analyst	OBFS, OD, NINCDS
	Kenneth Elsner	Systems Analyst	OBFS, OD, NINCDS
	Young Jack Lee	Mathematical Statistician	OBFS, OD, NINCDS
	Selma Kunitz	Chief, Computer Applications Section	OBFS, OD, NINCDS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Office of Biometry and Field Studies

SECTION
Computer Applications Section

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of the Medical Studies Database System (MSDS) is to provide a computerized system that facilitates data handling functions with a high degree of automation that minimizes data collection errors and computer programming, and provides forms-tracking, data updating with automatic audit-trail and user-friendly data retrieval. The methodology involves:

1) Entry of medical data from data collection forms onto Hewlett Packard 2647A Intelligent Terminal screens which mirror the data collection forms;

2) The transfer of the data to a database management system (DBMS), Hewlett Packard's Image, on an HP-1000 minicomputer under the RTE 6/VM operating system;

3) A forms-tracking system which records the validity status of the data;

4) Dictionary driven range and relational validity checks;

5) Easy-to-use time-oriented subsetting and retrieval utilities;

6) Terminal emulation for communication with other computers.

All of the above aspects of the system have been completed except dictionary-driven relational validity checks and terminal emulation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02516-03 OBFS

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Traumatic Coma: Epidemiological Characteristics

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Cynthia R. Gross	Biostatistician	OBFS, OD, NINCDS
Others:	Selma C. Kunitz	Chief, Computer Applications Section	OBFS, OD, NINCDS
	Eve K. Moscicki	Scientist	OBFS, OD, NINCDS
	Christine Wolf	Programmer	OBFS, OD, NINCDS

COOPERATING UNITS *(if any)*

Consultant (Rene K. Kozloff)

LAB/BRANCH

Office of Biometry and Field Studies

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Computer Applications Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.25	0.20	0.05

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The pilot Traumatic Coma Data Bank (NO1-NS-9-2306,7,8,9) collected information on 581 patients with severe head injuries, drawn from six centers in the United States. These data are being analyzed to identify patterns of injury and type of accident as they vary from center to center, by patient demographic characteristics, season and time of day. By profiling the characteristics of the 58 children in the data bank, it was found that pedestrian accidents (i.e., children who were struck by motor vehicles) were the most frequent cause of injury and that falls were most common among infants and toddlers. The case frequency sex ratio varied with age, being 2:1 (male excess) in children, almost 4:1 in the middle ages, and about 1:1 in the 60-and-older age group. Case fatality rates differed by age, but not by sex. Data analysis for a study of quality of life outcome of pediatric head injury patients is in progress.

A study which will shortly be submitted for publication focused on the age groups 15-24 years. The typical head injury victim was a young man between the ages of 15 and 24. Sex differences between injury victims in this age group include differences in mechanism of injury, role (driver, occupant, pedestrian) of the injured person and in alcohol use at the time of accident.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02595-02 OBFS

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Methodological Aspects of Data Banks

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Irene G. Fishman	Statistician	OBFS, OD, NINCDS
Other:	Selma C. Kunitz	Chief, Computer Applications Section	

COOPERATING UNITS *(if any)*

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Computer Applications Section

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NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Data Banks have been developed in Stroke and Traumatic Coma. Organizing the principles for establishing a data bank which describes a neurologic condition requires proposing and testing entirely new concepts of data management. This study analyzes the underlying organizational and methodological principles which are necessary for optimal functioning of a data bank.

The methodology employed by the data banks includes innovative techniques, such as interactive, on-site data entry, and local edit checking of data. Since the data banks consist of multiple clinical centers, which collaborate and pool data, stringent techniques are required to ensure consistent data collection. A data base management system is necessary to handle the hundreds of question parameters involved. Analysis of organizational principles is continuing. Information on this methodology is being disseminated by presentations at seminars, meetings and conferences. A Stroke Data Bank Workshop was sponsored at the Stroke Council of the American Heart Association in 1984.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02596-02 OBFS

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Data Bank Maintenance Center

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Barbara Nichols	Computer Specialist	OBFS, OD, NINCDS
Others:	Christine Wolf	Programmer	OBFS, OD, NINCDS

COOPERATING UNITS *(if any)*

Beth Israel Hospital, Computer Medicine Lab, Boston, Massachusetts

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NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.7	0.5	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Data Base Maintenance Center (DBMC) stores and maintains clinical data for the Stroke and Traumatic Coma Data Banks. The objectives of the Data Bank Projects are to efficiently collect, store, retrieve and manage clinical data in order to carry out research on cerebrovascular disease and to identify the course of traumatic coma and patterns of survival and recovery.

The DBMC receives the data via nighttime transmission from the data bank clinical centers. The maintenance center provides an existing, flexible computer system with a set of programs that examines trends and relationships among data items. Descriptive statistical programs such as frequency counts, scatter plots and cross-tabulations are provided as part of the software package. In addition, the DBMC provides utility programs for creation of files to interface with standard statistical packages such as the Statistical Analysis System (SAS) and the Statistical Package for the Social Sciences (SPSS).

The DBMC provides all program documentation and site training at the clinical centers on the use of the system and has staff available on a daily basis for any assistance needed by the clinical centers or OBFS.

OBFS staff direct the activities of the DBMC by setting priorities, over seeing work accomplishments, and consulting on future direction of effort. OBFS staff interact frequently with the DBMC. This project is continuing.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02597-02 OBFS

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Reliability and Validity of Data Collection Methodology in the Stroke Data Bank

PRINCIPAL INVESTIGATOR *(List other professionel personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	David Shinar	Psychologist	OBFS, OD, NINCDS
Others:	Cynthia R. Gross	Biostatistician	OBFS, OD, NINCDS
	Irene G. Fishman	Statistician	OBFS, OD, NINCDS
	Selma C. Kunitz	Chief, Computer Applications Section	OBFS, OD, NINCDS

COOPERATING UNITS *(if any)*
Depts. of Neurology in BU School of Medicine, Michael Reese Hospital, Univ. of Md. Hospital, and Univ. of So. Ala. College of Medicine, and the Dept. of Computer Medicine, Beth Israel Hospital, Boston, Mass.

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NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.8	0.7	0.1

CHECK APPROPRIATE BOX(ES)
- [X] (a) Human subjects
- [ ] (b) Human tissues
- [ ] (c) Neither
- [ ] (a1) Minors
- [X] (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Current human factors evaluation of data collection procedures and monitoring used in the pilot study resulted in changes implemented in the Full Phase Stroke Data Bank Study. Human factors studies concentrated on optimization of the man/computer/environment interfaces. Applications involved areas of forms design, formulation of questions, and data collection feedback and monitoring procedures.

The reliability of key data items was assessed and inter-center variability was measured. Criteria for accuracy were determined and used as objectives in data quality. Focus areas included neurological examination, stroke diagnosis, CT Scan and angiography readings. Recommendations for improvements in data collection methodology were made. The reliability assessments also involved inter-observer agreements. A publication describing the first study is to be published in the Archives of Neurology, and examines interobserver variability in the assessment of neurologic histories and examinations.

An algorithm has been developed for stroke diagnosis, which will be used in future validity studies.

Dr. Shinar completed his term as a special expert to OBFS in FY'84. Related work will continue under projects Z01-NS-02408-06 and Z01-NS-02498-04. This project has been completed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02598-02 OBFS

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Complications, Recurrence, and Outcome: Stroke Data Bank

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Cynthia R. Gross Biostatistician OBFS, OD, NINCDS

COOPERATING UNITS *(if any)*

Department of Neurology, Boston U. Medical Center, Boston, Massachusetts

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NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The majority of stroke patients will survive the acute episode; some will recover to their pre-stroke levels of functioning; and others will be disabled to some degree. Complications following stroke may result from the insult itself or be related to diagnostic or therapeutic methods used in stroke management. Complications may prolong a patient's hospital stay and affect his ultimate outcome. Data from the Stroke Data Bank (NO1-NS-2-2302, NO1-NS-2397, 98, 99), a prospective, multicentered, study of hospitalized stroke patients, will be used to profile the complications - prone patient. Socio-demographic and clinical data, including age, sex, location and type of stroke, severity and type of initial deficit(s) will be compared with occurrence of complications such as seizures, visceral bleeding and stroke recurrence to characterize those patients who experience complications, as well as to contrast their course with a similar group of stroke patients who differ in that they do not have complications. The clinical course of those patients with complications are being studied in order to determine the impact of complications on outcome. Data collection began in June 1983, and as of July 1984, over 700 cases were enrolled. Data analysis is scheduled to begin in FY 85.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02599-02 OBFS

PERIOD COVERED

October 1, 1983 through September 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Behavioral Factors Influencing Recovery from Stroke

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Selma C. Kunitz Chief, Computer Applications Section OBFS, OD, NINCDS

COOPERATING UNITS *(if any)*

Boston University (Philip Wolf); University of Maryland (Tom Price); Michael Reese Medical Center (Lou Caplan); University of South Alabama (Jay Mohr)

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NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.20	0.20	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In order to better comprehend the factors influencing recovery from Stroke, behavioral factors will be studied, utilizing the Stroke Data Bank population (N01-NS-2-2302, N01-NS-2-2397,98,99 OBFS). Specifically, two dimensions of social support will be looked at with respect to stroke outcome. The two dimensions are source, (family and institutional) and type, (affective and instrumental). Patients are stratified by stroke severity. Definition of outcome will include Activities of Daily Living (ADL) and social functioning. Data collection for this project began in July 1983. As of July 1984, data were entered on over 700 patients. Data analysis is in progress.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02411-06 OBFS

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Survey of Practice in the Management of Febrile Seizures

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Young Jack Lee	Mathematical Statistician	OBFS, OD, NINCDS
Others:	Jonas H. Ellenberg	Deputy Chief	OBFS, OD, NINCDS
	Deborah G. Hirtz	Pediatric Neurologist	DNB, CDNDP, NINCDS
	Karin B. Nelson	Chief, Cerebral Palsy and Other Motor Disorders Section	DNB, CDNDP, NINCDS

COOPERATING UNITS *(if any)*

Cerebral Palsy and Other Motor Disorders Section, DNB, CDNDP, NINCDS

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Office of Biometry and Field Studies

SECTION

Mathematical Statistics Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.30	0.15	0.15

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A survey of clinical practice in the management of febrile seizures is ongoing. The survey questionnaire was sent to 10,000 physicians. The data from the questionnaires have been entered into the DCRT/NIH computer. The analysis of the survey data is in progress and will determine which medical disciplines treat children with febrile seizures, the criteria physicians use to determine therapy, the regimens prescribed and the specific goals of therapy. The data have been edited, and the data analysis files have been created; analyses and reports will be completed in FY'85.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02415-06 OBFS

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 charecters or less. Title must fit on one line between the borders.)*
Cage Standards for Primates

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	James M. Dambrosia	Chief, Mathematical Statistics Section	OBFS, OD, NINCDS
Others:	William T. London	Chief, Experimental Pathology Section	IDB, IR, NINCDS

COOPERATING UNITS *(if any)*
Infectious Diseases Branch. IR, NINCDS

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Office of Biometry and Field Studies

SECTION
Mathematical Statistics Section

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.10	0.05	0.05

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Present cage assignments for primates are based solely on the animals' weight. Variation in shape between species of primates of the same weight indicate that the current weight-based standard may be inappropriate. A large number (410) of primates of four different species have been measured (arms, legs, chest, tail, crown to rump, crown to heel) in order to determine association of and variations in weight as functions of shape measurements. The results of this study provide for the assignment of cages based not only on animal weight, but also on allometric measurements and accounting for species differences. This project has been completed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02444-05 OBFS

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Statistical Coordinating Center for the Phenobarbital Clinical Study*

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Young Jack Lee	Mathematical Statistician	OBFS, OD, NINCDS
Others:	Jonas H. Ellenberg	Deputy Chief	OBFS, OD, NINCDS
	Karin B. Nelson	Chief, Cerebral Palsy and Other Motor Disorders Section	DNB, CDNDP, NINCDS
	Deborah G. Hirtz	Pediatric Neurologist	DNB, CDNDP, NINCDS
	Karlin Richardson	Programmer	OBFS, OD, NINCDS
	Kenneth Elsner	Systems Analyst	OBFS, OD, NINCDS

COOPERATING UNITS *(if any)*
Cerebral Palsy and Other Motor Disorders Section, DNB, CDNDP, NINCDS;
University of Washington

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Mathematical Statistics Section

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☒ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

During this fiscal year, two interim analyses of the phenobarbital clinical study data were performed for evaluation by the Performance and Safety Monitoring Committee. The system programs for monitoring patient status and data tracking have been modified to accommodate the need for more frequent and closer monitoring of the trial's progress. Accumulating data are continually being analyzed for consistency and for deviations from protocol using the OBFS H-P clinical trials computer management system. All edited data are transferred from the H-P to the DCRT, NIH computer where data analysis files are created and maintained.

*[This study supports the DNB/CDNDP/NINCDS contract study entitled: "Behavioral and cognitive side effects of phenobarbital used for prevention of febrile seizure recurrence." The project officer is Dr. Karin B. Nelson, DNB, CDNDP, NINCDS, and the contractor of the study is the University of Washington.]

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02446-05 OBFS

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Parkinson's Disease in Twins*

PRINCIPAL INVESTIGATOR *(List other professionel personnel below the Principal Investigator.) (Name, title, leboratory, and institute affiliation)*

PI:	James M. Dambrosia	Chief, Mathematical Statistics Section	OBFS, OD, NINCDS
Others:	Roswell Eldridge	Medical Officer	NES, IR, NINCDS
	Christopher Ward	Clinical Staff Fellow	ETB, IR, NINCDS

COOPERATING UNITS *(if any)*

Section on Neuroepidemiology, IR, NINCDS;
Experimental Therapeutics Branch, IR, NINCDS

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Office of Biometry and Field Studies

SECTION

Mathematical Statistics Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.20	0.15	0.05

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Twin pairs, discordant with respect to Parkinson's disease, were evaluated for zygosity and the presence of Parkinson's disease. Clinical, laboratory, historical, and psychometric data were obtained for both the proband and the co-twin. Statistical analysis of these matched pairs identified risk factors and examined differences between the probands and co-twins. This study has been completed, and two papers have been published.

*[This study is the OBFS/NINCDS portion of a larger study entitled: "Genetic Epidemiology Studies in MS and Other Multifactorial Neurologic Disorders: (Z01 NS 02167-09 ODIR). The principal investigator on the overall study is Dr. Roswell Eldridge, NES, IR, NINCDS.]

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02482-04 OBFS

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Optimization of Software for PET Scanner*

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Alan J. Talbert	Statistician	OBFS, OD, NINCDS
Others:	Rodney A. Brooks	Physicist	SNB, NINCDS

COOPERATING UNITS *(if any)*
Neuroradiology and Computed Tomography Section, Surgical Neurology Branch, IR, NINCDS

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Mathematical Statistics Section

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NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Image processing software has been modified with additional software, to markedly decrease the computing time required by the NIH PET Scanner. The software which has been written has been tested in regular use in clinical and research applications of the Neuro-PET. The programs have been refined, debugged, optimized, and documented. This project has been completed.

*[This study is the OBFS/NINCDS portion of a larger study entitled: Development of a High Resolution Positron Emission Tomograph. The Principal Investigator is Dr. Rodney Brooks, Neuroradiology and CT Section, SNB, IR, NINCDS.]

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02483-04 OBFS

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Predictive Value of the EEG in Febrile Seizures

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Lawrence V. Rubinstein	Mathematical Statistician	OBFS, OD, NINCDS
Others:	Jonas H. Ellenberg	Deputy Chief	OBFS, OD, NINCDS
	Karin B. Nelson	Chief, Cerebral Palsy and Other Motor Disorders Section	DNB, CDNDP, NINCDS
	Deborah G. Hirtz	Pediatric Neurologist	DNB, CDNDP, NINCDS

COOPERATING UNITS *(if any)*
Cerebral Palsy and Other Motor Disorders Section, DNB, CDNDP, NINCDS;
Pediatric Clinic, University of Skopje, Yugoslavia (Nikola Sofijanov)

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SECTION
Mathematical Statistics Section

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.55	0.20	0.35

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This study will evaluate the significance of the EEG as a predictor for recurrence of seizures in those children who have had a simple febrile convulsion. Outcome with respect to febrile seizure recurrence and afebrile seizure occurrence will be reported. The evolution of the EEG pattern will be described, and patterns will be correlated with the clinical outcome. The clinical study is being carried out in Skopje, Yugoslavia, at the Pediatric Clinic of the University of Skopje.

The study began in FY'82 and will be completed in FY'87. During FY'84 the data management and quality control systems were revised as needed. By the end of FY'84, approximately 500 patients were registered into the study and began the study protocol and follow-up. Data monitoring, editing and file creation are continuing. Statistical analysis of short-term outcomes will begin in FY'85. Accrual is scheduled to terminate during FY'85.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02486-04 OBFS

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Statistical Models of In Vitro Mutagenicity Assays

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Young Jack Lee	Mathematical Statistician	OBFS, OD, NINCDS
Others:	William J. Caspary	Biochemist	NTP, NIEHS

COOPERATING UNITS *(if any)*
National Toxicology Program, National Institute of Environmental Health Sciences

LAB/BRANCH
Office of Biometry and Field Studies

SECTION
Mathematical Statistics Section

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.05	0.05	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Chemically-induced genetic damages of cells (mammalian or submammalian) in vitro are observable by allowing the cells to express their DNA damage and the progenies with locus-specific mutation to be selected and form colonies.

During FY'84, work has been in progress on: (1) acute toxicity, (2) criteria for classifying assay results into the following categories: positive, negative, equivocal and not classifiable, and (3) statistical methods of data analysis. This work has been based on data of 200 chemical compounds. Reports describing the cell mutation assay and the statistical methods for analyzing the Ames mutation assay have been prepared. All scientific reports should be completed in FY'85.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02488-04 OBFS

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Interactive Computer System for Patient Entry and Randomization*

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: Young Jack Lee Mathematical Statistician OBFS, OD, NINCDS

COOPERATING UNITS *(if any)*
Personal Service Contract (Laurie Burch)

LAB/BRANCH
Office of Biometry and Field Studies

SECTION
Mathematical Statistics Section

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.05	0.05	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*
An interactive computer system has been developed. The system utilizes the TSO of the DCRT, NIH computer. The clinical trial operations office registers patients entering a clinical trial, checks the eligibility and performs random allocations of the treatment to eligible patients. The system is being used for the CDNDP phenobarbital clinical trial. This project has been completed.

*[Formerly titled "Interactive Computer System for Patient Entry and Randomization for Clinical Study."]

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02489-04 OBFS

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Evaluation of Communicative Disorders Information by MEDLINE*†

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Young Jack Lee	Mathematical Statistician	OBFS, OD, NINCDS
Others:	Christy Ludlow	Speech Pathologist	CDP, NINCDS
	Barbara Reiner	Expert	CDP, NINCDS
	Sylvia Edelstein	Systems Analyst	OBFS, OD, NINCDS
	Karlin Richardson	Programmer	OBFS, OD, NINCDS

COOPERATING UNITS *(if any)*

Communicative Disorders Program, NINCDS

LAB/BRANCH

Office of Biometry and Field Studies

SECTION

Mathematical Statistics Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.35	0.15	0.20

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Five information centers participated in an evaluation project in which over 900 participants were enrolled and received MEDLINE services. Information was gathered on the participants' characteristics, information needs and practices prior to participation. After training and receiving MEDLINE services, 80% of the participants completed a post-use evaluation questionnaire. The study demonstrated that among specialists in communicative disorders, those involved in research activities used MEDLINE services most frequently, were most satisfied and saw the greatest need for MEDLINE services. Those involved in clinical services saw less of a need for access to bibliographic services.

The study indicated that most participants used it infrequently, one to two times per year, and therefore forgot how to operate it effectively. Only those primarily involved in research used it frequently enough to report an interest in having direct access to MEDLINE services.

Recommendations were made for the NINCDS staff to encourage the development of self supporting direct access user groups within the scientific community in communicative disorders. A paper reporting the findings is in preparation.

*[This study is the OBFS/NINCDS portion of a larger contract study entitled: Evaluation of the Effectiveness of Information Services Provided to Specialists in Communicative Disorders by MEDLINE. The project officer is Dr. Christy Ludlow, CDP, NINCDS. Contract numbers are N01-NS-0-2342, N01-NS-0-2343, N01-NS-0-2344, N01-NS-0-2345 and N01-NS-0-2346.]

†Formerly titled "Evaluation of the effectiveness of information services provided to specialists in communicative disorders by MEDLINE."

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02490-04 OBFS

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Research in Statistics

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	James M. Dambrosia	Chief, Mathematical Statistics Section	OBFS, OD, NINCDS
Others:	Young Jack Lee	Mathematical Statistician	OBFS, OD, NINCDS
	Dallas W. Anderson	Mathematical Statistician	OBFS, OD, NINCDS
	Jonas H. Ellenberg	Deputy Chief	OBFS, OD, NINCDS
	Lawrence V. Rubinstein	Mathematical Statistician	OBFS, OD, NINCDS
	Richard F. Raubertas	Mathematical Statistician	OBFS, OD, NINCDS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Office of Biometry and Field Studies

SECTION
Mathematical Statistics Section

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.65	0.65	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project addresses statistical problems generated from collaboration with scientists in other program areas and general statistical problems of current interest. This project is a continuing activity of the Section on Mathematical Statistics. Work has been published in the following statistical areas: monitoring patient recruitment in clinical studies, sample size determination, tests of trends in categorical data, early stopping of clinical trials, sequential analysis, epidemiologic surveys, robust selection, and modeling of covariate dependence of binary sequences. Other work in progress includes space-time clustering of disease, modeling of residual drug effects, and the influence of missing data on variable selection.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02492-04 OBFS

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Stroke Diagnosis and Prognosis Based on the NINCDS Data Bank

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Lawrence V. Rubinstein	Mathematical Statistician	OBFS, OD, NINCDS
Others:	Selma C. Kunitz	Chief, Computer Applications Section	OBFS, OD, NINCDS

COOPERATING UNITS *(if any)*
Boston University Medical Center; New York Neurological Institute; University of Maryland Hospital; Michael Reese Hospital; Beth Israel Hospital

LAB/BRANCH
Office of Biometry and Field Studies

SECTION
Mathematical Statistics Section

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.35	0.30	0.05

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Pilot Stroke Data Bank project has developed operational diagnostic algorithms for the classification of stroke type. These algorithms are based on the type of laboratory evidence available (CT, angiography, etc.), its findings, and the severity of stroke as measured by the neurologic exam.

In this study, the usefulness of these diagnostic algorithms in differentiating etiology and predicting outcome is evaluated. The use of the algorithms as a supplement to the more traditional diagnoses is also explored, as well as correlations between diagnosis and various factors relating to initial stroke severity, subsequent neurological deficit, stroke recurrence, and mortality. Plans for data analyses have been formulated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02514-03 OBFS

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Review of Techniques for Sampling of Rare Populations

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: Dallas W. Anderson Mathematical Statistician OBFS, OD, NINCDS

COOPERATING UNITS *(if any)*
Institute for Social Research, University of Michigan, Ann Arbor, MI
(Graham Kalton)

LAB/BRANCH
Office of Biometry and Field Studies

SECTION
Surveys and Demographic Studies Section

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.10	0.09	0.01

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Special techniques of sampling are required for surveys of rare characteristics in populations, as ordinary approaches would be impractical. A comprehensive review of the literature has been undertaken. This investigation provides a compilation and assessment of sampling techniques used successfully in population studies of rare characteristics. This assessment has been made in light of the Institute's need for surveys of relatively rare neurological disorders. A paper has been prepared for publication. This project is now completed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02587-02 OBFS

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Utility of Diagnostic Tests in Predicting Stroke Mechanism and Outcome

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Dallas W. Anderson	Mathematical Statistician	OBFS, OD, NINCDS
Others:	Selma C. Kunitz	Chief, Computer Applications Section	OBFS, OD, NINCDS

COOPERATING UNITS *(if any)*
Michael Reese Hospital and Medical Center, Chicago, IL (Louis R. Caplan)

LAB/BRANCH
Office of Biometry and Field Studies

SECTION
Mathematical Statistics Section

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.10	0.10	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A variety of diagnostic tests (including angiography, CT scanning, and noninvasive cardiac and vascular tests) are available for the study of the stroke patient. These tests vary with respect to cost, discomfort, and risk of complication. We propose to investigate the utility of each of these tests in establishing stroke cause. Deciding on stroke cause is essential to planning effective therapy. Furthermore, we will examine the utility of these tests in predicting survival, rate and degree of recovery, and risk of stroke recurrence. We also propose to establish those circumstances in which each test is likely to be helpful and those instances in which the test should be deferred because of low ratio of benefit to either cost or risk of complications. Study designs and analysis plans have been formulated for this project.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02590-02 OBFS

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Evolving Stroke

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: James M. Dambrosia, Chief, Mathematical Statistics Section, OBFS, OD, NINCDS

Others: Selma C. Kunitz, Chief, Computer Applications Section, OBFS, OD, NINCDS

COOPERATING UNITS *(if any)*
University of Maryland; Boston University; Michael Reese Hospital; Columbia University

LAB/BRANCH
Office of Biometry and Field Studies

SECTION
Mathematical Statistics Section

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.15	0.10	0.05

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This study is one of a number of research components of the Stroke Data Bank Project. Based on prospectively collected patient data, a temporal description of stroke in evolution by type and site of lesion will be developed. The patients' clinical status determined by changes in Glasgow Coma Score, hemiparesis score, and stroke severity score identifies those cases that evolve. This study also attempts to identify factors that cause or contribute to stroke evolution. Some of these are: edema, shock, electrolyte imbalance, cardiovascular factors and cognitive problems. The accumulating patient data have been monitored periodically, and the first analyses of data will begin in FY'85.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02591-02 OBFS

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Reye's Syndrome Study

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Young Jack Lee	Mathematical Statistician	OBFS, OD, NINCDS
Others:	Anita Chu	Expert	IDB, IR, NINCDS

COOPERATING UNITS *(if any)*
Infectious Diseases Branch, IR, NINCDS

LAB/BRANCH
Office of Biometry and Field Studies

SECTION
Mathematical Statistics Section

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.15	0.05	0.10

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Infectious Diseases Branch is studying salicylate metabolism, other clinical chemistries and histocompatibility antigens in families with Reye's Syndrome patients who have completely recovered from the syndrome. OBFS was responsible for all statistical components of the study including design, data analysis and statistical modeling of the clinical chemistry data.

Five survivors and their unaffected family members were studied. This study showed significantly higher antibody levels to Influenza A and varicella, further supporting the importance of these viral infections in the etiology of the syndrome. It did not show an association between RS and 1) abnormal salicylate metabolism, 2) abnormal helper to suppressor T cell ratios and lymphocyte stimulation responses, 3) specific HLA type, and 4) permanent neuropsychologic sequelae.

A paper has been submitted for publication, and the study has been completed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02592-02 OBFS

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Central Nervous System Metastases from Lung Cancer*

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Lawrence V. Rubinstein	Mathematical Statistician	OBFS, OD, NINCDS
Others:	Mitchell H. Gail	Medical Statistician	BB, DCCP, NCI
	Steven Piantadosi	Medical Staff Fellow	BB, DCCP, NCI

COOPERATING UNITS *(if any)*
Biometry Branch, DCCP, NCI; Illinois Cancer Council; Mayo Clinic; Seattle Cancer Group; Toronto Cancer Group; UCLA Medical Center

LAB/BRANCH
Office of Biometry and Field Studies

SECTION
Mathematical Statistics Section

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.05	0.05	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Lung Cancer Study Group (LCSG) has determined that central nervous system, in particular brain, metastases account for approximately 25% of the first recurrences in Stage I lung cancer. OBFS is analyzing the LCSG data to determine the relationships of recurrence in the CNS to prognostic factors and the effect of treatment on recurrence. The outcome for patients with CNS metastases will be investigated also. A paper reporting the initial findings of CNS recurrences is in press.

*[In order to accomplish this study OBFS is using the data generated by NCI contract Z01-CP-04260-23B entitled: "Consultation on Clinical Trials."]

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02594-02 OBFS

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Factors Predictive of Reading and Writing Skills in the Congenitally Deaf*

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Richard F. Raubertas	Mathematical Statistician	OBFS, OD, NINCDS
Others:	Christy Ludlow	Speech Pathologist	CDP, NINCDS
	Judith Cooper	Speech Pathologist	CDP, NINCDS

COOPERATING UNITS *(if any)*
Central Institute for the Deaf, St. Louis, MO (Ann Geer);
Gallaudet College, Washington, D.C. (Donald Moores)

LAB/BRANCH
Office of Biometry and Field Studies

SECTION
Mathematical Statistics Section

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.3	0.2	0.1

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project consists of the statistical and data management aspects of this Communicative Disorders Program contract. Tasks include design of data collection and monitoring procedures, and statistical analysis of study data.

The study will examine factors that may be associated with development of reading and writing skills in the congenitally deaf. Study subjects will be three groups of deaf 16- to 17-year-olds, with 65 subjects in each group. Each group will include only subjects who received their preschool language training through one of three approaches: aural-oral, total communication, and American Sign Language. Data will be collected on the audiologic, familial, and educational background of the subjects, and on their present language skills. These data will be examined for their association with present reading and writing skills of the subjects.

*[This project is the OBFS/NINCDS support of the CDP contract study NIH-NINCDS-84-19. The project officer is Dr. Christy Ludlow, CDP/NINCDS.]

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02637-01 OBFS

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Stroke and Trauma Program Phase I-II Studies of Stroke Therapies*

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	James M. Dambrosia	Chief, Mathematical Statistics Section	OBFS, OD, NINCDS
Others:	Richard Raubertas	Mathematical Statistician	OBFS, OD, NINCDS
	Karlin Richardson	Programmer	OBFS, OD, NINCDS

COOPERATING UNITS *(if any)*

Stroke and Trauma Program, NINCDS; University of Pittsburgh; University of S. Alabama; University of Iowa; University of Cincinnati; New York University Medical Center

LAB/BRANCH

Office of Biometry and Field Studies

SECTION

Mathematical Statistics Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.8	0.5	0.3

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project includes all statistical aspects of design, planning, data coordination and management, and analysis for studies of interventional therapies initiated by task orders issued under the aegis of the STP Master Agreement. Currently these studies, each with two clinical centers, are in various stages of operation, i.e., the study of Naloxone in the treatment of acute cerebral infarction is ongoing, the study of the benefits of hypervolemic hemodilution (Dextran-40) for the treatment of stroke-in-evolution is awaiting final FDA clearance, and a study of calcium channel blockers for the treatment of SAH is in the planning and design stages.

*[This project supports the Stroke and Trauma Program contract entitled: Cerebrovascular Clinical Research Master Agreement. The Project Officer is Dr. Michael Walker.]

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02638-01 OBFS

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Survey of Major Neurological Disorders in Copiah County, Mississippi

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Dallas W. Anderson	Mathematical Statistician	OBFS, OD, NINCDS
Others:	Bruce S. Schoenberg	Chief, Neuroepidemiology Branch	IR, NINCDS

COOPERATING UNITS *(if any)*
University of Mississippi Medical Center, Jackson, MS (Armin F. Haerer)

LAB/BRANCH
Office of Biometry and Field Studies

SECTION
Surveys and Demographic Studies Section

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.35	0.25	0.10

CHECK APPROPRIATE BOX(ES)
- ☐ (a) Human subjects
  - ☐ (a1) Minors
  - ☐ (a2) Interviews
- ☐ (b) Human tissues
- ☒ (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The primary objective of the project is to establish the prevalence of major neurological and developmental disorders (cerebrovascular disease, convulsive disorders, cerebral palsy, psychomotor delay, Parkinson's disease, essential tremor, and dementia) in a well-defined population of southern blacks and whites. A secondary objective is to evaluate certain screening questions for possible use in other morbidity surveys.

The background information and methods employed in the study have been published. Prevalence of essential tremor and cerebral palsy, noting racial differences, have been published also. Manuscripts on dementia and Parkinson's disease have been submitted for publication. Work is in progress on the following: cerebrovascular disease, convulsive disorders, and psychomotor delay.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02639-01 OBFS

PERIOD COVERED
March 1, 1984 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Antecedents and Consequences of Premature Rupture of Membranes in Pregnancy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: Richard F. Raubertas Mathematical Statistician OBFS, OD, NINCDS

COOPERATING UNITS *(if any)*
George Washington University Medical Center (John Grossman, Goldie Gross)

LAB/BRANCH
Office of Biometry and Field Studies

SECTION
Mathematical Statistics Section

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project consists of the statistical aspects of a study initiated at the George Washington University Medical Center. Primary tasks include computerization and statistical analysis of study data.

Data have been collected on the mothers and infants involved in about 135 cases of premature rupture of membranes (PROM) seen at the GWU Medical Center. Information available includes demographic variables, some aspects of the mother's medical history, various aspects of the labor and delivery, and the immediate post-delivery course of the mother and infant. Those areas of particular interest are the demographic composition of the PROM patients, the relationship between PROM and maternal infection during pregnancy, and the relationship between length of interval from PROM to delivery and various post-delivery complications. These complications include intraventricular hemorrhage and respiratory distress syndrome in the infant, and infections in both mother and infant.

Information from this study will be used to plan possible clinical trials of medical interventions in PROM.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02404-06 OBFS

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

National Survey of Chronic and Debilitating Headache

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, end institute affiliation)*

PI:	Frederic D. Weinfeld	Chief, Surveys and Demographic Studies Section	OBFS, OD, NINCDS
Others:	Ta-Chuan Chen	Mathematical Statistician	OBFS, OD, NINCDS
	Dallas W. Anderson	Mathematical Statistician	OBFS, OD, NINCDS

COOPERATING UNITS *(if any)*

Nat'l. Center for Health Stat.; California Medical Clinic for Headache; Cleveland Clinic; Diamond Headache Clinic; Headache Research Foundation

LAB/BRANCH

Office of Biometry and Field Studies

SECTION

Surveys and Demographic Studies Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.10	1.00	0.10

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purposes of this study are to collect data on severe headache in order to measure the prevalence and to describe the demographic characteristics of the major types of headache. To this end a survey of the general population has been designed. A survey questionnaire, which includes sections on demography, descriptive headache features, medical information, and history, has been developed. The data will also be used to identify and assess the etiological and environmental factors associated with the major idiopathic headache types.

The study was designed in two parts: a feasibility study and an area survey. The feasibility study has been completed. Telephone interviews have been conducted with the patients from four headache clinics. The questionnaire data have been processed together with information abstracted from the physician records about the headaches. The planning and design of the area survey has been completed; however, the survey has not yet been funded. The first part of this study has been completed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02494-04 OBFS

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Prevalence of Multiple Sclerosis in Colorado

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, leboratory, and institute affiliation)*

PI:	Herbert M. Baum	Demographer	OBFS, OD, NINCDS
Others:	Sandra Calingo	Computer Aide	OBFS, OD, NINCDS

COOPERATING UNITS *(if any)*

The Rocky Mountain Multiple Sclerosis Center, University of Colorado School of Medicine

LAB/BRANCH

Office of Biometry and Field Studies

SECTION

Surveys and Demographic Studies Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.30	0.10	0.20

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Rocky Mountain Multiple Sclerosis Center is one of a few centers devoted solely to the care of patients with multiple sclerosis, and is the only center of its type in the State of Colorado. Using records from the Center, the local chapter of the National Multiple Sclerosis Society, hospital records, and physician records we will attempt to estimate the prevalence of multiple sclerosis for Weld and Larimer Counties.

The computer files have been constructed and duplicate cases identified. An analysis of the data on prevalence was completed and the draft of a journal article was prepared.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02495-04 OBFS

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Analysis of Data From the National Survey of Multiple Sclerosis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigetor.) (Name, title, laboratory, and institute affiliation)*

PI:	Herbert M. Baum	Demographer	OBFS, OD, NINCDS
Others:	Karlin Richardson	Programmer	OBFS, OD, NINCDS
	Sylvia Edelstein	Systems Analyst	OBFS, OD, NINCDS

COOPERATING UNITS *(if any)*

Demyelinating, Atrophic and Dementing Disorders Program, NIH (Emanuel Stadlan); National Analysts (Beth Rothschild); Albert Einstein College of Medicine (Alice Kornblith, Nicholas LaRocca, and Labe Scheinberg)

LAB/BRANCH

Office of Biometry and Field Studies

SECTION

Surveys and Demographic Studies Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.00	0.90	0.10

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The National Multiple Sclerosis Survey (NMSS) was a probability sample of all multiple sclerosis patients in the conterminous United States. The Survey gathered detailed data on the disease, employment, and social history of over 1200 cases. We have analyzed these data, with respect to incidence, prevalence, cost, mobility restriction, factors affecting employment, and symptomatology. An article on mobility restriction was published. The analysis with repect to employment was completed and a journal article submitted. The article on symptomatology has been drafted. No additional analyses or articles are planned.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02515-03 OBFS

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Study of Hearing Disorders Among the Aged

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Eve K. Mościcki	Scientist	OBFS, OD, NINCDS
Others:	Herbert M. Baum	Demographer	OBFS, OD, NINCDS

COOPERATING UNITS *(if any)*

Communicative Disorders Program; National Heart Lung and Blood Institute

LAB/BRANCH

Office of Biometry and Field Studies

SECTION

Surveys and Demographic Studies Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.10	1.00	0.10

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objectives of this project are to describe the prevalence of hearing loss in the Framingham cohort by demographic characteristics, to investigate the relationship between the severity of hearing loss and otologic risk factors, and to examine possible relationships between hearing loss and cardiovascular risk factors and events. Hearing data collected during Cycle 15 of the Framingham Heart Study (1978-1979) have been analyzed to estimate the prevalence of hearing loss among the Framingham cohort. The risk factors that might be associated with hearing loss found in this population have been examined.

Papers on the prevalence of hearing loss by demographic characteristics and on risk factors for hearing loss were presented at various scientific meetings. A journal article that describes the hearing status of the cohort, and examines otologic risk factors has been submitted. This project has been completed and is now terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02585-02 OBFS

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Survey of Rare Neurological Disorders

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Frederic D. Weinfeld	Chief, Surveys and Demographic Studies Section	OBFS, OD, NINCDS
Others:	Dallas W. Anderson	Mathematical Statistician	OBFS, OD, NINCDS
	Young Jack Lee	Mathematical Statistician	OBFS, OD, NINCDS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Office of Biometry and Field Studies

SECTION
Surveys and Demographic Studies Section

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.25	0.25	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this project is to design and conduct a survey which would measure the prevalence and describe the demographic characteristics of persons with rare neurological disorders and identify the clinical factors which are associated with these disorders. Initial plans were made for a relatively inexpensive survey of neurologists to ascertain the prevalence of some of the rare neurological disorders. It was expected that the survey would make use of membership lists of neurological associations. The planning of this survey has been completed. Funding for the project was not approved and the study has been terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02586-02 OBFS

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
An Examination of Multiple Cause of Death Data for Stroke

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Herbert M. Baum Demographer OBFS, OD, NINCDS

COOPERATING UNITS *(if any)*
Center for Population Studies, Duke University

LAB/BRANCH
Office of Biometry and Field Studies

SECTION
Surveys and Demographic Studies Section

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.40	0.30	0.10

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has three main goals. First to determine whether a change in the coding of a stroke on death certificates as underlying versus as an associated cause of death is partially responsible for the large decline in the rates of stroke mortality as calculated from the underlying cause of death. Next, to construct life tables and approximate the impact of eliminating stroke as a cause of death; and lastly, to examine the pattern of multiple causes of death which occur for stroke.

Computer tapes, issued by the National Center for Health Statistics, containing all death certificates in the United States for the period 1968-1978 were used. All certificates where stroke (ICDA-8 Codes 430-438) was listed as either an underlying or associated cause of death were selected for study. The data were then tabulated by age, race, and sex. Life tables were constructed to estimate the change in life expectancy if stroke were eliminated as a cause of death. An examination of disease pairs (underlying and associated) was also undertaken.

During the year an article on "CVD Mortality, 1968-1978; Observations and Implications" was published. Another article investigating the diseases which appear in conjunction with stroke on the death certificate was initiated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02636-01 OBFS

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Classification of Headache Types Based on Symptomatology and Features

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Frederic D. Weinfeld	Chief, Surveys and Demographic Studies Section	OBFS, OD, NINCDS
Others:	Robert Richter	Mathematician	OBFS, OD, NINCDS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Office of Biometry and Field Studies

SECTION
Surveys and Demographic Studies Section

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.10	1.00	0.10

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this study is to investigate the interrelationship between headache symptoms and features and their association with the four major types of headache, and to explore the development of a new classification system of headache based on objective criteria. The data for these analyses were collected in the feasibility study in Project Z01 NS 02404-06. In the feasibility study a detailed survey questionnaire was developed which included sections on demography, descriptive headache features, medical information, and history. Lengthy telephone interviews were conducted with patients from four headache clinics. Preliminary results show that the statistical technique of discriminant analysis can correctly classify most cases of headache into four major types. Factor and cluster analyses will be used to determine objectively, syndromes corresponding to the different headache types and to determine groups of related headache symptoms and features which can be used in an operational classification of headache types.

TAB 14 -- CLINICAL NEUROSCIENCES BRANCH -- (CNB)

ANNUAL REPORT

October 1, 1983 through September 30, 1984

Clinical Neurosciences Branch

National Institute of Neurological and Communicative Disorders and Stroke

Table of Contents

ANNUAL REPORT

October 1, 1983 through September 30, 1984
Clinical Neurosciences Branch
National Institute of Neurological and Communicative Disorders and Stroke

Paul Fedio, Ph.D., Acting Chief

Summary of Program Activity

The Clinical Neurosciences Branch formulates and conducts basic and applied clinical investigations to advance an understanding of normal and altered brain-behavior relations, utilizing electrophysiologic and neuropsychologic procedures, and patients with neurologic and neuropsychiatric disorders.

I. Clinical Diagnostic Service:

The clinical functions include standard electroencephalographic (EEG) consultative services, and computer-derived, evoked potential studies of epilepsy, brain tumors, neuromuscular, neuropsychiatric and developmental metabolic disorders. These diagnostic services are extended primarily to NINCDS and to other Institutes within NIH which are identified in the following table:

	Diagnostic Services			
Referral Sources	EEG	%	Evoked Potential	%
NINCDS	255	24.8	153	49.3
NIMH	160	15.6	9	2.7
NIA	119	11.6	4	1.4
NICHD	62	6.0	9	2.7
NHLBI	20	1.9	4	1.3
NCI	23	2.2	11	3.4
NIAID	30	2.9	19	6.1
NIADDK	34	3.3		
OP	315	30.6	103	33.1
MISC	11	1.1		
TOTAL (1341)	1029	100.0	312	100.0

The actuarial distribution of EEG and evoked potential evaluations indicate that 30% of the referrals were submitted by NINCDS physicians, the remainder, from other NIH sources. Services identified as miscellaneous represent bedside EEG recording in the CCU and electrocorticography (ECG) performed in the neurosurgical suite. Requests for EEG services and evoked potential studies (visual, brainstem auditory and somatosensory) have increased considerably during this reporting period. The latter procedure has proven especially useful in the diagnosis and management of demyelinating and related neurologic diseases.

The Branch also provides clinical opportunities and patient study materials for clinicians who intend to take training in Clinical Electroencephalography. Each year the Clinical Associate trainees become eligible for examination by the American Board of Qualification in EEG.

In addition to the EEG service, a team of neuropsychologists extends clinical consultation to patients in NINCDS and other Institutes. Standard and specialized examinations are performed to provide diagnostic information and to guide rehabilitative management of patients with neurologic and neuropsychiatric disorders. Special studies at preoperative and postoperative intervals have been developed to chart neurosurgical and pharmacological treatments of patients with brain tumors and epilepsy.

II. Research Activities:

Branch members actively conducted several independent research projects during this reporting period, and in addition, collaborated with investigators within NINCDS and other Institutes in electrophysiological and neuropsychological protocols.

Clinical seizure patterns elicited with different epileptic disorders continue to be a primary field of research interest. Branch members have utilized a standard EEG polygraph in tandem with video instruments to develop a unique monitoring system which allows the investigators to simultaneously record ictal, behavioral and EEG events. This procedure has greatly increased the reliability to correlate EEG and specific seizure patterns, and have documented rare electroclinical relations which occur incidentally during routine recordings with epileptic patients.

Analysis of these results showed at least one clinical seizure recorded in 17 (11%) of 159 consecutive outpatients. The referral diagnosis was changed in 13 of the patients as follows: undetermined seizure type was changed to complex partial seizures in 4 patients and to absence seizures in 4 patients, generalized tonic-clonic seizures to pseudoseizures in 2 patients, complex partial seizures to pseudoseizures in 2 patients, and pseudoseizures to complex partial seizures in 1 patient. The presenting diagnosis was confirmed in the remaining 4 patients; the regimen of anticonvulsant medication was changed in 15 patients. Although seizures were recorded in only 11% of the patients during routine EEG recording with video monitoring, the information improved patient management and avoided the need for prolonged and intensive monitoring on an in-patient basis.

In collaboration with the Epilepsy Section, positron emission tomography (PET) with 18flouro2deoxyglucose (FDG) and simultaneous EEG monitoring were conducted with patients with complex partial seizures. This noninvasive procedure provides reliable localization of focal abnormalities and is especially valuable in patients with medically intractable seizures and normal neurological and CT examinations who may be suitable candidates for neurosurgical treatment.

The localizing effect of anti-epileptic medication, Phenobarbital (Phb) and Phenytoin (Pht) on cerebral glucose metabolism was also evaluated. Focal peak glucose metabolic rates from frontal, parietal, and temporal regions were measured before and after Phb (8 patients) and Pht (6 patients) were administered and withdrawn. EEG was monitored continuously during and after FDG injection; scans were performed with eyes patched and ears plugged to reduce artifacts and to provide a uniform level of sensory stimulation. Mean metabolic rate before Phb withdrawal was 8.5 $\pm$ 1.9 mg of glucose per 100 ml tissues per minute, and after withdrawal, 11.0 $\pm$ 3.4 ($p<0.001$). Rates before and after Pht were 6.9 $\pm$ 1.6 and 7.7 $\pm$ 1.2, respectively ($p<0.05$). Patients showed increased activity in the epileptogenic, hypometabolic regions as well as surrounding structures, and in the contralateral hemisphere, after drug withdrawal. The effect appeared to be equal in frontal, parietal, and temporal regions. Thus, Phb, and to a lesser extent, Pht, appear to depress peak cerebral glucose metabolic activity.

In several neuropsychological studies, the role of temporal lobe mechanisms in perception was evaluated in epileptic patients following a unilateral left or right temporal lobectomy. Using tachistoscopic procedures, right temporal surgical patients required a longer exposure duration to detect the presence of a stimulus, but not to discriminate two versus single flashes. Left temporal patients, in contrast, exhibited the reverse pattern. These data suggest that right hemisphere mechanisms are optimally suited for summation of sensory input over time to yield heightened perceptual sensitivity, but at the expense of fine temporal resolution. Left temporal systems are better organized and suited to deal with fine temporal acuity, but at the expense of overall perceptual sensitivy. Additional studies of perceptual thresholds for recognizing material in the left, central and right visual field, indicate that spatial location is less dependent on the integrity of the anterior and medial temporal lobe.

The contribution of limbic structures in regulating emotional behavior was assessed, and revealed a functional dissociation of left and right brain participation. That is, left temporal lobectomy patients tended to neutralize judgements about emotional material, particularly items which were rated as pleasant by normal subjects. In a separate study, patients with left side removal did poorly in choosing approprite verbal descriptions for visually presented sequences of emotional behaviors. The same response bias held in judging photographs of faces displaying different emotional expressions. There appeared to be less disruption as a result of right temporal resection. Moreover, the left and right temporal patients were physiologically unresponsive while viewing affective material, as indexed by skin conductance responsivity. Unlike normal subjects, the temporal lobectomy patients were unable to take advantage of the emotional coloration of information in facilitating subsequent recall.

Relatedly, the left and right temporal patients produced different self-ratings on a behavioral inventory. In comparison with observations drawn from a previous study of nonoperative epileptic patients, the

present patients, following unilateral temporal removal, showed a marked decline in acknowledging behavioral difficulties. Nonetheless, within the context of this general improvement, specific personality profiles persisted and were dependent on the side of removal. The left temporal patients tended to view themselves as ideative, reflective and non-emotional, whereas, right temporal patients tended to view themselves as emotive.

In an effort to assess the value of compensatory strategies to deal with postoperative memory difficulties, the lobectomy patients were instructed in the use of mnemonic and encoding devices. The study confirmed the value of using visual imagery to improve memory; abstract, low imagery material was poorly recalled by left temporal patients. In a separate paradigm which manipulated phonemic, spatial and praxic cues, all groups, particularly the left temporal, did very poorly with phonetic encoding. In contrast, spatial and praxic mnemotechnics proved very beneficial, more so for left temporal patients. This encourages the use of motor or praxic cues as a valuable compensatory technique. The data also confirmed that left temporal mechanisms encode verbal information during initial learning, and that modest compensation for memory defects may be achieved with procedures which combine overt or covert imagery and praxic encoding.

In a collaborative project with the neurosurgical staff at the Brain Research Institute, UCLA, major modifications were developed with the intracarotid Sodium Amobarbital procedure (WADA) in lateralizing cerebral dominance for language functions. Expectedly, pharmacological anesthetization of the left or language-dominant hemisphere produced dysphasia and selective verbal memory losses. In addition, there was an inability to execute a segmented motor task. The improved diagnostic procedure showed that recovery of disrupted behavior was linked with laterality of epileptic focus. Injection of Sodium Amobarbital into the hemisphere, contralateral versus ipsilateral to the lesion produced greater behavioral changes and marked EEG slowing. The data implicate a negative functional effect of an epileptic lesion on the intact, contralateral hemisphere.

Extending these behavioral studies with computer-derived electrophysiological indices (P300 events), procedures were developed to analyze neural components of cognitive or judgmental processes. Following unilateral temporal lobectomy, P300 amplitude was found to be inversely proportional to stimulus probability. With auditory stimuli, P300 activity was essentially identical for both left and right temporal patients. In patients with left temporal surgery, smaller P300s were observed, owing to a negative shift which emerged approximately 90 msec after stimulus onset.

For the visual modality, right temporal patients manifested smaller P300s than left temporal or normal subjects. There were no consistent hemispheric asymmetries which distinguished the left and right temporal patients, suggesting that there is probably more than one neurogenerator of the P300 wave, independent of lateral or mesial temporal structures. Processing of auditory and visual material is at least, to some extent,

lateralized or hemispheric dependent.

P300 activity was also studied in a series of normal children and patients with Turner's syndrome. Wave forms from some of the 18- and 20-year old female patients with Turner's syndrome resembled those of normal children, however, much like younger children or those entering the age of puberty. These results underscore the role of sex hormones in the development of neuropsychological processes.

A detailed analysis of the developmental course of the P300 with normal children revealed a striking change in frontal negative slow wave across the age spectrum. The amplitude and duration of this negative waveform decreased with increasing age and was inversely related to stimulus event probability. Within conditions involving time and judgement, the P300 tended to become more broad and peaked with advancing age. The changes in frontal negative slow wave were consistent with data from other reports, suggesting a maturation of frontal negativity which continues over the entire lifespan, and parenthetically, is altered by presenile dementia.

In an independent study of neuropsychiatric patients with Alzheimer's or Huntington's Disease, pervasive and severe cognitive deficits were the benchmarks of these deteriorative disorders. Specifically, there was marked linguistic impairment in object naming and verbal fluency, except when the stimuli required emotional judgements. Qualitative analysis of the error patterns by Alzheimer patients revealed language disturbances and a loss of knowledge about specific object attributes. Knowledge for broad categorical information was seemingly preserved.

With Alzheimer patients, there was a sharp and progressive decline in memory and learning. However, no qualitative differences in memory processes were noted between demented and age-matched subjects. The patients showed similar patterns but at a reduced level of proficiency. The fundamental memory impairment in Alzheimer's disorder may be related to an inability by patients to effectively encode material. This faulty process results in poor retention regardless of the type of stimulus information. The defect also remains in sharp contrast with that seen with other amnesic populations, for example, Korsakoff and temporal lobectomy patients where the primary difficulty involves an inability to store and/or retrieve new information which is encoded in a normal fashion.

Visual, spatial, and constructional abilities were also examined with neuropsychiatric patients, and it was found that Alzheimer and Huntington patients did poorly. However, the pattern of deficits was different; Huntington patients exhibited relatively greater impairment on tests of spatial judgement (egocentric in comparison with extrapersonal spatial tasks) whereas Alzheimer patients showed the reverse pattern. These findings, viewed in the context of studies of patients with frontal vs parietal lobe lesions, implicate degeneration of frontal striatal mechanisms in Huntington's Disease, and the primary dysfunction in Alzheimer's Disease is associated with atrophy of cortical association regions.

A profile analysis of Alzheimer patients revealed a heterogenous disorder and differential losses. To analyze this hypothesis, data from verbal and nonverbal tests for 43 Alzheimer patients were analyzed. Several different patient groups were identified. One group exhibited marked spatial constructional difficulties and preserved verbal capabilities. A second group was characterized by severely impaired verbal abilities, but with intact perceptual and constructional skills. A third group exhibited relatively uniform deficits in both verbal and visual spatial sectors. In contrast to these different profiles, all patients exhibited memory defects, indicating that memory and learning defects, per se, were poor indicators of group membership.

Positron emission tomographic data from 19 Alzheimer patients with language and perceptual motor deficits were related to bilateral and symmetrical hypometabolic activity in the temporal and parietal cortex. Patients with primarily perceptual and constructional deficits evinced greater hypometabolism of the right temporal and parietal regions, while patients with selective language deficits showed metabolic decrements, primarily in the left temporal region. It is interesting to note that the frontal regions were less affected and did not reliably discriminate the Alzheimer subgroups.

In collaboration with investigators in NICHD, eight symptomatic longterm survivors of acute lymphoblastic leukemia (ALL) who had received CNS preventive therapy (cranial irradiation and intrathecal chemotherapy) were studied. On the basis of CAT scan findings, statistical relations were calculated between radiographic and behavioral abnormalities. In essence, patients with abnormal CAT scans showed impairment in attentional processes. Memory and learning were affected in all patients with abnormal scans, particularly those with evidence of calcification.

Neuropsychological indices of frontal regulatory mechanisms were investigated with children presenting severe and primary obsessive-compulsive symptomatology. In comparison with age and sex-matched normal children, the psychopathologic group experienced increased difficulty with spatial, and learning and memory procedures. Based on the configuration of neuropsychological deficits reported for patients with frontal dysfunction, it was speculated that an imbalance in the inhibitory function of the frontal lobes or frontally connected systems may form the basis of obsessive-compulsive disorders.

The temporal relationship between changes in heart rate and epileptic seizures in amygdala-kindled rats was examined. Heart rate was monitored by electrodes implanted in the shoulder area bilaterally. In 14 rats, a total of 47 seizures was studied, and the cardinal finding was slowing of heart rate which occurred several seconds after amygdala stimulation and was closely associated with clinical seizures, a few seconds before or after onset. The changes in heart rate lasted 4 to 28 seconds and ended before the clinical seizures terminated. Increased heart rate was not observed during the ictal period. These observations suggest that changes

in heart rate were produced by epileptic seizures but not by amygdala stimulation.

Seizure patterns produced by caudate and globus pallidus kindling were also studied in rats. Bipolar electrodes were implanted unilaterally in the amygdala, caudate, or globus pallidus of 28 adult Sprague-Dawley rats. There were no significant differences in the kindling rates between the caudate and globus pallidus animals, but these animals required twice as many stimulations as the amygdala group. Seizures originating from the globus pallidus consisted of initial rotation toward the stimulated side, followed by righting, chewing, forelimb clonus, rearing and falling. The caudate seizure started with opiothotonic posturing, followed by chewing and forelimb clonus. These nuclei are most likely a relay station for seizure propagation.

The effect of neonatal anoxia on kindling was studied in a group of 24 rats. Within 24 hours after birth, the experimental rats were placed in a chamber filled with pure nitrogen for 4 to 8 minutes and then returned to the mothers. Three of 4 rats exposed to 8 minutes of anoxia had respiratory distress after the implantation and only one survived. Bipolar electrodes were implanted in the right amygdala at about 3 months of age. One week later, one daily stimulation was given about the same time every day. The experimental group required an average of 28 stimulations to kindle, whereas the control animals required 19 stimulations. Moreover, the experimental group showed wider variability (SD=26.11) than the control group (SD=7.4). The afterdischarge duration during the last three days of stimulation averaged 60.0 and 62.7 secs respectively, again with greater variability for the experimental group (SD=26.2) than for the control group (SD=11.9). The groups did not differ in kindling rate.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01NS00200-30 CN

PERIOD COVERED: October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Cognitive and Emotional Profile of Neuropsychiatric Disorders

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.)* *(Name, title, laboratory, and institute affiliation)*

PI:	P. Fedio	Psychologist	CN	NINCDS
	A. Martin	Psychologist	CN	NINCDS
Other:	P. Brouwers	Psychologist	CN	NINCDS
	C. Cox	Psychologist	CN	NINCDS
	F. Lalonde	Psychologist	CN	NINCDS
	E. Mohr	Psychologist	CN	NINCDS
	T. Chase	Neurologist	ET	NINCDS

COOPERATING UNITS *(if any)*: Experimental Therapeutics Branch, IRP, NINCDS

LAB/BRANCH: Clinical Neurosciences, IRP, NINCDS

SECTION: Office of the Chief

INSTITUTE AND LOCATION: NINCDS, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.7	1.2	0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A neuropsychological profile of dementia was drafted for individuals with Alzheimer's Disease, Huntington's Disease and 'at risk' for Huntington's Disease. The evaluations extended into memory, learning and perceptual areas, utilizing standard and experimental tasks, also establishing normative references for functional changes accompanying the aging processes.

Although Alzheimer's Disease is accompanied by marked deficits in memory and learning, there were no qualitative differences between demented and age-matched subjects. The impairment also extended to object-naming and fluency, and AD patients performed poorly in perceiving meaning, except when the stimuli required emotional judgement. The data indicate that Alzheimer's patients may be unable to encode material; this is in sharp contrast with other amnesic disorders where the primary difficulty involves an inability to store and/or retrieve information.

Alzheimer's and Huntington's patients showed pronounced but dissimiliar deficits with visuospatial and constructional tasks. The behavioral data extend neuropathologic impressions of degeneration of the frontal striatal system in Huntington's Disease, and cortical involvement in Alzheimer's Disease.

The neuropsychological test profile of Alzheimer's patients yielded different clinical subgroups or populations. Memory and learning deficits, per se, were poor indicators of group membership. One group was characterized by severely impaired verbal abilities, but with intact perceptual and constructional skills. The second group was more impaired on perceptuomotor than verbal tasks. The third group showed comparable deficiencies in both linguistic and visual spatial sectors. Positron emission tomographic and EEG data confirmed corresponding changes in left, right or bilateral regions in the posterior cerebral quadrant, respectively.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01NS01245-19 CN

PERIOD COVERED: October 1, 1983 through September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.): EEG Learning Correlates Using Scalp and Intracranial Depth Electrodes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	P. Fedio	Psychologist	CN	NINCDS
	R. Johnson	Psychologist	CN	NINCDS
Other:	A. Martin	Psychologist	CN	NINCDS
	P. Brouwers	Psychologist	CN	NINCDS
	W. Meyer	Medical Officer	SN	NINCDS

COOPERATING UNITS (if any): Surgical Neurology Branch, NINCDS

LAB/BRANCH: Clinical Neurosciences, IRP, NINCDS

SECTION: Office of the Chief

INSTITUTE AND LOCATION: NINCDS, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Information processing was monitored and quantified by averaged evoked response techniques. The electrographic activity was recorded from left and right brain regions during memory and perception in normal subjects, patients with unilateral temporal lobectomy, and patients with neuropsychiatric disorders. Electroencephalographic disturbances in brain-behavior relations in psychiatric patients were also evaluated, relating left brain dysfunction to ideational disorders, and right brain activity to maladaptive emotional reactions.

In temporal lobectomy patients, P300 amplitude was found to be inversely proportional to stimulus probability in the same way as for normal controls, and larger P300s were elicited in reaction time. For visual material, right temporal patients manifested smaller P300s at frontal sites than either left temporal or normal individuals. Moreover, there were no consistent hemispheric asymmetries which distinguished the left or right temporal patients, or either group from normal subjects. These data discount the hypothesis that medial temporal structures, including the hippocampus, serve as a sole generator of P300. More specifically the data indicate that processing of auditory and visual is dependent to a great extent on the character of the material and the integrity of left and right brain mechanisms.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01NS01424-18 CN

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Behavioral Modulation by the Limbic System in Man

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	P. Fedio	Psychologist	CN	NINCDS
	A. Martin	Psychologist	CN	NINCDS
Other:	P. Brouwers	Psychologist	CN	NINCDS
	C. Cox	Psychologist	CN	NINCDS
	F. Lalonde	Psychologist	CN	NINCDS
	E. Mohr	Psychologist	CN	NINCDS

COOPERATING UNITS *(if any)*
Surgical Neurology Branch, NINCDS
Neuro-Opthalmology Section, Clinical Branch, NEI

LAB/BRANCH
Clinical Neurosciences, IRP, NINCDS

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.1	0.6	0.5

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Emotional and cognitive characteristics were studied in epileptic patients following unilateral left or right temporal lobe resection. The integrity of attentional and perceptual (visual, auditory, and tactile) systems were evaluated using standard and experimental procedures. Physiological events (skin conductance) were monitored and recorded during test performance. The research examined the role of the temporal lobe in establishing specific limbic associations between left and right hemispheres in regulating cognitive functions and emotional experiences in man.

Tachistoscopic studies identified a critical perceptual role for right temporal mechanisms, especially during the early stages of visual processing. The left and right temporal lobes contribute differentially to specifying the identity of a stimulus, but not to its position or orientation in space. Left temporal mechanisms encode verbal information during initial learning. Modest compensation for memory defects following temporal lobectomy may be achieved with strategy which combines overt and covert imagery with praxic encoding.

In affective sectors, left temporal patients tend to neutralize reactions to nuances with emotional coloration; right temporal patients, in contrast, rate these materials similar to normal individuals. Unlike normal individuals, however, the left and right temporal lobectomy patients were hyporesponsive to affective material as indexed by skin conductant measures. Moreover, both lobectomy groups failed to take advantage of the emotional characteristics of information to facilitate memory.

These data suggest that unilateral temporal lobectomy disrupts the normal linkage of cognitive-affective associations mediated by temporal limbic interaction. There were, however, beneficial effects to surgical treatment in that patients, following temporal lobe surgery, were less deviant from normal subjects in emotional behavior.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01NS01658-17 CN

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Hemispheric Development and Specialization of the Intellectual Functions

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	P. Fedio	Psychologist	CN	NINCDS
	P. Brouwers	Psychologist	CN	NINCDS
Other:	A. Martin	Psychologist	CN	NINCDS
	C. Cox	Psychologist	CN	NINCDS
	W. Meyer	Medical Officer	SN	NINCDS

COOPERATING UNITS *(if any)*
Surgical Neurology Branch, IRP, NINCDS

LAB/BRANCH
Clinical Neurosciences, IRP, NINCDS

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.6	0.6	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The disabling effects of chronic cerebral insult and neuropsychiatric disorders were evaluated by a broad range of neuropsychological tests evaluating brain-behavior relations in man.

Asymptomatic long term survivors of acute lymphoblastic leukemia (ALL) who received CNS preventive therapy (cranial irradiation and intrathecal chemotherapy) were studied. Based on CT scan findings, the patients were divided into three groups: normal scans, cortical atrophy; intracerebral calcifications. Memory and learning were significantly impaired in children with abnormal scans, more so for the patients with calcification. In addition, all patients with abnormal CT scans showed significant attentional dysfunctions.

Adolescents with obsessive compulsive features exhibited a cluster of neuropsychological deficits which correlated with ventricular enlargement. Deficits were identified in spatial judgement and spatial learning. It was suggested that an imbalance in the inhibitory functions of the frontal lobe and limbic systems may contribute to obsessive compulsive behavior.

Neuropsychological sequelae of Reye's syndrome were investigated 10 years after illness. Contrary to predictions, initial analysis revealed no adverse cognitive decline; the patients were of normal intelligence and performed well on select procedures.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01NS02269-08 CN

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Visual Evoked Potentials in Clinical Neurology and Neuro-Ophthalmology

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

S. Sato, M.D., Medical Officer, EB, NINCDS

V. Alexander, EEG Technologist, CN, NINCDS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Clinical Neurosciences, IRP, NINCDS

SECTION

Clinical Neurophysiology

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.2	0.3

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

An analysis of the morphology, amplitude and latency of visual evoked potentials to photic flashes and reversing checkerboard pattern is being conducted. Normative data have been collected from normal individuals, predominantly of 20-50 years. Visual evoked responses also have been examined in patients with various neurological disorders. Prolonged latencies of the major positive peak have been noted in patients with multiple sclerosis and neurological disorders. A half visual field stimulation is used to evaluate the retrochiasmatic visual pathway in normals and patients with various neurological disorders.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01NS02431-05 CN

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Experimental Epilepsy: Seizures Produced by Kindling in Rats

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigetor.) (Name, title, laboratory, and institute affiliation)*

S. Yamaguchi, Psychologist, CN, NINCDS
S. Sato, M.D., Medical Officer, EB, NINCDS
S. Walbridge, Laboratory Specialist, CN, NINCDS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Clinical Neurosciences, IRP, NINCDS

SECTION

Clinical Neurophysiology

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.8	0.6	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Seizures produced by chronic stimulation (Kindling) are a good model for human epilepsy. In rat, seizures are produced by daily electrical stimulation of amygdaloid complex complex and other central nervous system sites. In this project, Kindling of the various sites of the central nervous system, interictal epileptiform discharges and their propagation, and effects of sleep-wake cycles and maturation, and hypoxia on the epileptiform discharges are being investigated, and also the effect of kindled seizures on heart rate and respiration.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01NS02432-05 CN

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Brainstem Auditory Evoked Potentials in Clinical Neurology

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
S. Sato, M.D., Medical Officer, ETB, NINCDS
V. Alexander, EEG Technologist, CNB, NINCDS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Clinical Neurosciences, IRP, NINCDS

SECTION
Clinical Neurophysiology

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.2	0.3

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Analysis of the morphology, amplitude and latency of brainstem auditory evoked responses to clicks is being conducted. Normative data have been collected from normal subjects, predominantly of 20-50 years. The test has been carried out in patients with various neurological disorders. Prolonged latencies and distortion of morphology have been observed in patients with Multiple Sclerosis and Spinocerebellar Degeneration. The effect of pharmacological agents on the evoked responses is also being studied.

TAB 15 -- DEVELOPMENTAL METABOLIC NEUROLOGY BRANCH -- (DMNB)

ANNUAL REPORT

October 1, 1983 through September 30, 1984
Developmental and Metabolic Neurology Branch
National Institute of Neurological and Communicative Disorders and Stroke

Table of Contents

ANNUAL REPORT

October 1, 1983 through September 30, 1984
Developmental and Metabolic Neurology Branch, IRP
National Institute of Neurological and Communicative Disorders and Stroke

Roscoe O. Brady, Chief

The principal activities of the Branch concern the following areas of investigation: 1. Metabolism of complex lipids and mucopolysaccharides in normal and pathologic states. 2. Enzyme replacement therapy for the treatment of patients with hereditary metabolic disorders. 3. Development of cellular and animal models of human metabolic disorders. 4. Molecular basis of human lysosomal storage disorders. 5. Transmembrane signalling mechanisms and the role of glycolipids and glycoproteins in this process. 6. The involvement of myelin glycolipids and glycoproteins in the development of the nervous system, autoimmune pathologic phenomena, and demyelinating diseases.

I. HEREDITARY METABOLIC DISORDERS

A. International Conference on the Molecular Basis of Lysosomal Storage Disorders.

An extraordinarily successful symposium on the molecular basis of lysosomal storage disorders organized by DMNB and sponsored by NINCDS was held in Bethesda from September 12-14, 1983. Nobel Laureate Christian de Duve was the lead-off speaker and he set the stage for presentations and discussions regarding the state of the art in the field. Studies on the molecular biology of enzymes involved in the degradation of sphingolipids, mucopolysaccharides, and glycogen were presented along with preliminary indications of molecular pathology in certain lysosomal storage disorders. Evidence for the cloning of the genes for lysosomal enzymes was reported for the first time. A book emanating from the presentations will be published shortly by Academic Press.

B. Enzyme Replacement Therapy for Sphingolipid Storage Disorders.

The structures of the N-asparagine linked oligosaccharides of glucocerebrosidase, the enzyme involved in Gaucher's disease, have been determined in detail. We have developed procedures to modify and obtain large amounts of this enzyme specifically targeted to cells of the monocyte/macrophage system where the accumulating glucocerebroside is stored in patients with this disorder. A clinical trial of enzyme replacement using the modified enzyme has been initiated.

C. Tissue Culture Models of Human Lysosomal Storage Disorders.

Significant progress has been made in developing a model of Gaucher's disease in tissue culture through the use of conduritol-β-epoxide, a highly potent inhibitor of glucocerebrosidase, in human macrophage cultures. This system should prove extraordinarily helpful for determining the specific lectins on the surface of these cells that are involved in the endocytosis of

glucocerebrosidase. Use of this model for enzyme replacement investigations should markedly accelerate and improve our ability to deliver therapeutically effective quantities of glucocerebrosidase to patients with Gaucher's disease.

D. Animal Models of Human Lysosomal Storage Disorders

We have made significant progress in understanding the metabolic defect in the BALB/c mouse mutant that resembles Type C Niemann-Pick disease in humans. The depression of sphingomyelinase and glucocerebrosidase activities in these mice probably is a consequence of improper cholesterol metabolism. We have demonstrated that a basic metabolic defect in these mice is impaired esterification of exogenous cholesterol. Current research is directed toward obtaining an understanding of the biochemical basis of this defect and to elucidate the effects of membrane-bound cholesterol on the activity of lysosomal enzymes.

E. Molecular Genetics of Gaucher's Disease

The gene for glucocerebrosidase has been cloned in the section on Molecular and Medical Genetics. This is a major accomplishment concerning (1) the acquisition of knowledge of the molecular pathology in Gaucher's disease; (2) the possibility of producing glucocerebrosidase by recombinant DNA technology, and (3) the development of new diagnostic procedures involving DNA restriction fragment length polymorphisms in patients and carriers of this disorder. Future applications include considerations of possibilities of gene engineering or replacement.

II. MEMBRANE RECEPTORS FOR ENVIRONMENTAL SIGNALS

A. Role of Gangliosides as Recognition Molecules.

Novel techniques have been developed in the Section on Membrane Biochemistry that provide strong support for the concept that gangliosides are specific receptors for tetanus toxin on neurons. The molecular species of gangliosides involved in this process have been identified.

B. Importance of Ganglioside Localization.

Kidney epithelial cells grown in tissue in culture form tight intercellular junctions resulting in the separation of their plasma membrane into apical and basolateral portions. Exogenous gangliosides taken up by the apical membrane cannot pass through the tight junctions to the basolateral surface. Sodium channels in these cells are in th apical membrane. When exogenous gangliosides are incorporated into this surface, hormone, cholera toxin, and 8-bromo-cyclic AMP stimulation of sodium transport was enhanced. These results implicate gangliosides as cell surface modulators of sodium channels. This discovery may have an important neurophysiological implications since gangliosides are particularly concentrated in neural tissues.

C. Molecular Structure of Trophic Hormones.

Because many of these hormones are glycoproteins, the role of the carbohydrate portion of these molecules was examined in a critical fashion. When

oligosaccharides are removed from human chorionic gonadotropin (HCG), the deglycosylated hormone (dHCG) binds to the hormone receptor on Leydig tumor cells but does not stimulate adenylate cyclase and thus behaves as an antagonist. When cells containing bound dHCG were treated with an antibody to HCG, adenylate cyclase activity was stimulated, thereby converting dHCG from an antagonist to an agonist. The most likely explanation of this phenomenon is that the antibodies altered the conformation of the hormone critical for its antagonistic properties and imply that the carbohydrate moieties are involved in the spatial configuration of trophic hormones.

D. Studies on Hormone-induced Desensitization.

Phorbol esters desensitize cells whose adenylate cyclase activity has been raised by agents such as HCG and catecholamines. Phorbol esters are known to stimulate the phosphorylation of many cellular proteins through the activity of protein kinase C. These findings suggest that phosphorylation of protein(s) may be involved in hormone-induced desensitization of cells.

III. DEMYELINATING DISORDERS

A. Cytoarchitecture of Myelin-associated Glycoprotein (MAG).

The selective localization of MAG in the periaxonal region of the myelin sheath in the central and peripheral nervous systems was further confirmed in studies with Quaking mice mutants where a strict correlation was observed between the presence of MAG and the maintenance of a 12-14 nm periaxonal space as well as Schwann cell periaxonal cytoplasmic collars. Higher than normal apparent molecular weights of MAG were demonstrated in the peripheral nervous system in hypomyelinating Trembler mutant mice. This alteration in the structure of MAG may contribute to the neuropathology in these animals.

B. Role of MAG in Autoimmune Disorders.

1. Peripheral neuropathy in patients with benign gammopathies.

Monoclonal IgM antibodies are produced by a number of patients with peripheral neuropathy associated with paraproteinemias that react with the antigenic determinants in the carbohydrate portion of MAG. A similar epitope is also present in a ganglioside in human peripheral nerve myelin. It was further demonstrated that the monoclonal antibody known as HNK-1 that reacts with a determinant on the surface of a subset of human lymphocytes with natural killer and suppressor functions, binds to the same or a very similar epitope. This shared carbohydrate antigen on human lymphocytes and MAG and a ganglioside in the nervous system appears to be highly immunogenic and may play a role in demyelinating diseases. Other patients with gammopathy and neuropathy have been identified in which their paraprotein binds to different gangliosides in peripheral nerve.

2. Multiple sclerosis.

Peripheral blood lymphocytes in some multiple sclerosis patients are sensitized to MAG and other myelin proteins. This sensitization may be involved in the pathogenesis of this condition. We have discovered elevated

activity of a neutral proteolytic enzyme that partially degrades MAG in myelin isolated from the brain of multiple sclerosis patients. Current research is directed toward elucidating the cause of this increased catabolic activity in multiple sclerosis patients and to learn whether such autodegradation of MAG destabilizes the myelin sheath in this condition.

CONTRACT NARRATIVE

Developmental and Metabolic Neurology Branch
Intramural Research Program, NINCDS
October 1, 1983 through September 30, 1984

Contractor: GENZYME CORPORATION, BOSTON, MA. (NO1-NS-3-2346)

Title: Preparation of Ceramidetrihexosidase from Human Placental Tissue

Contractor's Project Director: Henry E. Blair

Current Annual Level of Support: $99,368

Objectives: To isolate human placental ceramidetrihexosidase in sufficient purity and quantity for use in enzyme replacement trials in patients with Fabry's disease.

Major Findings: A procedure has been developed for the large-scale purification of human placental ceramidetrihexosidase in sufficient purity and specific catlytic activity so that it can be safely administered to patients with Fabry's disease. The contractor has developed a satisfactory procedure to remove pyrogen(s) that previously prevented administration of large quantities of ceramidetrihexosidase to patients. We have begun enzyme replacement trials with this pyrogen-free enzyme preparation.

Significance to Biomedical Research and to the Program of the Institute: A principal mission of the Institute is to develop effective therapy to treat human diseases. If salutary clinical results can be obtained, an extraordinary milestone will have been accomplished regarding this type of a human genetic disease.

Proposed Course of the Contract: We are reinitiating enzyme replacement therapy in patients with Fabry's disease that has been in abeyance for a decade due to pyrogenic material(s) in the large-scale enzyme preparations that appear to be necessary to obtain a clinically beneficial response. We shall examine the effectiveness of the enzyme in patients with regard to clearance of accumulated ceramidetrihexoside in the liver and in the blood and monitor their clinical responses to this therapeutic agent.

CONTRACT NARRATIVE

Developmental and Metabolic Neurology Branch
Intramural Research Program, NINCDS
October 1, 1983 through September 30, 1984

Contractor: GENZYME CORPORATION, BOSTON, MA. (NO1-NS-3-2351)

Title: Preparation of Glucocerebrosidase from Human Placental Tissue

Contractor's Project Director: Henry E. Blair

Current Annual Level of Support: $390,712

Objectives: To isolate human placental glucocerebrosidase in sufficient purity and quantity for use in enzyme replacement trials in patients with Gaucher's disease.

Major Findings: A procedure has been developed for the large-scale purification of human placental glucocerebrosidase in sufficient purity and specific catlytic activity so that it can be safely administered to patients with Gaucher's disease. The intravenous infusion of this enzyme appears to have retarded the progression of enlargement of the spleen and liver in patients with this disorder, stabilized their blood platelet count, and caused an improvement in the general health and growth patterns of the recipients.

Significance to Biomedical Research and to the Program of the Institute: A principal mission of the Institute is to develop effective therapy to treat human diseases. If the results indicated in the preceding paragraph can be confirmed and extended, an unprecedented feat will have been accomplished regarding human genetic diseases.

Proposed Course of the Contract: We are seeking means to target of the enzyme to the specific cells in which toxic quantities of lipid accumulate. When a sufficient quantity of the modified enzyme is available, we shall examine its efficiency in patients. We shall also continue to attempt to develop methods to deliver the enzyme to the central nervous system for the treatment of patients with the neuropathic forms of the disorder.

CONTRACT NARRATIVE

Developmental and Metabolic Neurology Branch
Intramural Research Program, NINCDS
October 1, 1983 through September 30, 1984

Contractor: WEIZMANN INSTITUTE OF SCIENCE (NO1-NS-3-2349)

Title: Production of Radiolabeled Glycolipids and Other Sphingolipid Derivatives.

Contractor's Project Director: David Shapiro, Ph.D.

Current Annual Level of Support: $76,402

Objectives: To prepare glucocerebroside, sphingomyelin, and ceramidetrihexoside labeled with ^{14}C in critical portions of the molecule for diagnostic tests for Gaucher's disease, Niemann-Pick disease, and Fabry's disease.

Major Findings: The principal investigator is a world-recognized expert in the chemical synthesis of sphingolipids. He has developed procedures to incorporate radioactive carbon-^{14} into specific portions of sphingolipid molecules. These compounds are used to diagnose patients with the sphingolipid storage disorders listed above, to identify heterozygous carriers of these conditions, to diagnose these disorders prenatally, and to monitor enzyme isolation procedures for glucocerebrosidase, sphingomyelinase, and ceramidetrihexosidase.

Significance to Biomedical Research and to the Program of the Institute: The ability to diagnose patients, identify heterozygotes, and monitor pregnancies at risk for sphingolipid storage disorders represents major contributions to the control of the incidence of these diseases. These procedures are in wide use at the present time.

Proposed Course of the Contract: The contractor will provide radioactive sphingolipids necessary for diagnostic tests and for enzyme purification procedures. He will also develop analogues of sphingolipids for the development of animal models of the human disorders. He will also prepare specific sphingolipid derivatives for use as ligands in affinity column chromatography to expedite and improve the isolation of sphingolipid hydrolases.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 00706-25 DMN

PERIOD COVERED: October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Inborn Errors of Metabolism of Diverse Etiology

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

John A. Barranger, M.D., Ph.D.
Associate Chief, Developmental and Metabolic Neurology Branch, IRP, NINCDS

COOPERATING UNITS *(if any)*: None

LAB/BRANCH: Developmental and Metabolic Neurology Branch

SECTION: Clinical Investigations and Therapeutics/Molecular and Medical Genetics

INSTITUTE AND LOCATION: NINCDS, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.3	3.1	0.2

CHECK APPROPRIATE BOX(ES)

- [X] (a) Human subjects
  - [X] (a1) Minors
  - [ ] (a2) Interviews
- [X] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A better understanding of metabolic disorders which affect the nervous system is the goal of this project. In some phases, the studies are purely diagnostic and are applied to assist in identifying the less common or new disorders of metabolism. Other phases deal with biochemical observations in known disorders and are designed to elucidate the pathogenesis of the disease. In some poorly understood groups of neurologic disease, studies are conducted to draw biochemical correlations where none had previously been known or were poorly developed. Morphologic correlation is made by light microscopic and ultrastructural studies. Therapeutic trials are conducted in selected disorders. Disorders studied include the lysosomal storage diseases, the leukodystrophies, spinocerebellar degenerations, and amino-acidopathies.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 00815-24 DMN

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Metabolism of Complex Lipids of Nervous Tissue

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. O. Brady, Chief	DMN,	NINCDS
OTHER:	P. G. Pentchev, Biochemist	DMN	NINCDS
	A. E. Gal, Organic Chemist	DMN	NINCDS
	A. D. Boothe, Vet. Pathologist	DMN	NINCDS
	H. Weintroub, Visiting Fellow	DMN	NINCDS
	J. M. Quirk, Biochemist	DMN	NINCDS
	M. Comly, Biologist	DMN	NINCDS
	H. S. Kruth, Senior Investigator	EA, IR	NHLBI

COOPERATING UNITS *(if any)*
Weizmann Institute of Science, Rehovot, Israel
Laboratory of Experimental Atherosclerosis, NHLBI

LAB/BRANCH
Developmental and Metabolic Neurology Branch

SECTION
Enzymology and Genetics

INSTITUTE AND LOCATION
NINCDS. NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
8.6	7.6	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

1. We have identified the biochemical abnormality in a mutant strain of BALB/c mice which bear a certain resemblance to Niemann-Pick disease Type C as a specific impairment of esterification of exogenous cholesterol. This metabolic lesion causes organomegaly central nervous system damage and early death in this mouse analogue. Concomitant with this alteration, there is decreased activity of sphingomyelinase and glucocerebrosidase in the organs of affected animals. Activities of certain other lysosomal enzymes are increased similar to the situation often observed in humans with a lysosomal enzyme deficiency. The biochemical defect has also been demonstrated in cultured skin fibroblasts derived from affected mice and the molecular basis of this biochemical derangement is under investigation. This model should be useful to elucidate the effects of cholesterol on the activity of sphingolipid hydrolases and for developing therapeutic strategies to treat heritable metabolic disorders.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 01309-19 DMN

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Biosynthesis and Function of Glycosphingolipids and Other Glycoconjugates

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.)* *(Name, title, laboratory, and institute affiliation)*

PI:	P. H. Fishman, Ph.D., Chief, Membrane Biochemistry Section	DMN	NINCDS
OTHER:	D. R. Critchley, Ph.D., Visiting Scientist	DMN	NINCDS
	S. Spiegel, Ph.D., Visiting Fellow	DMN	NINCDS
	C. Freixas, M.S., Chemist	DMN	NINCDS
	R. O. Brady, M.D., Branch Chief	DMN	NINCDS

COOPERATING UNITS *(if any)*
Lab.of Cellular Metabolism, NHLBI; Lab.of Kidney and Electrolyte Metabolism, NHLBI; Lab. of Molecular Biology, NCI; Bacterial Toxins Branch, Center for Drugs and Biologics, FDA;

LAB/BRANCH
Developmental and Metabolic Neurology Branch

SECTION
Membrane Biochemistry Section

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, MD. 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Gangliosides appear to be important recognition molecules on the cell surface. Tetanus toxin binds to neuronal membranes. The binding components were identified by separating the membrane components by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. After transferring the components to nitrocellulose sheets, the transfers were overlayed with iodinated toxin. Toxin only bound to the region where lipids migrate. The lipids were separated by thin-layer silica gel chromatography and the chromatograms overlayed with labeled toxin. The toxin bound to specific gangliosides identified as GT1b and GD1b. Thus, gangliosides appear to be the specific receptors for tetanus toxin in neuronal membranes.

Kidney epithelial cells form tight intercellular junctions in culture with well-separated apical and basolateral plasma membranes. Exogenous gangliosides taken up by the apical membrane were unable to pass through the tight junctions to the basolateral surface. The kidney cells have a hormone-regulated active sodium transport system. The hormone receptors, adenylate cyclase and Na^+, K^+-ATPase are located in the basolateral membrane whereas the sodium channels are in the apical membrane. When gangliosides were incorporated into the apical surface, hormone-stimulated transport was enhanced. Transport is also stimulated by 8-bromo-cyclic AMP and cholera toxin which increases intracellular cyclic AMP. Ganglioside insertion also increased transport mediated by either agent. The effect of gangliosides on sodium transport was specific as GD1a and GM1 were stimulatory whereas GM3 a less complex ganglioside had no effect. These results implicate gangliosides as cell surface modulators of sodium channels.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 01457-18 DMN

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Chemical Synthesis of Radioactive Sphingolipids

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigetor.) (Name, title, laboratory, and institute affiliation)*

PI:	A. E. Gal, Chief, Neurochemical Methodology Section	DMN	NINCDS
OTHER:	Patricia J. Voorstad, Chemist	DMN	NINCDS

COOPERATING UNITS *(if any)*

None

LAB/BRANCH
Developmental and Metabolic Neurology Branch

SECTION
Neurochemical Methodology Section

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, MD. 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.4	0.2	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Sphingolipids containing radioactive isotopes were synthesized and used for metabolic studies and as diagnostic tools in sphingolipidoses. ^{14}C and ^{3}H labels were introduced by synthetic and semi-synthetic techniques, gas exposure, and a new approach: functional group exchange. These techniques were used for the syntheses of radioactive enantimorphic derivatives of sphingolipids. These products are not metabolizable. Experimentation with these in animals creates "animal models" for metabolic diseases and opens new areas for biomedical studies.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 01480-17 DMN

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 cherecters or less. Title must fit on one line between the borders.)*
Metabolism of Neurohumoral Substances in Marine Animals

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principel Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Dr. Norman Salem, Jr., Senior Staff Fellow, DMN, NINCDS

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Developmental and Metabolic Neurology Branch

SECTION
Physiology and Metabolism Section

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, MD. 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The above project has been terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 01481-17 DMN

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Studies on the Composition and Metabolism of Cellular Membranes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Norman Salem, Ph.D., Jr., Senior Staff Fellow, DMN, NINCDS

COOPERATING UNITS *(if any)*
LMI, BRM, NCI - Frederick Cancer Research Facility, Toxicology Branch, EPA.

LAB/BRANCH
Developmental and Metabolic Neurology Branch

SECTION
Physiology and Metabolism

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, MD. 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.3	2.3	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The above project has been terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 01808-15 DMN

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Glycoproteins of Myelin in Development and Disease

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Richard H. Quarles	Section Chief	DMNB, NINCDS
Others:	Roscoe O. Brady	Branch Chief	DMNB, NINCDS
	Takashi Inuzuka	Visiting Fellow	DMNB, NINCDS
	Michael Dobersen	Senior Staff Fellow	DMNB, NINCDS
	Antonio Noronha	Guest Researcher	DMNB, NINCDS
	Amjad Ilyas	Visiting Fellow	DMNB, NINCDS
	Daniel O'Shannessy	Visiting Fellow	DMNB, NINCDS
	Katsuhiko Yanagisawa	Visiting Fellow	DMNB, NINCDS

COOPERATING UNITS *(if any)* Neural and Molecular Ultrastructure Section, LMG, NINCDS; Clinical Hematology Branch, NHLBI; Childrens Hospital Medical Center, Boston,MA; Dept. Neurology, Ohio State Univ., Columbus, OH; Wisconsin School of Veterinary Medicine, Madison, WI; Anatomy Dept., University of Newcastle, Australia

LAB/BRANCH

Developmental and Metabolic Neurology Branch

SECTION

Section on Myelin & Brain Development

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
7.8	5.5	2.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The myelin-associated glycoprotein (MAG) is selectively localized in the periaxonal part of PNS and CNS myelin sheaths where it is likely to be involved in glia-axon interactions. This function was supported by recent immunocytochemical studies in Quaking mice showing a strict correlation between the presence of MAG and the maintenance of a 12-14 nm periaxonal space as well as a Schwann cell periaxonal cytoplasmic collar. Higher than normal apparent M_r's were demonstrated for MAG in the PNS of Trembler mice and in the CNS and PNS of Quaking mice suggesting that the abnormal MAG may contribute to the pathology in these hypomyelinating mutants. A panel of monoclonal antibodies reacting with polypeptide and carbohydrate sites on the MAG molecule has been produced by hybridoma techniques in mice. Monoclonal IgM produced in patients with paraproteinemia associated with peripheral neuropathy reacts with a carbohydrate epitope that is in human MAG as well as a ganglioside and other glycoconjugates of human peripheral nerve. HNK-1, a monoclonal antibody recognizing a surface determinant on a subset of human lymphocytes with natural killer and suppressor functions, binds to the same or a very similar carbohydrate epitope. This shared carbohydrate antigen between human lymphocytes and several glycoconjugates including MAG of the nervous system appears to be highly immunogenic and may be of significance with regard to demyelinating diseases. Other patients with gammopathy and neuropathy have been identified in which the paraprotein binds to different gangliosides of peripheral nerve. [^{3}H]Thymidine incorporation studies show that some multiple sclerosis patients have peripheral blood lymphocytes sensitized to MAG and other myelin proteins. A neutral protease that converts MAG to a lower molecular weight derivative, dMAG, and degrades myelin basic protein is elevated in myelin isolated from multiple sclerosis brains, suggesting that it may function in autodegradation of myelin in demyelinating diseases.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02162-10 DMN

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Synthesis of Compounds Analogous to Glycolipids

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Andrew E. Gal, Ph.D., Chief, Neurochemical Methodology Section, DMN NINCDS
OTHER: Patricia J. Voorstad, Chemist, Neurochemical Methodology Section

COOPERATING UNITS *(if any)*

None

LAB/BRANCH
Developmental and Metabolic Neurology Branch

SECTION
Neurochemical Methodology Section

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, MD. 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.4	0.7	0.7

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Work was continued on the syntheses of glycolipid analogures of sphingolipids that yield a chromogenic moiety on enzymatic hydrolysis. These compounds are used for the diagnosis and studies of Niemann-Pick, Gaucher's and Krabbe's disease.

Conduritol B epoxide, a saccharide that strongly inhibits -glucosidases, was synthesized by a method developed by this section that provides the produce in greater yield than previously available and permits the preparation of this compound containing a tracer with extraordinarily high specific radioactivity. Administration of conduritol B-epoxide to animals produces a syndrome that resembles Gaucher's disease in humans by inhibiting the enzyme glucocerebrosidase. Radioactive conduritol B-epoxide reacts with the active site of glucocerebrosidase isolated from normal human tissues and from patients with Gaucher's disease. This use of the radioactive conduritol ß-expoxide will materially accelerage the identification of the amino acid substitutions (or deletions) that occur in the glucocerebrosidase molecule in patients with Gaucher's disease.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02163-10 DMN

PERIOD COVERED: October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Development of Analytical Methods for the Use of Research of Sphingolipidoses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Andrew E. Gal, Chief, Neurochemical Methodology Section — DMN NINCDS

OTHER: Patricia J. Voorstad, Chemist — DMN NINCDS

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Developmental and Metabolid Neurology Branch

SECTION

Neurochemical Methodology Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, MD. 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.2	0.1	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

New analytical techniques were developed and used in enzymatic research and in clinical investigations of lipidoses. The lipid content in human tissues, the diagnosis of lipid storage diseases by gas, thin-layer chromatography and other techniques were studied at the microgram level. The techniques we developed previously were improved, modified and used in connection with ongoing projects related to lipidoses in our laboratories and also as joint projects with outside groups. Numerous analytical studies were undertaken by using these techniques. One of them had as its objective, the determination of gangliosides and other lipids in factor 8 protein fraction, a platelet constituent.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02366-06 DMN

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation of Hormone-Responsive Adenylate Cyclase

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: P. H. Fishman, Chief, Membrane Biochemistry Section, DMN,NINCDS

OTHER: R. V. Rebois, Ph.D., Senior Staff Fellow, DMN, NINCDS
S. Kassis, Ph.D., Visiting Associate, DMN, NINCDS
M. Schramm, Ph.D., Visiting Scientist, IRP, NINCDS
T. Zaremba, Ph.D., Pharmacology Associate, NIGMS
R. M. Bradley, B.S., Chief, DMN, NINCDS

COOPERATING UNITS *(if any)*

Laboratory of Clinical Science, NIMH.

LAB/BRANCH

Developmental and Metabolic Neurology Branch

SECTION

Membrane Biochemistry Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, MD. 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.3	4.3	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Deglycosylated human chorionic gonadotropin (DhCG) bound with high affinity to murine Leydig tumor cells but did not stimulate adenylate cyclase. DhCG blocked the binding and action of native hCG and therefore behaved as an antagonist. Whereas as exposure of the cells to hCG caused desensitization of hCG-stimulated adenylate cyclase and down-regulation of hCG-receptors, DhCG did not. Thus, receptor occupancy is not sufficient for these processes to occur. When cells containing bound DhCG were exposed to antibodies to hCG, adenylate cyclase was stimulated. No effect was observed by the antibodies alone or when added before DhCG. Fab, but not Fc, fragments of the antibodies also were effective. Thus, DhCG is converted from an antagonist to an agonist when certain antibodies bind to it. The most likely possibility is that the antibodies induce a change in conformation of the hormone that is crucial for its agonistic properties.

Exposure of murine Leydig tumor cells to tumor promoting phorbol esters caused desensitization of hCG-stimulated adenylate cyclase. The number and affinity of hCG-receptors remained unchanged. Inactive phorbol esters had no effect. The cells contained a large number of high affinity sites for phorbol esters. Others have implicated the phorbol ester receptor as the calcium-activated, phospholipid-dependent protein kinase C. This was tested by treating the cells with the endogenous activator of protein kinase C, diacylglycerol, which also caused desensitization. Finally, phorbol esters stimulated the phosphorylation of many cellular proteins. As desensitization mediated by phorbol esters was analogous to that mediated by hCG, phosphorylation by protein kinase C may be involved in the mechanism of hormone-induced desensitization. This possibility was supported by the observation that phorbol esters also caused desensitization of catecholamine-stimulated adenylate cyclase in rat glioma C6 cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02433-05 DMN

PERIOD COVERED: October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 chererters or lass. Title must fit on ona lina between tha borders.)*: Models of Lysosomal Storage Disease

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and instituta affiliation)*

John A. Barranger, M.D., Ph.D.
Associate Chief, Developmental and Metabolic Neurology Branch, IRP, NINCDS

COOPERATING UNITS *(if any)*: None

LAB/BRANCH: Developmental and Metabolic Neurology Branch

SECTION: Clinical Investigations and Therapeutics/Molecular and Medical Genetics

INSTITUTE AND LOCATION: NINCDS, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	2.0	0.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects
  - ☐ (a1) Minors
  - ☐ (a2) Interviews
- ☒ (b) Human tissues
- ☐ (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Tissue cultures of macrophages and naturally occurring animal mutants are used for these studies. Study of physiologic and biochemical parameters of these models is aimed at defining the milieu in which enzyme or gene replacement will be studied. Macrophages derived from circulating monocytes will survive in culture for approximately two weeks. Under special conditions, dividing cultures have been established without the use of transforming virus. These cells have survived more than six months. Alterations of lysosomal enzymatic activities have been recorded in both short and long term cultures. Estimation of lectin occurrence and function in these cells has been evaluated. The ability of cells to incorporate added lipids has been measured. Catabolism of added lipid has been compared in control and disease cells. Studies in a cat model of G_{M1} gangliosidosis have revealed that human placental β-galactosidase can be delivered to brain following blood-brain barrier opening. Study of animal models for the evaluation of enzyme and gene replacement is progressing.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02434-05 DMN

PERIOD COVERED: October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Studies of Lysosomal Function: Receptor-Mediated Pinocytosis of Lysosomal Enzymes.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

John A. Barranger, M.D., Ph.D.
Associate Chief, Developmental and Metabolic Neurology Branch, IRP, NINCDS

COOPERATING UNITS *(if any)*

None

LAB/BRANCH: Developmental and Metabolic Neurology Branch

SECTION: Clinical Investigations and Therapeutics/Molecular and Medical Genetics

INSTITUTE AND LOCATION: NINCDS, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

The uptake of active glycoprotein lysosomal enzymes occurs, in part, through the mechanism of adsorptive pinocytosis. Receptors for various parts of the enzyme molecule as ligands are present on the plasma and organelle membranes. It is the purpose of this project to study these receptors and utilize them for targeting enzymes to cells. These binding capacities may also play a role in localizing glycoproteins within the cell and thus may have a bearing on the survival of enzymes that have been incorporated into the cell. Studies are directed toward increasing the survival of exogenous enzymes within certain subcellular organelles. The goal is to increase the interaction of exogenous enzyme with stored material in the cell and increase the efficiency of enzyme replacement. Studies will be carried out in rats and later in human macrophages. Studies of the distribution of glucocerebrosidase confirm that infused enzyme can reach the lysosome and does not require the ligand mannose-6-phosphate (M-6-P).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02435-05 DMN

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies On The Mechanism of Pathogenesis Of The Mucopolysaccharidoses.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

George Constantopoulos, Ph.D., Research Biochemist, DMNB, NINCDS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Developmental and Metabolic Neurology Branch

SECTION

Clinical Investigations and Therapeutics

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The mucopolysaccharidoses (MPS) are a group of hereditary diseases characterized by defective metabolism of glycosaminoglycans (GAG). The disorders are usually associated with severe dysfunction of the nervous system as well as of liver, spleen, heart, bone, and other tissues. Objective of this project is the study of mechanism of pathogenesis of these diseases with emphasis on brain involvement and mental retardation. We are using a comparative approach. For this purpose we study the changes, in GAG, sphingolipids, and pertinent lysosomal enzymes in tissues of patients with various types of MPS and we make correlation in terms of clinical and ultrastructural findings. Our laboratory contributed significantly in understanding the chemical pathology and in particular the neurochemistry of MPS IH, MPS IS, MPS II, MPS III A and MPS III B. To complement the studies with human subjects, a drug (suramin) induced animal model of MPS has been developed and a canine model, (natural), of MPS I is being studied. Both animal models may prove useful for understanding the pathogenesis of MPS and in the development and assessment of therapeutic trials by enzyme replacement.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02453-04 DMN

PERIOD COVERED: October 1, 1983 through September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.): Gaucher's Disease: Biochemical and Clinical Studies.

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

John A. Barranger, M.D., Ph.D.
Associate Chief, Developmental and Metabolic Neurology Branch, IRP, NINCDS

COOPERATING UNITS (if any)

Joseph Tager, University of Amsterdam
Arnold Reuser, University of Rotterdam
Ann Erickson, Rockefeller Institute

LAB/BRANCH: Developmental and Metabolic Neurology Branch

SECTION: Clinical Investigations and Therapeutics/Molecular and Medical Genetics

INSTITUTE AND LOCATION: NINCDS, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS: 8.6	PROFESSIONAL: 8	OTHER: 0.6

CHECK APPROPRIATE BOX(ES)

- [X] (a) Human subjects
  - [X] (a1) Minors
  - [ ] (a2) Interviews
- [X] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Gaucher's disease is the most common lysosomal storage disorder. The carrier frequency has been estimated to be as high as 1 in 12 among Ashkenazi Jews. In addition, because of the unique situation in which the same enzyme deficiency leads to both neurologic and non-neurologic disease, the disorder provides an unusual opportunity for the study of biochemical pathology and the metabolic basis of neurologic disease. More importantly, Gaucher's disease like many other inherited disorders is presently untreatable.

The aim of these studies is to define the aberrant biochemistry in the group of disorders collected under the eponym of Gaucher's disease and to investigate methods of treating these disorders. As such, this disease serves as the prototype for this group of diseases. Results of these studies will be applicable to the whole group of lysosomal storage disorders.

Studies of the enzymology and protein chemistry of the enzyme deficient in Gaucher's disease, as well as the cellular and molecular biology and genetics will contribute significantly to construction of therapeutic modalities. The clinical disease will be studied by the most current methods. Enzyme and gene replacement will be studied as potential approaches to treatment.

The goal of this proposal is to apply basic scientific data to the treatment of this disorder.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02529-03 DMN

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 chrecters or less. Title must fit on one line between the borders.)*
Development of Enzymes That Inactivate Neurotoxic Agents

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Roscoe O. Brady, Chief	DMN	NINCDS
OTHER:	J. M. Poston	LB	NHLBI
	A. E. Gal	DMN	NINCDS

COOPERATING UNITS *(if any)*
Laboratory of Biochemistry, NHLBI

LAB/BRANCH
Developmental and Metabolic Neurology Branch

SECTION
Enzymology and Genetics

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, MD. 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

An enzyme that degrades barbital has been identified and partially purified from extracts derived from a soil micro-organism. The requirements for maximal catalytic activity are being determined. We are attempting to scale-up the production of this enzyme to examine its effectiveness in reversing the effects of lethal quantities of barbital in toxicological experiments with appropriate animals. If this approach proves successful, enzymes that inactivate other neurotoxins will be developed in this fashion.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02619-01 DMN

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Oxidative Metabolism in Patients with Inherited Neurological Diseases and in Mycoplasmas.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

George Constantopoulos, Ph.D., Research Biochemist, DMNB, NINCDS

COOPERATING UNITS *(if any)*

Gerard J. McGarrity, Ph.D., Institute for Medical Research, Camden, New Jersey

LAB/BRANCH

Developmental and Metabolic Neurology Branch

SECTION

Clinical Investigations and Therapeutics

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

An increasing amount of evidence points to a possible defect in oxidative metabolism in patients with certain inherited neurological disorders. Thus a defect in the pyruvate oxidation system has been shown in some patients with lactic acidemia and diffuse neurologic disease, of the mitochondrial malic enzyme in patients with Friedreich's ataxia, and a partial deficiency of glutamate dehydrogenase in some patients with olivopontocerebellar degeneration. However, there is much controversy about the exact enzymic defect(s). The objective of this project is the elucidation of the defect in some of these patients or in skin fibroblasts derived from such patients. For this purpose we are assaying a number of mitochondrial and non-mitochondrial enzymes in fibroblasts or leukocytes and we have initiated electron microscopic studies of the mitochondria. We became interested in the oxidative metabolism of mycoplasmas because mycoplasma contamination of fibroblast cultures interfered with the assay of pyruvate dehydrogenase complex in these cells. The oxidative metabolism of mycoplasmas is poorly understood. Hopefully, the elucidation of the defect in these diseases will help in the diagnosis and therapeutic intervention in these patients. Knowledge of the physiology of mycoplasmas may help in understanding the pathogenicity of these organisms.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02619-01 DMN

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Oxidative Metabolism in Patients with Inherited Neurological Diseases and in Mycoplasmas.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

George Constantopoulos, Ph.D., Research Biochemist, DMNB, NINCDS

COOPERATING UNITS *(if any)*

Gerard J. McGarrity, Ph.D., Institute for Medical Research, Camden, New Jersey

LAB/BRANCH

Developmental and Metabolic Neurology Branch

SECTION

Clinical Investigations and Therapeutics

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☑ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

An increasing amount of evidence points to a possible defect in oxidative metabolism in patients with certain inherited neurological disorders. Thus a defect in the pyruvate oxidation system has been shown in some patients with lactic acidemia and diffuse neurologic disease, of the mitochondrial malic enzyme in patients with Friedreich's ataxia, and a partial deficiency of glutamate dehydrogenase in some patients with olivopontocerebellar degeneration. However, there is much controversy about the exact enzymic defect(s). The objective of this project is the elucidation of the defect in some of these patients or in skin fibroblasts derived from such patients. For this purpose we are assaying a number of mitochondrial and non-mitochondrial enzymes in fibroblasts or leukocytes and we have initiated electron microscopic studies of the mitochondria. We became interested in the oxidative metabolism of mycoplasmas because mycoplasma contamination of fibroblast cultures interfered with the assay of pyruvate dehydrogenase complex in these cells. The oxidative metabolism of mycoplasmas is poorly understood. Hopefully, the elucidation of the defect in these diseases will help in the diagnosis and therapeutic intervention in these patients. Knowledge of the physiology of mycoplasmas may help in understanding the pathogenicity of these organisms.

TAB 16 -- EXPERIMENTAL THERAPEUTICS BRANCH -- (ETB)

ANNUAL REPORT

October 1, 1983 through September 30, 1984

Experimental Therapeutics Branch

National Institute of Neurological and
Communicative Disorders and Stroke

Table of Contents

ANNUAL REPORT

October 1, 1983 through September 30, 1984
Experimental Therapeutics Branch, IRP
National Institute of Neurological and Communicative Disorders and Stroke
Thomas N. Chase, M.D., Chief

The Experimental Therapeutics Branch directs its investigative efforts towards the rational development of improved pharmacotherapies for disorders of the human central nervous system. An integrated program of fundamental and applied research seeks to define relationships between clinical signs of brain dysfunction and specific alterations in neuronal transmission; based on a detailed understanding of synaptic mechanisms and of potential sites for pharmacologic intervention, novel therapeutic approaches are developed to modify the affected system and thus improve clinical function. Branch research, at both clinical and preclinical levels, remains focused on the dopamine system and closely interactive neuronal pathways in relation to extrapyramidal and dementing disorders.

The Branch is currently organized into four highly integrated components: Dr. John Kebabian's Biochemical Neuropharmacology Section carries out basic biochemical and pharmacologic studies of dopamine receptor mechanisms. Dr. Judith Walters' Physiological Neuropharmacology Section evaluates interactions between the dopamine system and other transmitter pathways within the basal ganglia. Dr. Thomas O'Donohue's Neuroendocrinology Unit investigates peptidergic systems involved in cognitive and motor function. Dr. Thomas Chase's Pharmacology Section explores transmitter abnormalities and pharmacologic interventions in dementing and extrapyramidal disorders. During the past year, Dr. Roger Porter's Clinical Epilepsy Section was transferred out of the Branch.

BIOCHEMICAL NEUROPHARMACOLOGY SECTION

During FY 84 the Section continued to focus upon two areas of experimental investigation, dopamine receptor pharmacology and pituitary gland cell biology. These are the two areas of traditional strength in the Section.

The pharmacological investigations of the Section focused attention upon three categories of dopaminergic drugs, benzazepines, tetralins and aporphines. The studies of the benzazepines were directed towards understanding the structure-activity relationship in this series containing both agonists and antagonists selective towards the D-1 receptor. The data obtained in FY 84 showed that replacement of a critical hydroxyl group can abolish efficacy but markedly increase the affinity of a benzazepine towards the D-1 receptor. The studies of the tetralins were directed towards developing potent D-2 agonists. Working in collaboration with Alan Horn in Holland, the Section was able to show that certain di-N-substituted 5-hydroxytetralins are extremely potent D-2 agonists. Using these molecules, it was possible to make inferences about the properties of the amine recognition site of the D-2 receptor as well as identify pharmacological differences between the catechol recognition site of the D-1 and the D-2 dopamine receptors. The studies with the aporphines were designed to elucidate the factors responsible for the affinity and efficacy of molecules towards dopamine receptors. The studies utilized the situation that R-aporphines (e.g. R-apomorphine) are dopamine receptor agonists while

S-aporphines (e.g. S-apomorphine or bulbocapnine) are dopamine receptor antagonists. Using a series of aporphines, the affinity and the efficacy of molecules towards dopamine receptors were characterized as separate and distinct properties. In addition, it was possible to attempt to account for the ability of certain ergots to block the D-1 receptor on the basis of their structural similarity with the S-aporphines.

In FY 84 the Section developed pertussis toxin as a biochemical probe of the D-2 dopamine receptor. The data obtained in the Section indicated that the toxin uncoupled the D-2 receptor from Ni, the inhibitory guanyl nucleotide regulatory protein linking the D-2 receptor to adenylate cyclase. This information was of general interest because it indicates the mechanism of the toxin which has been used to study many inhibitory receptors. However, the pertussis toxin was also used as a tool to investigate the participation of cAMP in the inhibition of hormone release from the intermediate lobe. Following pertussis toxin treatment, the dopaminergic inhibition of adenylate cyclase activity and the dopaminergic inhibition of hormone release were abolished.

In FY 84 the Section also studied the cell biology of the pituitary gland. Because of the Section's interest in the involvement of cAMP in the regulation of calcium-dependent hormone secretion, the presence and properties of this enzyme in the ACTH-secreting AtT-20 tumor were investigated. In addition, the presence of substrate proteins for this enzyme in these tumor cells was described. The physiological studies performed in parallel with these biochemical studies indicate that drugs increasing cAMP-dependent protein kinase activity can also stimulate hormone release in the same range of concentrations. However, it is possible to separate the activation of protein kinase from the enhancement of release. This supports the view that cAMP-dependent protein phosphorylation alters in some way the process of calcium-dependent hormone secretion.

In FY '84 the Section completed its experimental investigations of the dopaminergic regulation of the expression of genetic information in the intermediate lobe. In vitro studies performed in FY 84 complemented the in vivo studies of FY '83. The data showed that stimulation of the D-2 receptor in the IL diminished the capacity of the IL to synthesize pro-opiomelanocortin, the prohormone from which melanotrophic peptides are derived.

NEUROENDOCRINOLOGY UNIT

Pharmacology and Cellular Biology of Peptidergic Neurons

The most recently identified and the major known class of neurotransmitters in the central nervous system is comprised of neuropeptides. The goal of the Unit is to develop an understanding of the basic regulatory mechanisms in neurons which secrete peptide neurotransmitters, and through this understanding, develop novel pharmacotherapeutic approaches and agents for manipulating neuropeptidergic systems. Two investigations are ongoing. The first studies pre- and post-synaptic regulatory processes in peptidergic neurons which secrete multiple transmitters. The primary model under investigation is the opiomelanotropin containing neuronal and endocrine systems which secretes two peptides, α-MSH and β-endorphin. These peptides are derived from a single prohormone (pro-opiomelanocortin or POMC) and influence arousal and cognitive processes through interactions with MSH receptors and analgesia through

interactions with mu and delta opioid receptors. The second investigation is focused on determining if there is an endogenous peptide which interacts with the sigma opioid receptor, as is the case with endorphin, enkephalin and dynorphin interacting with mu, delta and kappa opioid receptors.

1. Studies of Co-transmitter Neurons

Two important questions regarding co-transmission concern: (a) whether there are presynaptic mechanisms for regulating ratios of transmitters synthesized and secreted and (b) whether there are post-synaptic interactions between the secreted co-transmitters. Developing an understanding of the regulatory mechanisms for peptide biosynthesis is also important for the development of strategies for antagonizing the synthesis of particular neuropeptides. In FY '84, studies of the POMC system indicated that chronic pharmacological stimuli co-induce prohormone and prohormone processing enzyme biosynthesis. Studies using specific cDNA probes to measure POMC mRNA indicated that the site of regulation of prohormone biosynthesis is pre-translational and preliminary results indicate that regulation occurs at the transcriptional level. Interestingly, biosynthesis of prohormone and prohormone processing enzyme can be dissociated. It is therefore possible that ratios of peptides derived from POMC can be altered in different physiological situations. Alterations of ratios of POMC-derived peptides synthesized and secreted may be particularly important as it was found that there were extentive post-synaptic interactions between α-MSH and β-endorphin and the interactions of the peptides were strictly dependent on the forms of peptides synthesized and modified by post-translational processing. The results of the studies of the POMC system indicate that there are numerous modulatory interactions between the secreted co-transmitters and that the post-synaptic target cell response may be determined by the ratios of peptides secreted by the POMC cell.

Interestingly, the Unit found quite a different situation in studies of the β-protachykinin (β-PROTAC) system which also secretes two peptides, substance P (SP) and substance K (SK) derived from a common prohormone. In this system, ratios of SP to SK were invariable in the central nervous system and there was no evidence for selective regulation of processing. Furthermore, studies of the post-synaptic actions of SP and SK indicated that these peptides have identical post-synaptic actions on gastrointestinal tract motility, elicitation of a spinal sensory response and alteration of single unit neuronal activity in the substantia nigra (performed in collaboration with the Physiological Neuropharmacology Section). The results of all these studies also indicated that the effects of SP and SK were additive and without the modulatory interactions that were observed in the POMC system. It therefore appears that both presynaptic and post-synaptic regulatory processing differ markedly between the POMC and β-PROTAC co-transmitter systems.

Although, SP and SK have identical post-synaptic actions, they appear to do so through different receptors as the Unit identified two distinct binding sites for SP and SK in FY '84. The PROTAC sensory afferents to the spinal cord have also been reported to contain a bombesin (BN)-like peptide and the Unit found that BN had identical actions to SP and SK but the effects were apparently mediated through separate BN binding sites. A

particularly interesting finding was that although SP, SK and BN receptors are distinct from one another and little cross-interaction between the transmitters and receptors occurs, several SP antagonists inhibited binding of all three peptides to their respective receptors. These data suggest the existence of three-dimensional conformational similarities among BN and PROTAC-derived peptides and may indicate an interesting evolutionary relationship between these two families of peptides and their receptors. This relationship may also have clinical relevance as we have found that the only SP antagonists that are effective spinal analgesics antagonize the binding of all three peptides. It is clear from these results that an important new strategy in developing neuropharmacotherapeutic agents will be synthesizing drugs which will interact with all co-transmitters of particular neuronal systems.

2. Studies of an Endogenous Peptide Ligand for the Sigma Opioid Receptor

In FY '83 the Unit identified a compound in porcine brain that inhibits receptor binding of phencyclidine (PCP), a sigma opioid agonist which produces the characteristic psychotomimetic actions of sigma opioids. In addition to displacing PCP binding rather selectively, the compound had similar behavioral and electrophysiological actions as PCP. The peptidase sensitivity and gel filtration behavior of this compound indicated it is a peptide with a molecular weight of approximately 3000 daltons. In FY '84, the Unit developed preparative procedures which allowed purification of bioactive material from 400 porcine brains in order to isolate quantities sufficient for final purification and structural determination studies. This material was used to purify the PCP-like peptide to homogeneity. Amino acid analysis of the purified compound confirmed the prediction of the molecular weight and peptidic nature of the compound, as 26 amino acid residues were identified in the putative endogenous sigma ligand.

A major question the Unit must address regards the specificity of the action of the isolated peptide. An advantage in isolating endogenous ligands for the mu, kappa and delta opioid receptors was the availability of an antagonist, naloxone, to prove that endorphins, enkephalins and dynorphins were exerting their actions by interacting with opioid receptors. Naloxone has also been particularly useful for predicting roles for endogenous opioid peptides. An antagonist was not available for sigma opioid sites. A sigma antagonist could have theoretically been produced by synthesizing derivatives of either the endogenous peptide or PCP. The development of a PCP-based antagonist was attempted because of the problems associated with penetration of peptides through the blood brain barrier and rapid peripheral degradation of peptides. These experiments have led to the development of the first sigma opioid receptor antagonist, Metaphit. Metaphit is a PCP receptor alkylating agent which effectively antagonizes PCP receptor binding and the behavioral effects of PCP. This compound will be useful for basic studies of the putative endogenous PCP-like peptide. The antagonist also has potential clinical utility for antagonizing the schizophreniform symptoms resulting from PCP abuse, eliminating the psychotomimetic actions of the sigma opioid anesthetic, ketamine and, perhaps for treating functional psychoses.

PHYSIOLOGICAL NEUROPHARMACOLOGY SECTION

1. Processes Involved in Regulation of Dopamine Cell Activity

In the past year, the Physiological Neuropharmacology Section has continued to examine the processes involved in regulation of substantia nigra dopamine cell activity. Studies of the role of glutamate and related amino acid-like compounds have suggested that there exist at least two types of excitatory amino acid receptors in the substantia nigra. Kainic acid (KA) and N-methyl-D-aspartate (NMA) induce qualitatively different patterns of excitation in these cells. In addition, specific excitatory amino acid antagonists block the effects of these substances with strong selectivity for either KA or NMA, further suggesting that these compounds act at separate sites. However, the role of these receptors in the substantia nigra is still unclear. Our studies suggest that glutamate-like input does not directly underlie tonic spontaneous discharge of dopamine or pars reticulata cells, although corticostriatal pathways may indirectly influence the activity of the latter neurons. The corticonigral glutamatergic pathway appears to be playing a phasic role rather than a tonic role, if any, in regulating neuronal activity in the substantia nigra.

Other related studies have included investigation of the effects of substance K on the activity of nigral neurons. This has been done collaboratively with the Neuroendocrinology Unit which has been interested in the relationship between this compound and substance P, peptides sharing a single precursor and apparently colocalized in striatonigral neurons. To date, we have not seen effects of substance K on dopamine cells. However, some non-dopamine cells in the substantia nigra pars reticulata are excited by this peptide. The excited cells are found in an area where the Neuroendocrinology Unit has recently detected substance K binding sites. Thus, the combined implications of the receptor binding studies and the iontophoretic studies have shed new light on the role of these peptides in the substantia nigra and focused our attention on the interactions between this striatonigral neurotransmitter system and a subpopulation of non-dopamine neurons within the substantia nigra.

We have also examined the effects of the compound, N-methyl-4-phenyl=1,2,3,6-tetrahydropyridine (NMPTP), known to be selectively toxic for dopamine cells in some species, in an attempt to better understand whether this substance affects dopamine release and how it may affect dopamine cell activity. We found no evidence from neurophysiological data of increased dopamine release with administration of NMPTP; if anything, this substance appeared to have a weak ability to reverse the effects of dopamine agonists, suggesting a weak dopamine antagonist effect, consistent with its molecular structure. In addition, dopamine cells were frequently stimulated and showed altered extracellular actions potentials of decreased amplitude and increased duration folowing systemic i.v. NMPTP injection. The mechanisms responsible for generating a distinct extracellular action potential are apparently affected by NMPTP.

2. Direct Effects of Dopamine at Postsynaptic Dopamine Receptor Sites

Previous studies in our section have demonstrated that dopamine exerts an attenuating effect on the actions of GABA in the globus pallidus and the

substantia nigra pars reticulata. Moreover, this ability of dopamine to act directly upon basal ganglia output neurons to lessen their responses to GABA represents a novel means by which nigral dopamine neurons can influence transmission of movement-related messages without directly involving the striatum. Demonstration of this GABA-dopamine interaction in these areas has raised the possibility that a modulatory function of dopamine might be a more general phenomenon which occurs in other areas of the CNS. We have continued our investigations of the neuromodulatory effects of dopamine and are currently examining the selectivity of the effects of dopamine for GABA in the globus pallidus. To date, studies indicate that dopamine produces mixed effects on the actions of glycine and glutamate in this region, while consistently attenuating the effects of GABA. These results suggest that the process involved in dopamine-induced modulation of GABA is not one which involves attenuation of the effects of all amino acid neurotransmitters, nor one which involves all chloride channels.

3. Effects of Systemic Dopamine Agonist Administration on Basal Ganglia Unit Activity

In previous years we have determined how classic dopamine agonists, such as apomorphine, administered systemically, affect the activity of dopamine neurons and neurons in the globus pallidus and substantia nigra pars reticulata, downstream from the cells. We have established that dopamine agonist-induced changes in dopamine cell activity and in the activity of neurons in the pars reticulata and globus pallidus can provide information about the effects of these drugs on dopamine autoreceptors and on postsynaptic dopamine receptors, respectively. Thus, we now have the tools for examining several currently interesting and well debated questions related to dopamine function and therapeutic pharmacology.

The first is the question of the relative roles of D-1 and D-2 receptors in mediating the effects of dopamine in the basal ganglia. Studies with selective D-1 and D-2 dopamine agonists have indicated that the dopamine autoreceptor-mediated inhibition of dopamine cell activity is a D-2 receptor phenomenon. However, we found that dopamine antagonist-induced increases in dopamine cell activity are apparently mediated by more complex receptor processes. The selective D-2 antagonists induced an increase in dopamine cell activity like that of haloperidol, but the D-1 antagonist, SCH 23390, also stimulates the activity of a subpopulation of dopamine cells. Additional selective D-1 antagonists will need to be found and studied to determine whether this observation is significant. It suggests, however, that blockade of D-1 receptors induces a indirect effect on the activity of some dopamine cells, presumably mediated through afferents to the pars compacta neurons and induced by an interaction of the antagonist with postsynaptic D-1 dopamine receptors.

When we examined the effects of selective D-2 agonists on globus pallidus activity, we found that LY 14865 and its active isomer, LY 171555, stimulate the firing of these cells, while the D-1 agonist, SKF 38393, has inconsequential effects. These results support the idea that D-2 receptors, and not the D-1 receptors, mediate the increase in pallidal activity observed with apomorphine and the ergot agonists examined to date. However, although the D-1 antagonist, SCH 23390, had no effect on the ability of apomorphine to inhibit dopamine neurons, it effectively blocked

the actions of apomorphine on pallidal activity. This suggests that postsynaptic D-1 and D-2 receptor subtypes may interact in some way to influence basal ganglia output. The results complement recent reports that the behavioral effects of apomorphine are blocked by pretreatment with a D-1 antagonist. Since these D-1 receptors have previously been thought to be neurophysiologically and behaviorally "silent", these observations showing effects of a D-1 antagonist on the activity of dopamine neurons and pallidal cells may provide the first clues to the functional significance of this dopamine receptor subtype.

The second question we have addressed with these techniques is whether there exist drugs which will selectively stimulate dopamine autoreceptors. Such compounds might have therapeutic advantages in the treatment of schizophrenia and tardive dyskinesia. We have recently examined the two isomers of (±) 3-PPP, a drug which has recieved considerable attention as a potential dopamine presynaptic receptor agonist. We found that the (+) form of 3-PPP acts like a dopamine agonist at both pre- and postsynaptic receptor sites, while the (-) form of 3-PPP has weaker dopamine agonist or partial agonist effects on the dopamine autoreceptors and acts like an antagonist at the postsynaptic receptor sites. These results suggest that there are differences in the sensitivites of the autoreceptors and the postsynaptic dopamine receptors to the relative agonist/antagonist effects of a drug, but they do not support the idea that 3-PPP is a useful selective agonist. It would not satisfy the therapeutic goal of decreasing dopamine cell activity without inducing the supersensitivity associated with postsynaptic dopamine receptor blockade. We will continue to test other candidates for selectivity at the autoreceptor site.

The third question we have explored involves examination of the changes occurring when dopamine receptors are denervated, as they are in Parkinsonism. Our previous studies have shown that the responses of cells in the globus pallidus and in the substantia nigra pars reticulata to systemically administered apomorphine are qualitatively altered in rats with supersensitive dopamine receptors. We have wondered whether some of the changes observed in the effects of apomorphine on these neurons in the supersensitive animal may be due to an alteration in the consequences of D-1 receptor stimulation. In normal rats, the selective D-1 agonist, SK&F 23390, has no effect on pallidal activity nor does it cause hyperlocomotion or stereotypy. However, this drug does induce an apomorphine-like rotation in animals with supersensitive dopamine receptors, and we have found that it also induces changes in the activity of neurons in the globus pallidus like those of apomorphine. These changes are more effectively reversed by a D-1 antagonist than a D-2 antagonist, suggesting that a qualitative change in the functional consequences of D-1 receptor stimulation is involved in mediating the behavioral and neurophysiological effects of SK&F 38393 in the supersensitive rat. However, other selective D-1 antagonists will need to be studied before an action of SK&F 23390 on supersensitive D-2 receptors can be ruled out as the mechanism behind the pallidal increases and rotational behavior induced by this drug, and a change in the expression of D-1 receptor stimulation ruled in.

PHARMACOLOGY SECTION

The Section conducts clinical and laboratory studies linking the Branch's basic research efforts with the neurologic patient. Clinical investigations seek to associate the status of a particular transmitter system with specific signs of extrapyramidal or cognitive dysfunction. Evidence bearing on such relationships provides the basis for preclinical studies of pathophysiological mechanisms and novel pharmacotherapeutic interventions, especially those involving the dopamine system and interacting peptidergic pathways. Pathophysiologic hypotheses and drug therapies deriving from these laboratory studies are then submitted to clinical evaluation.

1. Dementing Disorders

a. Cerebral Imaging Studies. Results from positron emission tomography (PET) scans following fluorodeoxyglucose (FDG) confirmed and extended earlier findings of a relatively focal pattern of cortical dysfunction in Alzheimer's disease: although most of the cerebral cortex is abnormal, greatest involvement occurs in the parietal association area. Comparison of patients with relatively early dementia with those with more advanced disease suggests that a substantial metabolic decline occurs before cognitive impairment becomes evident; once this threshold has been passed, a marked deterioration in intellectual function attends small metabolic reductions. The present results further indicate that cerebral degeneration begins long before symptoms appear, a possibility that could have important implications for the design of future etiologic studies.

The observation that dementia severity correlates with the degree of hypometabolism supports the view that FDG uptake rates provide a semi-quantitative index to synaptic activity. Preponderant involvement of the parietal association cortex is consistent with our finding that certain tests of aphasia, apraxia, agnosia, and other commonly used measures of parieto-temporal lobe function are more abnormal than tests of attention, orientation, and affective state, which are often used in the assessment of frontal lobe function.

b. Biochemical Studies. The discovery of a relative focal pattern of cortical involvement in Alzheimer's disease has helped focus biochemical probes aimed at an improved understanding of the pathophysiology of this disorder. In particular, PET-directed biochemical investigations have been used in the search for cortical transmitters contributing most significantly to the cognitive decline. A study, carried out with the Neuroendocrinology Unit, comparing transmitter levels in the relatively spared frontal cortex with the relatively severely involved posterior parietal cortex suggested no significant abnormality in neuropeptide Y. In addition, we found high cortical concentrations of neuropeptide Y, partially in neurons also containing somatostatin. Since somatostatin abnormalities are consistently observed in Alzheimer's disease, these findings could indicate that neurons containing both somatostatin and neuropeptide Y are relatively spared in this disorder.

Other clinical investigations have addressed the pathogenesis of Alzheimer's disease through studies of neuropeptide levels in cerebrospinal fluid. We have confirmed previous reports of spinal fluid somatostatin reductions in Alzheimer's disease. The magnitude of this decrement correlates mainly with glucose hypometabolism in the posterior parietal and temporal regions. There also appears to be a close relation between the degree of

somatostatin reduction and the severity of such primary symptoms as apraxias, agnosias and aphasias. Although these results are consistent with the view that cortical somatostatin-containing neurons contribute to the pathophysiology of Alzheimer dementia, the precise origins of this peptide in lumbar fluid remain to be established. Indeed, related studies of spinal fluid gradients for somatostatin, cholecystokinin and neurotensin have failed to provide evidence that lumbar CSF reflects levels of these peptides in the cerebrum only.

c. GABA Agonist Therapy. As a test of whether disordered GABA-mediated transmission contributes to Alzheimer dementia, a therapeutic trial of orally administered THIP, a potent and specific $GABA_A$ receptor agonist, has been completed. Alzheimer patients with low spinal fluid GABA levels received THIP at maximum individually tolerated doses. Cognitive function did not improve. Since adverse effects appeared centrally mediated and resembled those associated with other GABA-mimetics, THIP was probably administered in doses sufficient to stimulate cerebral GABA receptors. The drug's lack of therapeutic efficacy supports the view that GABA system dysfunction in Alzheimer's disease may be a secondary rather than primary deficiency.

d. Cortical Localization Studies. Further analysis of PET data from right-handed Alzheimer patients and their controls provided new information concerning the cortical representation of cognitive function. One study sought to relate subtest performance on the Boston Diagnostic Aphasia Examination with local rates of cortical glucose metabolism. Scores on the subtest depending mainly on visual discrimination localized to the left posterior parietal cortex, while naming tasks consistently related to metabolism in the left parasylvian area. Reading and writing subtests localized to similar portions of the left superior frontal and parietal lobes; writing also involved the right posterior parietal lobe.

A second cortical mapping study accrued from scores on 75 tests of apraxia. The results failed to correlate with degree of overall dementia and appeared unrelated to ideational content, complexity, or parts of the body used. On the other hand, performance on apraxia tests to spoken command correlated closely with scores on psychometric tests dependent on verbal proficiency, while the ability to imitate correlated best with performance on tests of visual-spatial skill. The cortical distribution of significant correlations between local metabolic rates and apraxia scores fell into two patterns: tests involving imitation localized to an area in the right posterior parietal lobe, while responses to command correlated with a region in the left inferior frontal and superior temporal lobes.

2. Extrapyramidal Disorders

a. On-off Phenomena. Parkinson's disease research has continued to focus on the on-off phenomena. One approach has involved PET scanning following FDG administration during drug free and drug treatment periods as well as during on and off periods of response. Initial studies in untreated, hemiparkinsonian patients using the high resolution NEUROPET scanner suggest lenticular hypermetabolism contralateral to the side of motor disability, possibly reflecting the increased activity of neurons released from inhibitory dopaminergic inputs.

Earlier Branch findings suggested that parkinsonian patients with various on-off phenomena stabilized during intravenous infusions of L-dopa. To further evaluate the consistency and durability of this response, patients with mild to severe on-off reactions received L-dopa infusions for up to two weeks. Since none failed to stabilize, each has now been treated with an oral, sustained release formulation of L-dopa. Results to date indicate that certain individuals disabled by on-off phenomena on standard L-dopa-carbidopa preparations improve substantially on a slow release formulation.

b. Cholecystokinin Pharmacology. Cholecystokinin-octapeptide (CCK-8) containing neuronal systems may contribute to the pathophysiology of Parkinson's disease. In rats, peripherally administered CCK-8 has been found to produce centrally mediated pharmacologic effects, in part apparently reflecting interactions with the dopamine system: CCK-8 alters local glucose utilization rates in brain areas containing dopamine cell bodies or projections; CCK-8 also modifies such motor behaviors as apomorphine-induced stereotyped movements and contralateral turning in unilaterally lesioned animals. The precise mechanisms by which these effects occur are now being evaluated, in view of our recent finding that CCK-8 induced changes in certain operant behaviors reflect vagal stimulation.

c. Caerulein Therapy. The foregoing observations as well as reports of nigral CCK-8 reductions in Parkinson's disease prompted attempts to treat this disorder by stimulating cerebral cholecystokinergic transmission. Although injections of the CCK-8 analog, caerulein, markedly increased plasma caerulein levels, no consistent motor effects were observed. A parallel evaluation of caerulein in schizophrenic subjects revealed no antipsychotic activity. These negative results may be attributable to the rapid degradation of CCK-8 related peptides as well as their limited access to the central nervous system.

d. Cholecystokinin Proteolysis. In the search for alternative approaches to the manipulation of central CCK-mediated transmission, preclinical studies have concentrated on the development of drugs to inhibit the inactivation of synaptically released CCK-8. Since this process largely depends on enzymatic hydrolysis, we have begun studies of CCK-8 proteolysis by rat brain synaptic membranes. Available results indicate an initial cleavage at the Met^{3}-Gly^{4} bond. The responsible enzyme requires a metal ion and sulphydral groups for activity. Further characterization of this metalloendopeptidase as well as a determination of its specificity for CCK-8 degradation in vivo and its contribution to the regulation of CCK-8 mediated synaptic function are now being actively pursued.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02263-08 ET

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the bordars.)*
Biochemical and Pharmacological Studies of Dopamine Receptors

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
John W. Kebabian, Chief, Biochemical Neuropharmacology Section, ETB, NINCDS
Koji Miyazaki, Visiting Fellow; Yoshiharu Itoh, Visiting Associate; Robin Felder, Guest Researcher; Elizabeth Frey, Senior Staff Fellow; Thomas Cote, Senior Staff Fellow; Michele Beaulieu, Visiting Fellow; Simon Guild, Visiting Fellow; Anita Sidhu, Guest Researcher, Biochemical Neuropharmacology Section, ETB, IRP, NINCDS; Terry Reisine, Staff Fellow, LCB, ADAMAHA; John Neumeyer, Graduate School, Northeastern University; Carl Kaiser, Department of Medicinal Chemistry, SKF Laboratories

COOPERATING UNITS *(if any)*
Laboratory of Cell Biology
National Institute of Mental Health
ADAMAHA, Bethesda, MD

LAB/BRANCH
Experimental Therapeutics Branch

SECTION
Biochemical Neuropharmacology Section

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
9.5	9.5	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The two dopamine receptor hypothesis (which was formulated in ETB) provided a rational basis for studying the biochemical and physiological effects of dopamine. This project investigates the pharmacology and biochemistry of the D-1 and D-2 dopamine receptors. The knowledge gained about these receptors may facilitate the development of drugs effective in the treatment of Parkinson's disease, endocrine and psychiatric disorders; hypertension and antiemetics.

The pharmacology of the D-1 and D-2 dopamine receptors was investigated in experiments using apomorphines, tetralins and benzazepines. Aporphines with R and S configurations at position 6a have divergent pharmacologies: the S-aporphines are dopamine receptor antagonists while certain R-aporphines are dopamine receptor agonists. Because the aporphines have relatively rigid structures in which a limited number of conformations are possible, it was productive to compare their structures with those of other, more flexible molecules to gain insight into how drugs stimulate or block dopamine receptors. Certain tetralins were potent, selective D-2 agonists; these molecules permitted differences between the pharmacological properties of the D-1 and D-2 dopamine receptors to be identified. Certain benzazepines are selective towards the D-1 receptor; from an understanding of the structure-activity relationship between these molecules, it was possible to begin the development of new research tools for the D-1 receptor.

The cell biology of the pituitary gland and the presence of cAMP-dependent protein kinase in normal and malignant pituitary tissue was investigated. The tumors were especially convenient because they provide much more tissue than is routinely available from the intermediate lobe. The data obtained supports the view that cAMP modulates calcium-dependent hormone release. The ability of the D-2 receptor to regulate the synthesis of proopiomelanocortin and the melanotrophic peptides derived from this large prohormone was investigated. A series of in vitro experiments using drugs discriminating between the D-1 and D-2 receptor could markedly inhibit the synthesis of the melanotrophic peptides.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02578-02 ET

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Pharmacology and Cellular Biology of Peptidergic Neurons

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Thomas L. O'Donohue, Head, Neuroendocrinology Unit, ETB, IRP, NINCDS

Elizabeth Burcher, Thomas N. Chase, Bibie M. Chronwall, Patricia C. Contreras, Michael D. Hirsch, Thomas H. Lanthorn, William R. Millington, Terry W. Moody, Remi Quirion, Clifford W. Shults, Judith R. Walters, Nadav Zamir, Experimental Therapeutics Branch, IRP, NINCDS

COOPERATING UNITS *(if any)*

NIGMS PRAT, NIADDK LC, NIADDK DDB, NHLBI LC, Uniformed Services University of the Health Sciences (USUHS), University of Maryland

LAB/BRANCH

Experimental Therapeutics Branch

SECTION

Neuroendocrinology Unit

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
11.3	8.5	2.8

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The goal of this project is to develop an understanding of the basic regulatory mechanisms in neurons which secrete peptide neurotransmitters, and through this understanding, develop novel pharmacotherapeutic approaches and agents for manipulating neuropeptidergic systems. Two investigations are ongoing. The first studies pre- and post-synaptic regulatory processes in peptidergic neurons which secrete multiple transmitters. The primary model under investigation is the opiomelanotropin-containing neuronal and endocrine system which secretes two peptides, α-MSH and β-endorphin. These peptides are derived from a single gene and influence arousal processes through interactions with MSH receptors and analgesia through interactions with mu and delta opioid receptors. Our investigations indicate that there are extensive post-synaptic interactions between these co-transmitters and that the ratios of peptides secreted presynaptically can influence the post-synaptic action. We have studied the presynaptic regulation of synthesis of the different forms of POMC-derived peptides and found that regulation of prohormone and probably peptide processing enzyme occurs at the transcriptional level. These studies also indicate that effective antagonism of the actions of a particular peptidergic system requires the development of drugs which antagonize all the co-transmitters secreted.

The second investigation is focused on determining if there is an endogenous peptide which interacts with the sigma opioid receptor. We have isolated a peptide that binds to sigma opioid sites and has similar behavioral and electrophysiological actions as sigma agonists such as phencyclidine (PCP). We have also developed the first antagonist for the sigma opioid receptor which effectively blocks the actions of PCP. This antagonist may be particularly useful for determining the roles of endogenous systems which secrete PCP-like peptides and may be clinically useful for treating PCP-induced psychoses, hallucinations resulting from therapeutic use of ketamine, a PCP-like anesthetic and, perhaps, functional psychoses.

Projects Z01 NS 02577 and Z01 NS 02579 have been incorporated into this one.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02139-10 ET

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Pharmacology and Physiology of the Substantia Nigra and Basal Ganglia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Judith R. Walters, Ph.D., Chief, Physiological Neuropharmacology Section, Experimental Therapeutics Branch, NINCDS

Debra Bergstrom, Ph.D.
Thomas H. Lanthorn, Ph.D.

COOPERATING UNITS *(if any)*
Pharmacology Section, Experimental Therapeutics Branch, NINCDS
Neuroendocrinology Unit, Experimental Therapeutics Branch, NINCDS

LAB/BRANCH
Experimental Therapeutics Branch

SECTION
Physiological Neuropharmacology Section

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER:
5.1	3	2.1

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The role of specific neurotransmitters in regulating neuronal activity in the basal ganglia and related areas has been investigated in order to develop a basis for designing improved pharmacological treatments for neurological disorders involving these brain regions. Current topics under investigation are:

1) Modulation of substania nigra (SN) dopamine cell activity. Although dopamine and SN pars reticulata neurons possess at least two types of specific excitatory amino acid receptors, excitatory amino acid antagonists do not block spontaneous discharge of these cells. The neuropeptide, substance K, which shares a precursor molecule with the excitatory peptide, substance P, appears present in striatonigral neurons but, like substance P, exerts an excitatory effect on only a subpopulation of reticulata neurons, located in the region where substance K receptors have been demonstrated. Thus, known excitatory inputs to the SN do not appear to contribute to the tonic activity of the dopamine neurons.

2) Stimulation of dopamine receptors subtypes. To better understand which dopamine receptors play the most significant roles in determining the effects of dopamine agonist administration, the effects of stimulating different subtypes of dopamine receptors have been examined. D-2 dopamine receptor-mediated processes exert different effects on the tonic activity of cells in the globus pallidus as compared with the SN pars reticulata. They also differentially affect subpopulations within these two regions in a manner dependent upon the state of sensitivity of the dopamine receptors. Blockade of D-1 receptors does not affect the actions of D-2 agonists on the SN pars compacta dopamine neurons, nor does stimulation of D-1 receptors affect tonic dopamine cell activity. However, blockade of D-1 receptors does appear to markedly attenuate the D-2 mediated effects of dopamine agonists on neuronal activity in the globus pallidus. These results suggest heretofore unappreciated but potentially significant interactions between striatal D-1 and D-2 receptors may be involved in regulating basal ganglia output.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02265-08 ET

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 charectars or less. Title must fit on one line between the borders)*
Pharmacology, Biochemistry and Physiology of Central Neurotransmitters

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Thomas N. Chase, M.D., Chief, Pharmacology Section, Experimental Therapeutics Branch, NINCDS

N. Foster, C. Shults, J. Juncos, M. Knight, L. Steardo, G. Bruno, P. Barone, C. Tamminga

COOPERATING UNITS *(if any)*
Dept. of Psychiatry, Univ. of Maryland; Dept. of Psychiatry, Karolinska Institute; Dept. of Neurobiology, Weizmann Institute; Dept. of Psychology, Bloomsburg University; Tissue Research Center, Harvard University.

LAB/BRANCH
Experimental Therapeutics Branch, IRP, NINCDS

SECTION
Pharmacology Section

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
7.5	6.0	1.5

CHECK APPROPRIATE BOX(ES)
- [X] (a) Human subjects
  - [X] (a1) Minors
  - [X] (a2) Interviews
- [X] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The goal of this project is to develop improved pharmacotherapies for central nervous system disease based on the relation between transmitter mechanisms and clinical function. Investigations focus on the dopamine system and closely interacting pathways in relation to extrapyramidal and dementing disorders:

1. Positron emission tomography (PET) - fluorodeoxyglucose (FDG) studies of Alzheimer's disease confirmed a predominant involvement of the parietal association cortex. Cortical dysfunction substantially precedes dementia onset; once cognitive impairment becomes evident, a marked intellectual decline attends relatively small decrements in cortical function.

2. PET directed studies of cortical transmitters revealed no neuropeptide Y abnormality in Alzheimer's disease; spinal fluid somatostatin levels are consistently reduced, to a degree which correlates with the severity of parietal lobe signs and with the magnitude of the parietal metabolic deficit.

3. The cortical representation of language function, as suggested by correlations between FDG metabolism and Boston Aphasia Examination scores, largely involves the left cerebral hemisphere, but with characteristic differences in the localization of naming, reading and writing. Apraxia to spoken command primarily localized to the left inferior frontal and superior temporal regions, while apraxia to visual imitation related mainly to the right posterior parietal lobe.

4. Parkinsonian patients, disabled by on-off responses to dopaminomimetic therapy, stabilized indefinitely on intravenously infused L-dopa, and often maintained stability on an orally administered sustained-release dopa preparation.

5. Cholecystokinin octapeptide (CCK-8) and related peptides injected systemically produced centrally mediated pharmacologic effects, apparently related to an interaction with the dopamine system. Neither parkinsonian nor psychotic symptoms, however, improved with caerulein therapy. As part of the search for alternative approaches to modifying central CCK-8 transmission, an enzyme mediating this peptide's initial proteolytic step has been identified and partially characterized.

TAB 17 -- INFECTIOUS DISEASES BRANCH -- (IDB)

ANNUAL REPORT

October 1, 1983 through September 30, 1984

Infectious Diseases Branch
National Institute of Neurological and Communicative Diseases and Stroke

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ANNUAL REPORT

October 1, 1983 through September 30, 1984
Infectious Diseases Branch, IRP
National Institute of Neurological and
Communicative Disorders and Stroke

John Louis Sever, M.D., Ph.D., Chief

I. RESPONSIBILITY OF THE BRANCH

The responsibility of the Infectious Diseases Branch is to carry out planned, coordinated research programs concerned with infections which damage the human nervous system. The Branch is divided into three sections: 1) Immunochemistry and Clinical Investigations; 2) Experimental Pathology; and 3) Neurovirology. These sections utilize the techniques of immunology, clinical investigations including human volunteers and clinical trials, experimental pathology with nonhuman primates, virology, bacteriology, mycoplasmaology, neurovirology, human tissue culture and electron microscopy.

II. PROGRAM SEGMENTS

The program segments are: a) perinatal; b) acute; and c) chronic. In each segment we are concerned with: 1) etiology and diagnosis; 2) treatment; and 3) prevention.

The research areas in the program segments include:

A. Perinatal

Develop and utilize serological and isolation methods to study the relation between viral, bacterial, mycoplasmal and protozoal infections in the perinatal period and birth defects, related abnormalities and pediatric neurological diseases. Investigate approaches to early diagnosis, treatment and prevention using combined laboratory and clinical studies.

B. Acute

Investigate agents which may be responsible for acute neurological diseases such as meningitis, encephalitis, Reye's syndrome, Bell's Palsy, and tic douloureux as well as possible methods for rapid diagnosis, treatment and prevention.

C. Chronic

Study chronic neurological diseases such as multiple sclerosis, amyotrophic lateral sclerosis, progressive multifocal leukoencephalopathy, Parkinson's disease, subacute sclerosing panencephalitis, Alzheimer's and Pick's disease, polyneuropathies, polymyositis, postpolio muscular atrophy and epilepsy using combined tissue culture, immunological, serological, genetic, electron microscopic and clinical approaches for possible infectious etiologies. Whenever possible, explore methods for early diagnosis, treatment and prevention.

III. SECTION ACTIVITIES

A. Section On Immunochemistry and Clinical Investigations (ICI)

1. Perinatal

The Section is responsible for the completion of the broad, overall analysis of Collaborative Perinatal Project sera and data for infection in 60,000 pregnancies. The approaches being used include: 1) clinical infections - correlation with pregnancy outcomes; 2) serological investigation of 8,000 abnormals and 8,000 controls; and 3) high IgM among 30,000 children as a method to identify infected children.

Additional specific studies are in progress and include toxoplasma infections in pregnancy and genital herpes infections. New methods are developed and evaluated for these studies.

2. Acute

New tests for the detection and diagnosis of genital herpes virus infections are being developed and evaluated in patients with this disease. The methods used employ a biotin-avidin reaction to provide high sensitivity and specificity.

The ELISA tests are being used in studies of CSF and serum of patients with a number of different neurological diseases. Group B streptococcal meningitis infections are being studied in experimental monkeys in our laboratories.

3. Chronic

Oligoclonal IgG has been found in the CSF of patients with several different types of neurological diseases. New methods for detecting oligoclonal bands in CSF are being evaluated.

Patients with various chronic neurological diseases are being studied for virus antibodies and antigens. These diseases include: postpolio ALS, ALS, polymyositis, and peripheral neuropathy. Patients with ALS are being evaluated with PET scan techniques.

Animal models of chronic CNS infection (coronaviruses) and autoimmunity (EAE) are being used in studies of possible therapeutic materials for MS.

Cellular immune and humoral immune studies of Simian AIDS (SAIDS) and human AIDS are being conducted in collaboration with other sections.

B. Section On Experimental Pathology (EP)

1. Perinatal

This Section is conducting studies using nonhuman primates as models to investigate the effects of in utero infection of several common human pathogens. Current studies include Group B streptococcal disease.

2. Acute

New methods of treatment and prevention of Group B streptococcal meningitis are being studied using the monkey model developed in this section.

3. Chronic

The neuro-oncogenic studies continue with the owl and squirrel monkey models inoculated intracerebrally with JC virus, a human polyomavirus. Experimental Allergic Neuritis (EAN) is being studied in rhesus monkeys.

Studies of simian AIDS (SAIDS) are being conducted to determine the cause of this disease and methods for prevention and treatment. Specimens from AIDS patients are being studied in subhuman primates

C. Section On Neurovirology (NV)

1. Perinatal

The possible role of immune complexes in influencing the initiation of the immune response in recurrent infections is being investigated.

2. Acute

Studies of acute herpes infections are being conducted jointly with the Section on Immunochemistry and Clinical Investigations.

3. Chronic

Immunologic studies were continued to determine the role of immune response to viruses in multiple sclerosis. These investigations included responses to measles virus, rubella viruses, herpes simplex virus, cytomegalovirus and Epstein-Barr virus.

Studies of the pathogensis of JC virus infection to sub-human primates and humans were extended. Molecular probes were prepared and used to demonstrate JC viral DNA sequences located in tumor tissue but not in non-tumor tissue. Structural organization, sequence and function of JC viral DNA in these tumors is under study. Antibody to JC viral and "T" antigen demonstrated a transient active viral infection preceding tumor initiation.

Differences between acute and persistent infections are being sought via use of the patas monkey - simian hemorrhagic fever virus model. Virological and immunological techniques are being used to determine the mechanism of elimination of persistent SHF virus infection by superinfection. Physical-chemical differences between acute and persistent strains of SHF virus are being sought by monoclonal antibody and molecular biology techniques. Cellular immunology techniques are being used to elucidate the cellular interactions involved in restricting the immune response and maintaining tolerance of persistent SHF virus infection. Immune enhancement of death is being studied in macaque monkeys.

Studies of AIDS specimens are directed at identifying the causative agent. Investigation of simian AIDS (SAIDS) have demonstrated the transmissability of the disease and the etiologic agent is a Group C-D retrovirus.

IV. Findings

A. Perinatal

1. Group B Streptococcal Antigens Detected In Amniotic Fluid of Monkeys

Tests of amniotic fluid using a rapid latex agglutination method detected the presence of Group B streptococcal infection. This method may be of value in early diagnosis of this infection in utero.

2. Reproduction Of Patas Monkeys Excellent In Laboratory Conditions

A 5½ yr. study showed that excellent breeding and high rates of live births could be achieved under laboratory conditions.

3. Maternal Antibody And ISG Protect Against Experimental Group B Streptococcal Infection

Studies in monkeys showed that maternal antibody and passive use of immune serum globulin gave some protections to newborn monkeys with Group B streptococcal infection.

4. Herpes Virus Survives On Warm, Moist Surfaces

Experimental studies showed that HSV can survive 4.5 hrs. on plastic surfaces in warm, humid locations. This may be a possible route for nongenital spread of HSV.

5. Genital Herpes Near Term

A study of 215 pregnant women near term showed that 25 had a history of genital herpes and 10 were infected and shedding virus near term. Eight of the ten were in the high risk group. There were no cases of neonatal herpes.

B. Acute

1. Evaluation Of Tests For Herpes Infections

Short term tissue culture and staining was compared to tissue culture for detection of HSV. The 24 hr. TC plus staining with Biotin Avidin was as sensitive and specific as 7-day tissue culture.

2. Rapid Detection Of Herpes Simplex Infection By Capture ELISA

A new test was developed using biotin-streptavidin with ELISA and provided a sensitive and specific 4½ hr. method for detecting HSV antigens in clinical specimens.

3. Aerosolized Measles Vaccine Successful

Inhalation of aerosolized measles vaccine was immunogenic in 100% of 4- and 6-month old and older children. This provides a new method for mass immunization and demonstrates that young children can be successfully immunized with potent vaccines.

C. Chronic

1. New Technique For Separating Monkey Lymphocytes

A new technique for separating monkey lymphocytes has been developed using Percoll. This is of considerable value for immunological virological studies of SAIDS and AIDS.

2. SAIDS Monkeys Have Late T & B Cell Changes

Studies of SAIDS monkeys (Simian AIDS) with OK T4/T8 monoclonal antibodies did not show changes until near the time of death of the animals. These findings differed from those seen with AIDS. Antibody levels and B cell populations decreased during the course of SAIDS disease.

3. Transmission Of SAIDS With Tissue Homogenates

SAIDS (Simian AIDS) was experimentally transmitted by the use of tissue homogenates, from naturally infected animals at Davis, California to monkeys at NIH. This demonstrated the transmissability of the disease.

4. Transmission Of SAIDS With Filtered Plasma

SAIDS was transmitted by filtered plasma and serum thus establishing that the infectious agent was filterable.

5. Isolation Of SAIDS Retrovirus - The Cause Of SAIDS

The SAIDS agent was isolated in tissue culture and shown to be a retrovirus. This virus was inoculated into monkeys and the disease was transmitted. The virus had the characteristics of a type C - D retrovirus.

6. AIDS Patients Antibody Levels

Serologic studies of human AIDS patients and various comparison groups with specimens from UCLA showed that 96% of AIDS patients had antibody to cytomegalovirus and 94% had antibody to EBV virus.

7. Molecular Studies Of JCV Infections In Simian Glioblastomas

Monkey glioblastoma cells which maintain the JC DNA genome demonstrated tumor phenotypes of viral proteins, cytoskeletal changes and secretion of cellular proteases. This confirms that the JC virus is the etiologic agent of the tumors.

8. Viral Genetic Analysis In Nonpermissive JCV Infections

The pattern of JC genomic integration in the chromosomes of owl monkey tumor cells was most commonly found in a tandem "head to tail" confirmation which is typical for papovavirus transformed tumor cells. This indicates a common mechanism of virus-host interaction in oncogenesis by this group of viruses.

9. JC Gene Products In Productive Infections

During the course of productive infection in human glial cultures JC virus was shown to synthesize an early protein which comigrates with similar proteins of SV40 and BKV. This indicates that JC virus produces a functional early gene product of the same size as other members of the papovavirus group.

10. Detection Of JCV In Human Astroglial Cells

JC virus and an adapted strain of JC virus (for human epithelial cells) is able to replicate its DNA in human astroglial cells as well as oligodendroglial cells. This can be detected by in-situ hybridization.

11. Synthesis Of Small T Protein Not Necessary For JCV Infection

Successful infection of human glial cells with JC virus may proceed without the synthesis of the viral small T protein.

12. Evaluation Of Silver Stain For Oligoclonal Band In CSF

The silver stain method was considerably more sensitive than Coomassie blue stain. It detected more bands and required a smaller amount of protein for assay.

13. High Measles Antibodies In MS Twins

A study of MS twins showed that measles antibody titers and ratios were increased in MS twins which were DW-2 positive.

14. Corona Virus (MHV-A59) Causes Demyelination In Mice

The MHV-A59 strain of coronavirus caused demyelination in the CNS of infected mice 4 weeks after inoculation and viral antigen is in the same areas of the brain.

15. Oligoclonal IgG Bands In The CSF Of Monkeys With EAE

Oligoclonal bands were detected in the CSF of monkeys before the onset of EAE.

16. Treatment Of Polyneuropathy In Waldenstrom's Macroglobulinemia

The severe polyneuropathy in one patient with IgM paraproteinemia and IgM antibodies specific for myelin-associated glycoprotein, was successfully treated with chemotherapy. The improvement has been sustained for 5 years.

17. Late Postpoliomyelitis Muscular Atrophy: Clinical, Virologic And Immunologic Studies

Patients with old poliomyelitis who developed new symptoms were studied clinically, immunologically and virologically. A group of 7 patients had only musculoskeletal complaints without new weakness whereas the rest of 10 patients developed late postpoliomyelitis muscular atrophy characterized by new denervation in some muscle groups, abnormal immunoregulatory ratio of peripheral lymphocytes and oligoclonal IgG bands in the spinal fluid.

18. Paraproteins In The Spinal Fluid Of Patients With Paraproteinemic Polyneuropathies

An IgM band was identified and characterized in the spinal fluid of patients with IgM paraproteinemic polyneuropathy indicating an abnormality in the blood and nerve-CSF barrier.

19. Tremor As A Feature Of Chronic Relapsing And Dysgammaglobulinemic Polyneuropathies

The presence of tremor was found, studied and treated in patients with immune neuropathies. The tremor was indicative of disease activity and improved in most patients when the neuropathy responded to immunosuppressive therapy.

20. IgM In A Human Neuropathy Related To Paraproteinemia Binds To A Carbohydrate Determinant In The Myelin-associated Glycoprotein And To A Ganglioside

The IgM in 3 patients with IgM paraproteinemic polyneuropathy was found to react specifically with a carbohydrate determinant of the human peripheral nerve myelin; the carbohydrate part of this myelin antigen was shared between myelin-associated glycoprotein and peripheral nerve ganglioside.

21. Motor Deficits In Patients With Large-fiber Sensory Neuropathy

Study of the deafferentated humans due to large fiber sensory neuropathy, demonstrated a critical role for somatesthetic feedback in the regulation of centrally generated levels of motor output.

22. Human Peripheral Blood Lymphocytes Bear Markers For Thymosin a_1, a_7, b_4

A small subset of peripheral blood lymphocytes in normal humans have surface markers for thymic hormones (thymosin a_1, a_7, b_4) and may play a role in the immunoregulatory mechanisms.

23. Thymosin b_4 Is Present In A Subset Of Oligodendrocytes In The Normal Human Brain

Thymosin b_4 was found to immunoreact with a subset of human oligodendrocytes. We speculate that these thymosins b_4-positive oligodendrocytes are 1a+ cells and may play a primary role in the immune surveillance of the CNS.

24. Experimental Transmission Of Simian Acquired Immunodeficiency Syndrome (SAIDS) And Kaposi-like Skin Lesions

Some monkeys with immunodeficiency (SAIDS) developed muscle weakness and wasting which we found to be due to polymyositis. Antibodies to retrovirus D immunoreacted with these inflammatory cells that infiltrated the muscle fibers.

CONTRACT NARRATIVE

Infectious Diseases Branch, IRP, NINCDS

Fiscal year 1984

Bio Tech Research Laboratories Inc. (NO1-NS-1-2351)

TITLE: Provide Special Tissue Culture Cells and Reagents to NINCDS

Contractor's Project Director: Dr. Anton F. Stewen

Current Annual Level: $78,333.00

Objective: This is a service contract to produce a variety of cells and reagents not available under other mechanisms for use in the research programs of the Branch.

Major Findings: A number of satisfactory lots of special tissue culture cells have been submitted to the Branch for use in our studies of the JC virus in owl monkeys, the study of herpes, CMV and rubella virus in neurological disease and virus isolation attempts in acquired immunodeficiency syndrome (AIDS) in man and simian acquired immunodeficiency syndrome (SAIDS) in monkeys. The reagents supplied have helped identify the herpes and CMV in a variety of diseases.

Significance to the NINCDS Program and Biomedical Research: The cells and viruses produced by this contract have been utilized in the research programs of the Branch. The reagents supplied have helped to identify the role of the "T" and "t" antigens in tumors of owl monkeys.

Proposed Course: This contract will be discontinued July, 1984.

Publications: None

CONTRACT NARRATIVE

Infectious Diseases Branch, IRP, NINCDS

Fiscal Year 1984

Microbiological Associates: (NO1-NS-3-2316)

TITLE: Development and Delivery of Antigen, Antisera, and Viral Diagnostic Reagents.

Contractor's Project Director: Dr. David A. Fuccillo

Current Funding: $328,500.00

Objectives: This is a service contract to provide research reagents for the papovavirus studies, acquired immune deficiency syndrome (AIDS), simian study of AIDS (SAIDS) and other neurological disease investigations with possible infectious etiology.

Major Findings: Viral diagnostic reagents have been provided for herpes viruses types I and II, cytomegalovirus, measles, rubella, influenza, Coxsackie A and B and varicella. These antigens are used in an attempt to identify the etiology of perinatal and other neurological infections. Evaluation of reagents and materials required to produce successful enzyme-linked immunosorbent assays (ELISA) was accomplished. ELISA tests for detection of IgM to CMV, rubella and toxoplasma have been developed. Reagents for acquired immune deficiency syndrome (AIDS) are being developed to study this highly fatal disease. A similar outbreak of simian AIDS-like disease (SAIDS) has occurred in rhesus monkeys. Reagents to study rhesus monkey CMV and its relationship to SAIDS have been prepared. Large quantities of a retrovirus are being prepared for comparison studies to be done against a similar virus found in SAIDS. Reagents for ELISA tests have been developed for the JC papovavirus. Reagents have been prepared for studies on the molecular genetics of the BK and JC virus.

Significance to the NINCDS Program and Biomedical Research: This contract provides the Infectious Diseases Branch with reagents which are made under standard protocols and with similar cells and strains of viruses from one production lot to another. This allows us to test sera for antibodies from the Collaborative Perinatal Research Project with viruses that were prevalent from 1964 to 1970. Similar production techniques permit data obtained several years ago to be combined with current data. To date, over 80 publications have resulted from analysis of data from these studies. Many of the reports helped establish the frequency of disease during pregnancy, syndromes that develop and information on which to base rational therapeutic and preventive measures. The causative agent of AIDS is now thought to be a retrovirus. An animal disease model such as SAIDS would greatly help in understanding its pathogenesis and neurological consequences.

Papovavirus studies provide basic information as to the initiation of viral growth in brain tissue and eventual production of malignancy. These studies may help to explain the host-related mechanism of persistent infection for progressive multifocal leukoencephalopathy (PML) and other slow viral infections.

Proposed Course: The contract will be continued for the next year.

Publications:

Sabin, A.B., Arechiga, A.F., de Castro, J.F., Sever, J.L., Madden, D.L., Shekarchi, I. and Albrecht, P. Successful immunization of children with and without maternal antibody by aerosolized measles vaccine. I. Different results with undiluted human diploid cell and chick embryo fibroblast vaccines. JAMA 249(19):2651-2662, 1983.

Shekarchi, I.C., Sever, J.L., Lee, Y.J., Castellano, G. and Madden, D.L. Evaluation of various plastic microtiter plates with measles, toxoplasma, and gamma globulin antigens in enzyme-linked immunosorbent assays. J. Clin. Microbiol. 19(2):89-96, 1984.

Sabin, A.B., Arechiga, A.F., de Castro, J.F., Albrecht, P., Sever, J.L., and Shekarchi, I. Successful immunization of infants with and without maternal antibody by aerosolized measles vaccine. II. Vaccine comparisons and evidence for multiple antibody response. JAMA 251:2363-2371, 1984.

Leinikki, P., Shekarchi, I.C., Iivanainen, M., Taskinen, E., Holmes, K.V., Madden, D. and Sever, J.L. Virus antibodies in the cerebrospinal fluid of multiple sclerosis patients detected with ELISA tests. J. Neurol. Sci. 57:249-255, 1982.

CONTRACT NARRATIVE

Infectious Diseases Branch, IRP, NINCDS

Fiscal Year 1984

Microbiological Associates: (NO1-NS-3-2386)

TITLE: Preparation and Delivery of Special Tissue Culture Cells, Media and Immunological Reagents.

Contractor's Project Director: Norma Parker

Current Level of Funding: $99,500.00

Objectives: This is a service contract to provide special tissue culture cells, media and immunological reagents for use by the Branch.

Major Findings: Several large lots of media were obtained for use in cellular immunity studies and production of viral antigens. These lots were non-stimulated to human lymphocytes and did not contain substances which stimulated non-specific antigens. Antigens for use in the various types of cell immunity and serological studies were grown in cells produced with a lot of fetal calf serum previously obtained on this contract in order to reduce non-specific cell stimulation. Large lots of pretested microelisa plates have been obtained. Several large lots of high quality alkaline phosphatase labeled anti-human IgG or IgM have been produced which are significant to NINCDS programs and biomedical research.

Production of antigens for cell immunity studies in pretested media and lots of serum and the use of these reagents in the test itself reduces the nonspecific reactions. This allows us to determine more accurately the specific reactions. Use of specialized equipment and the knowledge of highly qualified individuals on this contract allow us to be far more flexible in purchase of equipment and hiring of personnel. Thus this contract permits us to obtain good reagents at a reasonable price and to maintain a high commitment to research on neurological disease.

Proposed Course: The contract will be discontinued September, 1984.

Publications: None

CONTRACT NARRATIVE

Infectious Diseases Branch, IRP, NINCDS

Fiscal Year 1984

Meloy Laboratories, Inc.: (NO1-NS-7-2375)

Title: Isolated Housing and Care of Animals Used in Several Studies of Infectious Diseases of the Central Nervous System.

Contractor's Project Director: Dr. John L. Cicmanec

Current Annual Level: $263,387.00

Objectives: To provide isolated housing and care for laboratory rodents and a colony of nonhuman primates consisting of several genera. The animals are on experimental studies directed by written protocols. They require monitoring daily for clinical signs of disease and biological specimens are collected as prescribed by protocols. The aims of the project are the use of animal models to develop methods of early diagnosis, treatment and prevention of several neurological diseases affecting man.

Major Findings: This contract satisfactorily provides housing and care for most of the laboratory animals used for research in the Infectious Diseases Branch. The animals are used in several infectious diseases studies of the central nervous system (CNS). These studies require the prescreening of the animals for the presence of antibody, followed by inoculation of the animals by a variety of routes. The infected animals are then held in individual isolation units, monitored and tested as directed in written protocols. All experimentally sick animals are identified and treated with supportive therapy as needed. The investigators on the contract provide overall daily clinical care for the entire colony, with strict isolation procedures carried out at all times. The Contractor's Project Director makes modifications of studies when necessary to achieve the overall goals of the contract.

Significance to the NINCDS Program and Biomedical Research: The goal of the NINCDS is to carry out planned, directed research programs concerned with the diseases which damage the human nervous system. This contract provides the backup source in housing and monitoring laboratory animal models used to study infectious neurological diseases.

Proposed Course: This contract will be continued for the following year to provide the isolated housing and care of a colony of nonhuman primates and rodents inoculated with various infectious agents of the CNS.

Publications: None. All publications from this contract are listed in each area of study of the Experimental Pathology Section.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-NS-00402-28-ID

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Perinatal Infections Causing Damage to the Children in the CPP

PRINCIPAL INVESTIGATOR *(List other professionel personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

John L. Sever		Chief	IDB, IRP, NINCDS
David L. Madden		Veterinary Director	IDB, IRP, NINCDS
Other:	Jonas Ellenberg	Deputy Chief,	OB & FS, OD, NINCDS
	Anita C. Ley	Microbiologist	IDB, IRP, NINCDS
	Nancy Tzan	Microbiologist	IDB, IRP, NINCDS
	Dorothy M. Edmonds	Clinical Nurse	IDB, IRP, NINCDS

COOPERATING UNITS *(if any)*

Johns Hopkins University; Univ. of CA, Los Angeles; Kaiser Hospital George Washington University Medical School; OB & FS, OD, NINCDS

LAB/BRANCH

Infectious Diseases Branch

SECTION

Immunochemistry and Clinical Investigations

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this study is to determine insofar as possible the role of perinatal infections in the production of fetal damage. To accomplish this, clinical data and a large number of serial serum specimens have been obtained from the 58,000 women and their children in the Collaborative Perinatal Project. Now that the project is complete, the "core" study of perinatal infections involves three main approaches: 1) clinical infections; 2) subclinical infections detected serologically using abnormals and matched controls; and 3) high risk children with elevated IgM levels. Special supplemental investigations included the epidemiology of infections and the frequency of congenital toxoplasmosis. Serum antibody titers, IgM values, plus clinical findings are being used to identify infected infants at risk for perinatal damage. Specific tests are then applied for identification of the infection. The data indicate that congenital toxoplasmosis is rare. Special studies of specific infections are also in progress including: hepatitis, infectious mononucleosis, pneumonia, varicella-zoster, and condylomata.

The frequency of clinically recognizing infections during pregnancy was determined and geographic variation was demonstrated. Serological tests were used to document certain diseases. The frequency of confirmed clinical cases per 10,000 were: rubella, 8; rubeola, 0.6; mumps, 10; varicella-zoster, 5. The epidemiology and clinical findings associated with infections were studied using serological methods. This has provided data on the frequencies of infections such as cytomegalovirus, herpes simplex, mumps, rubeola, respiratory syncytial virus, and others. The study of abnormal pregnancies and matched controls is in progress using serological techniques. A number of specific studies have been reported on infections including rubella, neonatal meningitis, cytomegalovirus, maternal urinary tract infections, and toxoplasmosis. Further testing is now being completed employing more sophisticated laboratory methods and more complete data analysis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-NS-02532-02-ID

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Study of AIDS and SAIDS Neurological Findings and Etiology

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	John L. Sever	Chief	IDB, IRP, NINCDS
Others:	Sidney A. Houff	Neurologist	IDB, IRP, NINCDS
	William T. London	Veterinary Director	IDB, IRP, NINCDS
	Maneth Gravell	Research Microbiologist	IDB, IRP, NINCDS
	David L. Madden	Veterinary Director	IDB, IRP, NINCDS
	Lata Nerurkar	Special Expert	IDB, IRP, NINCDS
	Delia Budzko	Special Expert	IDB, IRP, NINCDS
	Marinos Dalakas	Senior Staff Fellow	IDB, IRP, NINCDS
	Barbara J. Potts	Staff Fellow	IDB, IRP, NINCDS
	Gail Scherba	Staff Fellow	IDB, IRP, NINCDS
	Marta Monzon	Visiting Associate	IDB, IRP, NINCDS

COOPERATING UNITS *(if any)*
California Primate Research Center, Davis, CA; Drs. Henry Masur and Abe Macher, Department of Critical Care Medicine, CC, NIH; Dr. Gopal Murti, St. Jude Children's Research Hospital, Memphis, TN.

LAB/BRANCH
Infectious Diseases Branch

SECTION
Immunochemistry and Clinical Investigations

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
13.80	6.25	7.55

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Clinical and laboratory studies were conducted to determine the etiological agents and neurological manifestations of acquired immunodeficiency syndromes in man (AIDS) and in nonhuman primates (SAIDS). Patients with neurological complications of AIDS have been admitted to the Neurology Service of the NIH Clinical Center for study. Patients admitted to other Institutes have been seen by the Infectious Diseases Branch Consultation Service. Patients have been evaluated to determine the spectrum of neurological illnesses found in AIDS. Appropriate virological and immunological studies are being conducted by IDB and collaborating laboratories.

Findings from studies of nonhuman primates with SAIDS are being compared with those obtained through our AIDS protocols. Clinical and immunological parameters of SAIDS are being evaluated. Transmission of SAIDS to normal, uninfected rhesus monkeys using filtered serum and plasma, tissue homogenates or filtered plasma or serum from monkeys with SAIDS has been successfully completed.

A type D retrovirus related to Mason Pfizer monkey virus has been found to be the etiologic agent of SAIDS.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01NS01985-13-ID

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Viral and Nonviral Antigens or Antibodies in Perinatal and Neurological Diseases

PRINCIPAL INVESTIGATOR *(List other professional personnal below the Principal Investigator.) (Name, title, leboratory, and institute affiliation)*

PI: David L. Madden, Veterinary Director, IDB, IRP, NINCDS		
John L. Sever	Chief	IDB, IRP, NINCDS
Lata Nerurkar	Special Expert	IDB, IRP, NINCDS
Mary Ann South	Medical Officer	IDB, IRP, NINCDS

COOPERATING UNITS *(if any)*
Microbiological Associates, Inc.

LAB/BRANCH
Infectious Diseases Branch

SECTION
Immunochemistry and Clinical Investigations

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL	OTHER:
1.5	.75	.75

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Special emphasis has been placed upon development of techniques to improve existing methods for rapid viral diagnosis. The use of a short term tissue culture technique (24 hours) followed by staining of the cells using an anti herpes antibody linked to biotin with the fluoroscein labeled avidin conjugate is a highly efficient system for detecting herpes antigen and is much quicker than detection by conventional tissue culture methods. The correlation of this method with conventional tissue culture was 100%. Changing the parameters of the staining techniques did not help reduce the culture time needed. Several techniques were examined in an attempt to identify the viral antigen directly in the specimen without culture in tissue culture. Staining of the specimen with the biotin-avidin fluoroscein technique was not highly successful. An enzyme-linked immunosorbent assay (ELISA) was developed. Briefly, anti-herpes antibody was attached to the surface of wells in a 96 well plate; plates of the Immulon II or Costar brand were the best. The clinical specimen was incubated and rewashed and anti-herpes antibody linked to biotin was added. After incubation and washing and alkaline phosphatase avidin conjugate was added. After incubation and washing the appropriate substrate was added and the color read. This method has excellent sensitivity (95.6%) and specificity (91.4%) when compared to conventional tissue culture techniques. The results of this test were obtained within 4½ hours after initiation of the test procedure. Infectious particles were not needed for the test so that samples not handled correctly for infectivity studies could still be examined for presence of antigen. Routine contamination of tissue cultures for experimental virus studies for mycoplasma contamination continues.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01NS02038-12-ID

PERIOD COVERED: October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Combined Clinical, Viral and Immunological Studies of Peripheral and CNS Diseases

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Marinos C. Dalakas,	Senior Staff Fellow,	IDB, IRP, NINCDS
John L. Sever,	Chief,	IDB, IRP, NINCDS
David L. Madden	Veterinary Director	IDB, IRP, NINCDS
Maneth Gravell	Research Microbiologist	IDB, IRP, NINCDS
Sidney A. Houff	Neurologist	IDB, IRP, NINCDS
Anita Chu	Visiting Associate	IDB, IRP, NINCDS
J. Woyciechowska	Medical Staff Fellow	IDB, IRP, NINCDS

COOPERATING UNITS *(if any)*: VA Hospital, George Washington Univ. Med. Ctr., Georgetown Univ. Med. Sch., Children's Hospital, Washington, D.C.; Nat. Naval Med. Ctr. (NNMC), Bethesda, MD

LAB/BRANCH: Infectious Diseases Branch

SECTION: Immunochemistry and Clinical Investigations

INSTITUTE AND LOCATION: NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.5	1.5	3.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Clinical and laboratory studies are conducted to determine etiology (infection, immunity and/or genetics) for chronic diseases of the peripheral and central nervous system. Current studies include amyotrophic lateral sclerosis, (ALS), polymyositis/dermatomyositis, demyelinating polyneuropathies, Reye's syndrome, multiple sclerosis, progressive multifocal leukoencephalopathy, subacute sclerosing panencephalitis and myasthenia gravis. Combined clinical data, genetic information, HLA and MLC typing virus serology and virus isolation studies are obtained for these studies. The nature of oligoclonal bands found in the CSF of patients with chronic neurological diseases is under investigation. A neuromuscular disease that occurs in patients who have had poliomyelitis at an early age has been clinically defined; the possibility that this might be due to a late polio virus infection or an abnormal immunoregulation and an immune reaction to neuronal cells is under investigation. IgM monoclonal band has been identified in the spinal fluid of patients with paraproteinemic polyneuropathies and an abnormal blood-CSF and nerve barrier were found. The metabolic activity of the cortex in ALS patients is being studied using the PET scan and ^{18}F 2-deoxy-D-glucose; hypometabolism was demonstrated not only in the motor but also in the paramotor and sensory cortex, suggesting that ALS is a rather generalized process affecting many cortical regions. Patients with bulbar ALS are now studied with Nuclear Magnetic Resonance (NMR) which, from our preliminary findings, appears to demonstrate structural defects in the medulla. Using recombinant DNA alpha$_2$-interferon, an experimental therapeutic trial was conducted in 5 ALS patients. No improvement or beneficial change in the course of the disease was noted. Virological studies were performed in patients with Reye's syndrome and their families and their ability to handle a salicylate challenge was investigated. The effect of aging on the neuromuscular systems is being investigated electrophysiologically and morphologically.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-NS-01731-16-ID

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Isolation, Characterization and Diagnosis of Infectious Agents from Chronic Diseases

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	Maneth Gravell	Research Microbiologist	IDB, IRP, NINCDS
Other:	Rebecca S. Hamilton	Biologist	IDB, IRP, NINCDS
	Marta Monzon	Visiting Associate	IDB, IRP, NINCDS

COOPERATING UNITS *(if any)*
Section on Experimental Pathology, IDB, IRP, NINCDS

LAB/BRANCH
Infectious Diseases Branch

SECTION
Neurovirology Section

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.3	.2	.1

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Simian hemorrhagic fever (SHF) virus is an unclassified togavirus which resembles most closely the flaviviruses in its mode of replication. Four strains of virus have been identified. Two of the strains produce persistent infections in patas monkeys and the others, acute infections. All strains produce fatal infections of monkeys of the genus Macaca.

By in vitro translation studies using rabbit reticulocyte lysates, we have obtained evidence that only non-virion polypeptides are coded from SHF virus genomic RNA. Four polypeptides with molecular weights of 40K, 25K, 15K and 12K daltons were synthesized from genomic RNA. Immunoprecipitation studies indicate that these polypeptides were not contained in mature virions. Polypeptides with these molecular weights were found in infected cells early in infection (up to three and one-half hours after infection). About five hours after infection, polypeptides contained in mature virions were found in infected cells. The factor(s) which control the regulation of synthesis of viron and non-virion polypeptides are currently unknown.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01NS01983-13 ID

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 charecters or less. Title must fit on one line between the borders.)*
Chronic Viral Infections - Molecular Biology of Human JC Virus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principel Investigator.) (Name, title, laboratory, and institute affiliation)*

Eugene O. Major,	Special Expert,	IDB, IRP, NINCDS
Allen Aksamit	Medical Staff Fellow	IDB, IRP, NINCDS
William T. London	Veterinary Director	IDB, IRP, NINCDS
Sidney Houff	Clinical Associate	IDB, IRP, NINCDS
Nancy Miller	Special Expert	IDB, IRP, NINCDS
Rene Traub	Microbiologist	IDB, IRP, NINCDS
Craig Cummins	Senior Staff Fellow	SNB, IRP, NINCDS
Dominick Vacante	Dept. of Biology, U of Illinois, Chicago	

COOPERATING UNITS *(if any)*
Surgical Neurology Branch, NINCDS
University of Illinois at Chicago, Chicago, IL
Microbiological Associates, Bethesda, Maryland

LAB/BRANCH
Infectious Diseases Branch

SECTION
Unit on Molecular Virology and Genetics

INSTITUTE AND LOCATION
NINCDS, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	2.1	1.4

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standerd unreduced type. Do not exceed the space provided.)*

The association of the human papovavirus, JCV, with a demyelinating disease described as progressive multifocal leucoencephalopathy (PML) has been firmly established from isolation of virions from PML brain tissue and detection of viral antigen in PML plaque lesions. Seroepidemiological studies have established that the infecting virus, JCV, is widespread in populations throughout the world even though PML is a rare disease, suggesting that JCV establishes a latent or persistent infection. We have undertaken the study of the molecular pathology of JCV and its interaction with human glial cells in culture and as a potential cause of demyelination or tumor production in simians. Our experiments are focused at the intracellular level, designed to assay the molecular nature of JCV infection, and its effect on its host cells as a model for viral persistence. Our current findings now suggest that JCV induced owl monkey glioblastomas when explanted in culture lose the integrated JCV genome from the cell chromosome resulting in loss of the viral T protein and tumor cell phenotypes. We have shown that the JCV T protein is necessary for such glioblastoma cells to continue to grow in culture and that cell morphology changes characterized by microfilament disorganization is under viral gene control. Further, such cells in culture require the JCV T protein, presumably, to secrete serine proteases into their environment, a characteristic of many human astrocytomas. We have further identified the molecular size of the JCV T protein in productive infections of human glial cells and that successful infection does not require either an association with host p53 proteins or synthesis of the viral small t protein. We have also observed that JCV and a mutant strain of JCV adapted to human kidney cells is able to replicate their DNA and produce virions in astroglial as well as oligodendroglial cells derived from human fetal brain.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-NS-02602-01-ID

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Border Disease Virus: Structure, Replication and Pathogenesis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Barbara J. Potts, Staff Fellow, IDB, IRP, NINCDS

COOPERATING UNITS *(if any)*
University of California at Davis, Department of Pathology, School of Veterinary Medicine, Davis, California;
United States Diagnostic Services, USDA, Ames, Iowa

LAB/BRANCH
Infectious Diseases Branch

SECTION
Neurovirology Section

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1	1	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Border disease (BD), the result of a congenital infection of sheep, is caused by a virus in the genus Pestivirus and the family Togaviridae. Abortion or congenitally affected lambs may result when pregnant ewes, in their first trimester, develop a primary infection with BD virus. This noncytopathic virus which is antigenically related to hog cholera and bovine diarrhea viruses, causes congenital malformations of many systems of the fetal lamb. Affected lambs are small, weak, have long straight fleece with abnormal pigment, have cerebellar tremors and a low survival rate. The only histopathological lesions reported in this disease are a reduction in CNS myelin (dysmyelination) and glial proliferation in the newborn lambs. In our present work we have studied lambs congenitally infected with BD virus and sheep exposed to BD virus as adults and studied them for one year to determine the pathogenesis of a congenital exposure compared to an adult exposure to the virus.

Persistent BD virus was isolated in tissue culture and detected by immunofluorescence (FA) from the peripheral white blood cells, urine and CSF of the lambs with congenital BD for up to one year of age. These animals had no detectable serum neutralizing antibody response for the same time period. BD viral antigen was also detected by FA in many CNS tissues of these lambs with BD. In comparison, sheep infected with BD virus as adults had normal antibody levels against the virus, and no detectable virus isolated from similar tissues.

A reduction in myelin content (dysmyelination) and glial proliferation in the CNS and microencephaly were noted in the lambs with congenital BD and these lesions appeared to remain the same over a 12 month period.

This virus in sheep is an excellent animal model for the study of microencephaly and dysmyelination in humans and as a tool for investigating CNS cell migration and maturation and for determining mechanisms for viral invasion and persistence.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01NS02531-03-ID

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Studies in Neuromuscular and CNS Diseases and Their Experimental Models

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Marinos C. Dalakas, Senior Staff Fellow, IDB, IRP, NINCDS		
John L. Sever	Chief	IDB, IRP, NINCDS
David L. Madden	Veterinary Director	IDB, IRP, NINCDS
Maneth Gravell	Research Microbiologist	IDB, IRP, NINCDS
William T. London	Veterinary Director	IDB, IRP, NINCDS
Joanna Woyciechowska	Medical Staff Fellow	IDB, IRP, NINCDS

COOPERATING UNITS *(if any)*
VA Hospital, George Washington University Med. Ctr., Georgetown University Med. Sch., Children's Hospital, Washington, D.C.; Naval Med. Ctr. (NNMC), Bethesda, MD

LAB/BRANCH
Infectious Diseases Branch

SECTION
Immunochemistry and Clinical Investigations

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.5	1.5	3.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the spece provided.)*

Enzyme histochemistry in muscle and nerve biopsies is carried out for diagnostic purposes in patients with several neuromuscular disorders. Immunocytochemical studies were conducted using specific antibodies to thymic peptides to investigate changes in the distribution of epithelial cells and thymocytes in the thymus of patients with myasthenia gravis. Using the cytofluorograph, specific subsets of lymphocytes that carry thymic markers (thymosin alpha$_1$, alpha$_7$, beta$_4$) were defined. The interaction between cells of the lymphoid and central nervous system was investigated searching for common antigenic markers on their cell surface. Thymosin beta$_4$, an immunomodulating polypeptide, was found to be a common antigen shared by macrophages, dendritic lymphoid cells and oligodendrocytes. The immunoglobulin of certain patients with paraproteinemic polyneuropathies has been identified as a specific antibody to myelin associated glycoprotein; nerve biopsies from these patients are studied by electron microscopy and immunocytochemically with specific antimyelin antibodies. The nature of amyloid protein in 15 patients with "sporadic" amyloid polyneuropathy was identified immunocytochemically using specific antibodies to amyloid proteins; the immunocytochemical findings were confirmed biochemically on the extracted amyloid tissue. Immune cellular markers were investigated during evolution of EAN and EAE induced in rhesus monkeys and therapies were attempted using some novel immunomodulating agents. The mechanism of mouse hepatitis virus-induced demyelination was investigated in mice inoculated with mouse hepatitis virus; the distribution of viral antigens was demonstrated in the demyelinated regions. Inflammatory myopathy was identified in monkeys with immunodeficiency (Simian AIDS) and antibodies to retrovirus D immunoreacted with the infiltrating inflammatory cells and muscle capillaries.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-NS-00972-13-ID

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Animal Models for CNS Infections in Normal and Immunocompromised Hosts

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	William T. London	Veterinary Director	IDB, IRP, NINCDS
Others:	Maneth Gravell	Research Microbiologist	IBD, IRP, NINCDS
	Val G. Hemming	Associate Professor	USUHS
	Gerald W. Fischer	Professor	USUHS
	John L. Sever	Chief	IDB, IRP, NINCDS
	Sidney A. Houff	Neurologist	IDB, IRP, NINCDS
	Marinos C. Dalakas	Senior Staff Fellow	IDB, IRP, NINCDS
	Blanche L. Curfman	Biologist	IDB, IRP, NINCDS
	Robert L. Brown	Biological Lab Technician	IDB, IRP, NINCDS

COOPERATING UNITS *(if any)*
University of Pittsburgh Presbyterian Hospital, Department of Neuropathology, Pittsburgh, Pennsylvania; Meloy Laboratories, Inc., Springfield, Virginia; Uniformed Services University of the Health Sciences, Bethesda, Maryland

LAB/BRANCH
Infectious Diseases Branch

SECTION
Experimental Pathology Section

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.10	.80	2.30

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Group B streptococci (GBS) are a major cause of neonatal sepsis and meningitis. Early diagnosis of GBS infections in neonates would allow extensive therapeutic intervention with possible increases in survival rates. Using a commercial latex particle agglutination test, we detected GBS antigen in the amniotic fluid, gastric fluid and serum of rhesus monkey infants as early as 24 hours following bacterial challenge.

Additional studies with our model have failed to demonstrate inhibitory factors in normal rhesus monkey amniotic fluid. Rhesus amniotic fluid is an excellent growth media for this bacteria. These findings in rhesus monkeys are in conflict with human studies in which the growth of GBS appears to be inhibited by normal amniotic fluid.

Utilizing our rhesus model for perinatal GBS infection, we have been able to demonstrate the safety and efficacy of intravenous immunoglobulin to provide GBS specific IgG to newborn babies. This has prompted the use of intravenous immunoglobulin as a method of producing significant increases in GBS IgG in amniotic fluid which enhances bacterial opsonization and protection from neonatal disease.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-NS-01986-13-ID

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 charecters or less. Title must fit on one line between the borders.)*
Inoculation of Animals with Tissue Culture Grown Materials from Patients with Chronic Neurological Diseases

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	William T. London	Veterinary Director	IDB, IRP, NINCDS
Others:	Marinos C. Dalakas	Senior Staff Fellow	IDB, IRP, NINCDS
	John L. Sever	Chief	IDB, IRP, NINCDS
	Blanche L. Curfman	Biologist	IDB, IRP, NINCDS
	Robert L. Brown	Biological Lab Technician	IDB, IRP, NINCDS

COOPERATING UNITS *(if any)*
Meloy Laboratories, Springfield, Virginia

LAB/BRANCH
Infectious Diseases Branch

SECTION
Experimental Pathology Section

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standerd unreduced type. Do not exceed the spece provided.)*
The remaining aspects of this project are being transferred to Project Z01-NS-00972-13-ID.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-NS-02136-10-ID

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Control of Acute Infectious Diseases in Experimental Animals Using Biologicals and Chemotherapeutic Agents

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	William T. London	Veterinary Director	IDB, IRP, NINCDS
Others:	Maneth Gravell	Research Microbiologist	IDB, IRP, NINCDS
	Marinos C. Dalakas	Senior Staff Fellow	IDB, IRP, NINCDS
	John L. Sever	Medical Director, Chief	IDB, IRP, NINCDS
	Blanche L. Curfman	Biologist	IDB, IRP, NINCDS
	Robert L. Brown	Biological Lab Technician	IDB, IRP, NINCDS

COOPERATING UNITS *(if any)*
Meloy Laboratories, Springfield, Virginia

LAB/BRANCH
Infectious Diseases Branch

SECTION
Experimental Pathology Section

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)
- ☐ (a) Human subjects
  - ☐ (a1) Minors
  - ☐ (a2) Interviews
- ☐ (b) Human tissues
- ☒ (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*
The remaining aspects of this project are being transferred to Project Z01-NS-00972-13-ID

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-NS-02271-08-ID

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Papovaviruses in Nonhuman Primates

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	William T. London	Veterinary Director	IDB, IRP, NINCDS
Other:	Sidney A. Houff	Research Associate	IDB, IRP, NINCDS
	Eugene Major	IPA	IDB, IRP, NINCDS
	John L. Sever	Chief	IDB, IRP, NINCDS
	Nancy R. Miller	Expert Consultant	IDB, IRP, NINCDS
	Blanche L. Curfman	Biologist	IDB, IRP, NINCDS
	Robert L. Brown	Biological Lab. Technician	IDB, IRP, NINCDS

COOPERATING UNITS *(if any)*
University of Wisconsin Medical School, Departments of Medical Microbiology and Pathology, Madison, Wisconsin
Meloy Laboratories, Inc., Springfield, Virginia

LAB/BRANCH
Infectious Diseases Branch

SECTION
Experimental Pathology Section

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The remaining aspects of this project are being transferred to Project Z01 NS 01983-13 ID

TAB 18 -- MEDICAL NEUROLOGY BRANCH -- (MNB)

ANNUAL REPORT

October 1, 1983 through September 30, 1984

Medical Neurology Branch, IRP
National Institute of Neurological and Communicative Disorders and Stroke

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ANNUAL REPORT
October 1, 1983 through September 30, 1984

Medical Neurology Branch, IRP
National Institute of Neurological and Communicative Disorders and Stroke

Chief, Roger J. Porter, M.D.

The Medical Neurology Branch was officially re-established within the Intramural Research Program of NINCDS on June 27, 1984. The Branch conducts research on human epilepsy, including new approaches to diagnosis and treatment, investigates basic questions related to normal and abnormal neuronal excitability, performs studies on human motor control and speech, and conducts research on Alzheimer disease and related disorders including autonomic dysfunction.

The Branch is divided into four approved sections. Roger J. Porter, M.D., is chief of the Clinical Epilepsy Section, Dr. Mark Hallett is chief of the Human Motor Control Section, and Dr. Ronald J. Polinsky is chief of the Clinical Neuropharmacology Section. The position of Chief, Neuronal Excitability Section is vacant.

CLINICAL EPILEPSY SECTION

The Clinical Epilepsy Section has developed and tested new techniques to achieve improved seizure control, reduce drug-induced side effects, and achieve better rehabilitation in patients with severe epilepsy. These include simultaneous video and telemetered EEG recording of seizures, daily determination of antiepileptic drug serum concentration. Recent advances include use of positron emission tomography, magnetic resonance imaging, and magnetoencephalography.

The use of positron emission tomography (PET) may greatly alter our understanding of localized brain lesions in patients with partial seizures. Three groups of patients have been studied, with complex partial, absence, and atonic seizures. In patients with partial seizures, metabolic evaluations using F^{18}-2-deoxyglucose, demonstrate focal hypometabolic cerebral areas corresponding to the interictal seizure EEG focus. During a seizure, this region is converted from a hypometabolic to hypermetabolic focus. Focal PET lesions may be identified in some patients even if the EEG abnormality itself is not well localized. In other cases, an ictal PET scan may clarify the results of an equivocal interictal scan. These studies allow more definitive overall identification of the localization of the epileptic lesion and permit a more precise surgical approach to patients with partial seizures, patients who are often refractory to medical therapy. The PET scan is noninvasive and lesions are often documented in patients whose neurological examinations and CT scan are normal. PET may also help to elucidate the effect of seizures on the metabolism of areas outside the seizure focus itself. Clinical correlations can be made with associated data such as the results of neuropsychologic tests and evoked potentials. Patients with absence seizures show normal interictal scans, but diffuse rate increases during seizures. Patients with atonic seizures have been divided into two groups, one with normal, the other with reduced, interictal metabolic rates.

Thus, PET has shown metabolic differences among patients with clinically similar seizure disorders. PET is also being used to study the effect of antiepileptic drugs on cerebral metabolism. Preliminary results suggest that sedative-hypnotic antiepileptic drugs decrease glucose metabolic rates. We have also begun an evaluation of magnetic resonance imaging in patients with focal seizure disorders, in an attempt to learn more about the structural basis of changes in brain electrophysiology and metabolism.

Intensive monitoring with simultaneous video and EEG recordings continue to elucidate new areas of seizure classification and differentiation. A study of complex partial seizures has been recently concluded. A study of generalized tonic-clonic seizures is underway; in both cases the differential diagnosis is very important to appropriate therapy. Intensive monitoring has been useful in an on-going study of secondary generalization, and its effectiveness in intractable epilepsy has been documented.

The study of evoked responses in patients with epilepsy has new implications for patients with intractable seizures. Early studies have shown that the dominant eye may greatly influence the amplitude of the visual evoked response, an important feature to recognize in all patients. In addition, patients with complex partial seizures are currently being evaluated for abnormalities of the visual evoked response, auditory and brainstem evoked potentials, and the somatosensory evoked potentials. Evoked potentials are also being utilized in the evaluation of new drugs. Serial spectral analysis of tape recorded EEGs in patients are being studied to help analyze the effects seizure frequency and drugs have on cerebral electrical activity. Evoked potentials and EEG have also been used to study children with precocious puberty, some of whom may suffer from cerebral dysrhythmias or clinical seizures. A study of magnetoencephalography is underway. This technique has great potential for localizing epileptiform discharges in the depths of the brain. Conventional EEG may not provide adequate spatial discrimination of spikes, and gives little information about depth beneath the cortical surface of potential generators. Magnetoencephalography may become an important investigative tool.

Clinical Pharmacology of Antiepileptic Drugs

Pharmacologic studies in epilepsy continue to concentrate on studies of drug interactions and of new antiepileptic drugs.

A new potential antiepileptic agent, flupirtine, is in the final stages of testing in Germany as an analgesic. The structure of the compound is completely different from currently marketed antiepileptic drugs. The drug is effective in animal models of epilepsy which suggests that it may be effective in both partial seizures and absence seizures. The Clinical Epilepsy Section is studying both of these seizure types in different patients in an open pilot study of intensive design. Preliminary results show a promising decrease in seizure frequency in some patients. Patients with both seizure types appear to have benefitted. Neuropsychological tests are being performed to help assess the effects of the drug. Initial pharmacologic studies have been performed to derive data on absorption, distribution, and metabolism of flupirtine.

Studies of new drugs in refractory epilepsy are especially difficult because of the requirement of using patients not controlled on standard medication. These so called "refractory" patients are a difficult test for any new potential antiepileptic drug, and usually require that maximal doses of the new drug be administered to see any effect. A special problem with flupirtine is the uncertainty of the magnitude of the maximal tolerated dose; establishing the appropriate chronic dose is an important goal of this study. Patients are now tolerating twice the dose that was previously thought likely to be tolerated, allowing clinical studies to proceed with greater likelihood of appropriate evaluation in epileptic patients.

Another unique aspect of this study is the intensive design. This study design has not been previously tested in clinical trials in epilepsy, and though it requires patients with a very high seizure frequency (3 or 4 per day), seizure data are collected much more rapidly and the drug may be evaluated for its potential in ways which may be less expensive of both time and money.

One of the most widely used antiepileptic drugs is phenytoin, which is effective against several seizure types in many patients. Phenytoin, however, often confronts the physician with difficult management problems because; of its non-linear kinetics--blood levels tend to go up much more rapidly at higher doses, and small dose increases can quickly cause toxicity. Furthermore, when changes in phenytoin dose are made, blood levels may rise or fall to reach a deceptive "pseudo-steady state", followed by further fluctuations before a true steady state is reached. Failure to appreciate this effect may initiate the most assiduous therapeutic efforts. The Clinical Epilepsy Section has studied this phenomenon and has, from clinical data, provided theoretical pharmacokinetic hypotheses for the cause of this difficult clinical management problem.

The controversial issue regarding which drugs to use for epilepsy has concentrated on 1) the use of single drugs or more than one drug for severely affected patients, and 2) the use of sedative-hypnotic agents in the routine management of patients. The Clinical Epilepsy Section addressed the first question several years ago, and is now completing efforts in the second area, in which sedative drugs have been documented as unnecessary and potentially toxic, even for patients with intractable seizures. Appropriate means for withdrawal of these drugs, including outpatient withdrawal, have been designed with these investigations.

HUMAN MOTOR CONTROL SECTION

The Human Motor Control Section is composed of the Speech Pathology Unit, which has been on-going for several years, and a Motor Disorders Unit, which is yet to be organized. The mission of the Section is to understand normal principles of motor control in man and the pathophysiology of motor disorders including both deranged voluntary movement and involuntary movements. The major goal of the Speech Pathology Unit is to understand the neurologic and physiologic bases of speech and phonation through study of the patterns of breakdown of these functions in various disorders. One major goal of the Motor Disorders Unit is to elucidate physiologic

mechanisms of normal limb voluntary movement and pathophysiology in cerebellar ataxia, parkinson bradykinesia and hemiplegia. A second major goal of the Motor Disorders Unit is to analyze the pathophysiology of involuntary limb movements such as tremor, myoclonus and dystonia. A report of the detailed objectives and recent findings of the Speech Pathology Unit follows.

The following objectives are being addressed:

1. To develop objective techniques for quantitative measurement of speech production and phonation in both normal and pathological states.

2. To identify changes in speech and phonation associated with neurological disorders.

3. To identify the separate functions independently affected in speech and phonatory disorders as an indication of the neurological components of the speech production and phonatory systems.

4. To determine the neurological organization of the speech production and phonatory systems, through the study of speech and language disorders associated with well defined neuroanatomical lesions of the CNS.

5. To determine the degree to which independent components of speech and phonation can be altered by behavioral or neuropharmacological treatment conditions.

Advances in the study of speech production and phonation will be reviewed separately.

In the project aimed at identifying "Patterns of Speech Breakdown in Neurological Disease", we completed a measurement system for assessing speech timing and phonatory control. Spectrographic measurement and analytic procedures were developed providing 30 independent measures. A study of 24 normal males and 23 normal females between 20 and 80 years of age was completed to determine sex and age effects. Increased age had an adverse effect on five measures: fast speaking rates; laryngeal reaction times; speech intensity; range in intensity; and, use of fundamental frequency to differentiate between stress conditions. These reductions all indicate a reduced control of laryngeal timing and competency with age. Increased performances by male speakers were found on measures of: speaking rate; rate change; rate of laryngeal adduction-abduction in syllables; mean intensity; range in intensity; and maximum intensity level.

To determine whether the measurement system is sensitive to changes in speech with treatment alterations, a Parkinson disease patient was recorded in two different drug conditions. Comparisons between recordings made during maximum L-Dopa therapy and during a maximal dosage of bromocriptine, demonstrated significant improvements on most measures during L-Dopa treatment. These treatments are now being compared in other patients.

To determine whether the assessment system can differentiate between different types of neurological disease, two groups of patients, one with idiopathic orthostatic hypotension (IOH) and another with early signs of multiple systems atrophy, Shy-Drager Syndrome, (SDS) were compared on speech production tasks with age- and sex-matched controls. No speech production deficits were found in the IOH group. Statistically significant differences were found between the controls and SDS patients on seven of the 30 speech production measures demonstrating that phonatory control for speech was most affected early in the disease process.

In the project, "Rate Manipulation and Neuropharmacological Effects on Speech", astudy of the reaction times of the various speech articulators was conducted in two different diseases of the basal ganglia to examine whether differential effects on articulator movements could be found. Patients with Parkinson's disease (PD) and Huntington's disease (HD) were contrasted on measures of speech reaction time (to an acoustic signal) to determine whether different effects would be found in a disease affecting the substantia nigra from another affecting the caudate. Neither group was impaired in simple laryngeal reaction time nor the maintenance of rate during syllable repetition, while both groups were impaired in maximum rate of repetition. The HD patients were slower than normal on rate and movement coordination tasks involving each of the articulators while the PD patients were particularly impaired in laryngeal movement control. Normal relationships between speech reaction time and rate and between rate of laryngeal adduction/abduction and repetition rate were not found in either patient group. Also, the pattern of relationships found between performances on the various tasks differed in the two patient groups. Thus these two diseases had selective effects on different aspects of articulator movement control.

In the project, "Acoustic Analysis of Vocal Fold Vibration in Phonatory Pathology", a study of frequency perturbation in the normal population determined what subject and phonatory characteristics are related to frequency perturbation. In 96 normal subjects, a multiple regression model including fundamental frequency, maximum phonation length, duration of periodic phonation, and phonatory intensity, was able to predict frequency perturbation with 70% accuracy in females and with 60% accuracy in males. A 90% confidence interval for normalcy was used to detect individuals with laryngeal pathology in a study of the validity of using this measure for non-invasive screening for laryngeal pathology. Patients with various laryngeal pathologies including carcinoma, nodules, polyps, unilateral paralysis and edema were tested. Only 35% of the patients were identified as outside the normal range, although all carcinoma cases were detected. Although frequency perturbation is significantly greater than normal in groups with laryngeal pathology, it was not significantly greater than normal in the majority of individual patients and could not be used for screening. These results are not in agreement with the commonly held assumption that morphological alterations in vocal fold tissue will disrupt the regularity of vocal fold vibration. Our future research will be aimed at determining the vibratory mechanism responsible for frequency perturbation and other acoustic attributes of phonation.

In the project, "Optimum Phonatory Functioning in Various Types of Laryngeal Pathology", a preliminary investigation indicated that efficiency was impaired in a patient with vocal fold nodules while it exceeded normal levels in a patient with unilateral paralysis. Improved procedures for assessing the physiology of phonation have been developed. Additional patients sustaining unilateral damage to the recurrent laryngeal nerve during thyroidectomy are being followed, to determine how different degrees of vocal fold closure alter the efficiency of phonation.

In the project "Characteristics of Voice Disorders of Unknown Etiology", we have been studying an idiopathic phonatory disorder, spasmodic dysphonia. For many years this disorder was thought to be psychological in origin. Recent studies have demonstrated signs of neuropathologies in such patients. Some speculate that an abnormally sensitive reflex occurs in this disorder which is stimulated by increases in subglottic pressure with vocal fold closure, causing a hypertonicity of the vocal folds. An alternate hypothesis is that this may be a highly specific dystoniaconfined to the laryngeal musculature. We have completed two studies this year bearing on these issues. One study was aimed at determining which phonatory gestures were impaired in patients with spastic dysphonia. Comparisons with normal controls on experimental tasks indicated that the patients were impaired only on tasks reflecting recurrent laryngeal nerve function and not those reflecting superior laryngeal nerve function. Laryngographic tracings in patients demonstrated excessive changes in laryngeal height and vocal fold position prior to the onset of vocal fold adduction for phonation. Thus, movement abnormalities did not seem to be dependent upon increases in subglottic pressure.

The effects of unilateral recurrent laryngeal nerve resection on phonatory control were studied in two patients prior to surgery, immediately following surgery and one year post surgery. In both cases, the symptoms of the underlying movement disorder persisted and were unaltered by resection of one of the recurrent laryngeal nerves. Our preliminary interpretations are that spastic dysphonia is an upper motor neuron disorder affecting laryngeal adduction gestures during speech. Experimental tasks are planned to examine whether these motor control problems are present only for speech or whether the same movements are affected during non-speech actions.

Our collaboration with the Vietnam Head Injury Study has continued. Over 500 veterans with penetrating head injuries and 100 controls will have completed CT scanning and speech and language testing by September 1984. A small number of cases exhibit classic speech and/or language syndromes 12 to 15 years post-injury. For the project entitled, "Location and Size of Brain Lesions Associated with Speech Deficits", lesion locations associated with long term residuals in language, such as Broca's and Wernicke's aphasia, are being compared with those associated with verbal dyspraxia, speech dysprosody and dysarthria. A syndrome of expressive and receptive syntactic deficits was identified in 12 cases and the CT scan lesions compared with those of 26 cases who recovered from aphasia. In all cases with residual syntactic deficits, the lesion involved both Broca's and Wernicke's area in the left hemisphere as well as the underlying white matter and the neostriatum. In contrast, patients with verbal dyspraxia, a speech articulation disorder without Broca's aphasia, had left hemisphere lesions involving the sensory-motor cortex and the supplementary motor area.

In the project entitled, "Relationships Between Language and Speech Deficits in Neuropathologies", language deficits previously found in patients with Parkinson's disease are currently being reexamined to determine whether they are related to the location and degree of disease progression.

Subject testing has been completed in two projects, "Speech and Language Abnormalities in Tourette Syndrome", and "Auditory Processing and Language Skills in Behavioral Disorders." Both will be discontinued this year following the completion of data analysis and the submission of manuscripts for publication. The first project is evaluating the effects of haloperidol on language learning disorders, in monozygotic twins with Tourette Syndrome and auditory attention deficit disorders. The other project involves studies of the effects of stimulants on auditory processing and language expression in boys with attention deficit disorder (ADD). Only a few remaining subjects with delayed language and ADD need to be tested for the completion of a double blind cross-over study of the effects of d-amphetamine on auditory perception and language encoding.

CLINICAL NEUROPHARMACOLOGY SECTION

The Clinical Neuropharmacology Section has been developing clinical, physiological, biochemical and pharmacological methods for assessment of autonomic nervous system function in man. Since norepinephrine is the neurotransmitter released by most post-ganglionic sympathetic nerve endings and is also an important central nervous system neurotransmitter, these investigations have focused primarily on the noradrenergic system. High performance liquid chromatography, liquid scintillation spectrometry, and mass spectroscopy are used to measure neurotransmitter and metabolite levels in plasma, urine, and cerebrospinal fluid under basal conditions and after a variety of stimuli have been applied to elicit a sympathetic response. Two groups of patients with chronic autonomic failure have been studied in order to elucidate the biochemical and pharmacological differences between central and peripheral autonomic dysfunction. Patients with idiopathic orthostatic hypotension (IOH) have pure or isolated autonomic failure in contrast to patients with multiple system atrophy (MSA) in whom the autonomic dysfunction is attended by a central nervous system disorder. Cerebrospinal fluid levels of neurotransmitter metabolites can be used as an index of central nervous system metabolism. The major brain metabolite of norepinephrine is 3-methoxy, 4-hydroxyphenylglycol (MHPG). Since MHPG readily crosses the blood-brain barrier, a substantial portion of CSF MHPG is derived from the plasma. We previously developed a method for correcting CSF MHPG levels for the contribution from plasma by subtracting 90% of the plasma level of free MHPG from the total CSF level of MHPG. The "corrected" CSF MHPG level is an index of central nervous system norepinephrine metabolism. Both MSA and IOH patients have low total CSF MHPG levels. The amount of CSF MHPG due to central nervous system norepinephrine metabolism is diminished in MSA whereas in IOH the decreased CSF MHPG is due to the low plasma MHPG levels. These findings indicate that central nervous system noradrenergic activity is diminished only in MSA.

Homovanillic acid (HVA) and 5-hydroxyindole-acetic acid (5-HIAA) are the major metabolites of dopamine and serotonin respectively in man. IOH patients have slightly decreased CSF HVA levels and normal CSF 5-HIAA levels. Both CSF HVA and 5-HIAA levels are extremely low in MSA patients. The decreased CSF HVA levels in MSA reflect diminished central nervous system dopamine turnover, probably due to striatonigral involvement in MSA. There may be a reduction in spinal cord dopaminergic activity in IOH, possibly related to a decrease in inhibitory sympathetic efferent pathways. The diminished 5-HIAA levels in MSA suggest that there is involvement of serotonergic neurons. Serotonergic neurons are known to play an important role in the central nervous system control of blood pressure.

Our previous studies have shown that supine plasma norepinephrine levels are low in IOH, but normal or slightly elevated in MSA. Although plasma levels of norepinephrine reflect the responses of the peripheral sympathetic nervous system it is necessary to consider removal rates of the catecholamine. A study of the disappearance kinetics of levo- and dextro-norepinephrine was completed in order to assess neuronal uptake in patients with orthostatic hypotension. Following a constant rate infusion of the catecholamine mixture to steady-state levels, there is a slower decline of the isoproterenol in plasma compared to the norepinephrine isomers in normal subjects. This supports the results of animal and tissue studies which indicate that isoproterenol is not taken up into sympathetic neurons. There is no difference between the removal rates of the norepinephrine isomers which suggests that neuronal uptake in man is not stereospecific. The removal rate of norepinephrine in IOH is similar to isoproterenol. This indicates that neuronal uptake is severely compromised in IOH, presumably due to a reduction in the number of peripheral sympathetic neurons. Norepinephrine kinetics and clearance are normal in MSA. Examination of the differential labelling pattern of urinary norepinephrine metabolites suggests that vesicular uptake and metabolism are stereoselective. There is a decreased ratio of levo/dextro-MHPG in MSA which might result from increased protection of levo-norepinephrine in vesicles with decreased peripheral sympathetic activity. A higher levo/dextro-MHPG ratio in IOH may reflect an increased rate of norepinephrine release and reuptake in remaining sympathetic neurons.

We have studied beta-endorphin and enkephalin responses during insulin-induced hypoglycemia in patients with chronic autonomic failure. There is no consistent pattern of enkephalin responses during hypoglycemia. However, the beta-endorphin response is essentially absent in MSA patients. Since this deficiency may be related to central nervous system dysfunction, a study of the ACTH levels is in progress. Patients with MSA and IOH also develop hypotension during insulin-induced hypoglycemia. This might result from vasodilation due to stimulation of beta-adrenergic receptors by insulin. In order to test this hypothesis, a series of insulin tolerance tests is being conducted in IOH and MSA patients after administration of propranolol. We are also continuing our investigations of peptide and hormonal responses during feeding.

Our therapeutic endeavors have focused on further development and testing of the sympathetic neural prosthesis. This device is an example of closed-loop feedback-controlled infusion therapy. An arterial blood pressure transducer relays blood pressure measurements to a microprocessor that controls an infusion pump which administers a short-acting pressor drug at a rate sufficient to maintain the blood pressure at a pre-set level. The device has been tested in two MSA patients for 72 hours. These patients did not experience any symptoms due to hypotension during this ambulatory trial. The device also prevented post-prandial hypotension which is a significant clinical problem encountered in managing orthostatic hypotension patients. Thus, it appears that closed-loop feedback-controlled infusion therapy is a viable option which can be successfully used to treat orthostatic hypotension without causing increased supine hypertension. One potential problem with this approach is the development of apparent temporary subsensitivity to norepinephrine which occurred after continued administration of the drug. We are now investigating the mechanism and time course of this phenomenon.

The Clinical Neuropharmacology Section has undertaken the study of familial Alzheimer disease as a major priority within the scope of its research efforts. This project has evolved from an initial investigation of a large family with histologically confirmed Alzheimer disease from New Brunswick, Canada. Alzheimer disease is a major medical and social problem since it is the most common cause of irreversible, chronic dementia. The studies of Alzheimer disease are significantly limited by both accuracy and timing of diagnosis. Unfortunately, the diagnosis of Alzheimer disease must be left to the neuropathologist. This complicates clinical research studies since more than 20% of clinically diagnosed cases do not have Alzheimer disease at autopsy. Although Alzheimer disease may be inherited in less than 10-20% of all cases, the main justification for studying familial cases lies in the accuracy of diagnosis which may be inferred through post-mortem examination of other affected family members.

There are two major aims of our studies. One is to investigate genetic linkage in order to define the chromosomal abnormality in familial Alzheimer disease. This may ultimately allow identification of the gene product which will elucidate the underlying pathophysiology and hopefully stimulate more rational therapeutic approaches. The second aim is to define the clinical and biochemical progression of the disease through a longitudinal investigation of affected and at-risk subjects using detailed neuropsychological testing, PET scanning, NMR imaging, neurotransmitter studies and pharmacological strategies. Neuropathological and neurochemical studies of post-mortem specimens from these families will also be conducted. These studies will hopefully provide clues for earlier and more accurate diagnosis that will facilitate research on sporadic cases.

Much of this work is currently in progress. We have completed several field expeditions to perform clinical evaluations and obtain skin biopsies and blood samples on selected members of these large families. Skin fibroblast and peripheral blood lymphoblast cultures are being established by the Institute for Medical Research, Camden, New Jersey. These cultures will

serve as a renewable source of DNA and cell lines which can be used for genetic linkage, viability, and biochemical studies. Collaborative arrangements have been made with several laboratories to investigate genetic markers including DNA restriction fragment length polymorphisms. We are also planning fieldwork to investigate a very large kindred with Alzheimer disease from southern Italy.

NEURONAL EXCITABILITY SECTION

The Neuronal Excitability Section will be studying temporal lobe sections from patients with epilepsy. These studies will involve the evaluation of putative neurotransmitter levels in this tissue, the distribution of proteins in this tissue as well as electrophysiologic studies in slices. In addition, the release of putative neurotransmitters from slices of temporal lobe foci will be studied, in an effort to distinguish differences between normal tissue and an epileptic focus.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02318-07 MNB

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Clinical Pharmacology of Antiepileptic Drugs

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.: Roger J. Porter, M.D. Neurologist, Chief,MNB,IRP,NINCDS and Head,CES,MNB,IRP,NINCDS

COOPERATING UNITS *(if any)*

Epilepsy Branch;
CDNDP, NINCDS

LAB/BRANCH

Medical Neurology Branch, Intramural Research Program

SECTION

Clinical Epilepsy Section (CES)

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Clinical Epilepsy Section continues to study the clinical pharmacology of old and new antiepileptic drugs. Special emphasis has been placed on studies of new antiepileptic compounds, such as flupirtine. Flupirtine is being evaluated both clinically and pharmacologically in patients with either complex partial or absence seizures. Flupirtine is especially promising in models of epilepsy and preliminary clinical results are encouraging. A new protocol for the use of progabide, as well as gamma vinyl GABA, in children with the Lennox-Gastaut syndrome has been approved by the NINCDS-ICRS, but studies of both these compounds are awaiting FDA approval. These drugs, although chemically unrelated, are thought to act by increasing CNS levels of gamma amino butyric acid (GABA), a putative inhibitory neurotransmitter. Measurements of changes in CSF GABA levels due to the drugs will be correlated with their effects on seizure control. Studies have been undertaken on the unusual pharmacokinetics of phenytoin, establishing a "pseudo steady-state" phenomenon based on clinical observation made possible by the intensive monitoring unit of the Clinical Epilepsy Section. This phenomenon helps explain some of the difficulties physicians have when they use this drug, and provides a theoretical framework for future investigations of this difficult pharmacologic problem. The pharmacologic evaluation of antiepileptic drugs is coupled with efficacy studies, carried out by intensive monitoring techniques including videotape analysis of epileptic seizures with simultaneous telemetered EEG recording, and daily determination of antiepileptic drug levels. Future studies are planned to evaluate the specific patterns of hepatic microsomal enzyme metabolism of antiepileptic drugs by evaluating antipyrine metabolites.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02236-09 MNB

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 cherecters or less. Title must fit on one line between the borders.)*
Diagnostic and Therapeutic Reevaluation of Patients with Intractable Epilepsy

PRINCIPAL INVESTIGATOR *(List other professionel personnel below the Principal Investigetor.) (Name, title, leboretory, and institute affiliation)*

PI: Roger J. Porter, M.D. Neurologist, Chief,MNB,IRP,NINCDS and
Head,CES,MNB,IRP,NINCDS

COOPERATING UNITS *(if any)*
Epilepsy Branch; CDNDP,NINCDS
Office of Administrative Management; Clinical Center, NIH

LAB/BRANCH
Medical Neurology Branch, Intramural Research Program

SECTION
Clinical Epilepsy Section (CES)

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the spece provided.)*

The Clinical Epilepsy Section has been developing and testing new techniques to improve seizure control, medication tolerance, and rehabilitation in patients with severe epilepsy. Patients with uncontrolled seizures are admitted for a complete evaluation, including simultaneous video and telemetered EEG recording of seizures, daily determinations of antiepileptic drug serum concentrations, positron emission tomography (PET), magnetic resonance imaging (MRI), and magnetoencephalography (MEG). A specific seizure diagnosis is established allowing each patient to be assigned to an appropriate research protocol and therapy.

PET in patients with localized brain lesions has demonstrated focal hypometabolic cerebral areas corresponding to the interictal seizure EEG focus. In some patients, PET has been able to detect a focus when other methods have failed. Studies of patients during partial seizures have shown a change from hypo to hypermetabolism at the site of the focus. In the Lennox-Gastaut syndrome, PET has revealed the existence of two separate metabolic patterns despite clinical seizure similarity.

PET studies allow more definitive overall identification of the epileptic lesion and suggest new avenues of investigation into the basic mechanisms of the epilepsies. MRI is being used in conjunction with PET and EEG in order to study the structural basis of glucose hypometabolism and EEG epileptiform discharges. MEEG may have the potential to accurately localize the subsurface origin of spikes. EEG provides little information on the spatial distribution of epileptiform in cortical depths; MEEG may be superior.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02561-02 MNB

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Optimum Phonatory Functioning in Various Types of Laryngeal Pathology

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	Christy Ludlow, Ph.D.	Speech Pathologist Chief	SPU, HMCS, MNB	IRP NINCDS
Others:	Ralph Naunton, M.D.	Otolaryngologist Director	CDP	NINCDS
	Nadine Connor, M.A.	Speech Pathologist	SPU, HMCS, MNB	NINCDS
	Edward G. Movius, M.D.		CE	NIADDK

COOPERATING UNITS *(if any)*
Communicative Disorders Program, NINCDS

LAB/BRANCH
Medical Neurology Branch, Intramural Research Program

SECTION
Speech Pathology Unit, Human Motor Control Section (HMCS)

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.55	.25	.30

CHECK APPROPRIATE BOX(ES)

☑ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objectives are: 1) to determine how phonatory efficiency is altered in various types of laryngeal pathology, and 2) whether changes in laryngeal structure and neural control alter phonatory efficiency. A preliminary study was completed of the alterations in phonatory efficiency in two patients in comparison with normal; one with vocal fold nodules, the other with unilateral paralysis. The results indicated that efficiency was impaired in the patient with vocal fold nodules while it exceeded normal levels in the patient with unilateral paralysis. The validity of the concept of an optimum phonatory efficiency at a particular fundamental frequency, was assessed in the normal controls and each of the patients. Efficiency increased as fundamental frequency increased in the normal speakers while no systematic relationship with fundamental frequency was found in the patients. Methods of studying phonatory efficiency have been improved and instrumentation developed for measuring physiological aspects of phonatory function. Studies of phonatory efficiency are continuing in additional numbers of patients with the improved experimental and measurement techniques. Patients sustaining unilateral damage to the recurrent laryngeal nerve during thyroidectomy for carcinoma are being followed to determine whether phonatory efficiency changes with the return of nerve function. Also, when there is no change in nerve function, phonatory efficiency will be monitored to examine whether there are compensatory changes in the mode of phonation which alter phonatory efficiency.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02440-05 MNB

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Acoustic Analysis of Vocal Fold Vibration in Phonatory Pathology

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	Christy Ludlow, Ph.D.	Speech Pathologist Chief	SPU, HMCS, MNB	IRP NINCDS
Others:	Ralph Naunton, M.D.	Otolaryngologist Director	CDP	NINCDS
	Celia Bassich, M.A.	Speech Pathologist	SPU, HMCS, MNB	IRP NINCDS
	Young J. Lee, Ph.D.	Biostatistician		OBFS NINCDS

COOPERATING UNITS *(if any)*

Communicative Disorders Program, NINCDS

LAB/BRANCH
Medical Neurology Branch, Intramural Research Program

SECTION
Speech Pathology Unit, Human Motor Control Section (HMCS, MNB)

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.55	.25	.30

CHECK APPROPRIATE BOX(ES)

☑ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The project objectives are: 1) to develop quantitative measures of phonation in normalcy and patients with laryngeal pathology: 2) to determine which measures of phonation are sensitive to laryngeal pathologies; and 3) to determine the patterns of vocal fold movement associated with particular acoustic attributes of phonation. A study was completed assessing the validity of using an acoustic measure of frequency perturbation (random variations in cycle length) for the non-invasive detection of laryngeal pathology. Ninety-five normal subjects were tested to determine the relationship of age, sex, phonatory intensity, fundamental frequency, maximum phonation length and drinking and smoking histories with frequency perturbation. A multiple regression model employing 4 factors was able to predict frequency perturbation in the normal population with 70% accuracy. This model was used to predict the 90% normal confidence interval for normal expected of frequency perturbation for 31 patients with laryngeal carcinoma, nodules, polyps, unilateral paralysis and edema. The measured frequency perturbation exceeded the normal expected level in only 35% of patients and was accurate only for the detection of carcinoma. Thus, frequency perturbation was not a good measure of phonatory abnormalities due to laryngeal pathology. These results suggest that random variations in frequency do not result from morphological changes in the vocal folds in the majority of cases. Other acoustic measures are now being examined for their sensitivity to laryngeal pathology. Investigations will be initiated to determine the relationship between patterns of vocal fold vibration and acoustic attributes of phonation in normalcy and laryngeal pathology.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02337-07 MNB

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Auditory Processing and Language Skills in Behavioral Disorders

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	Christy Ludlow, Ph.D.	Speech Pathologist Chief	SPU HMCS MNB	IRP NINCDS
Others:	Judith Rapoport, M.D.	Chief, Section on Child Psychiatry	LCS	NIMH
	Thomas Insel, M.D.	Psychiatrist	CNB	NIMH

COOPERATING UNITS *(if any)*
Section on Child Psychiatry, LCS, CNB, NIMH

LAB/BRANCH
Medical Neurology Branch, Intramural Research Program

SECTION
Speech Pathology Unit, Human Motor Control Section (HMCS, MNB)

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.25	.15	.10

CHECK APPROPRIATE BOX(ES)
☑ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☑ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this project was to determine the effects of behavioral disorders on language processing, speech perception and auditory processing skills in children. Two studies have been completed this year with final manuscripts in preparation. Obsessive-compulsive adolescents were examined for signs of language processing deficits and laterality on speech perception testing to determine whether deficits were suggestive of left hemisphere dysfunction as has previously been suggested. The pattern of impairments did not confirm this hypothesis. Rather, the results suggested that performance on tasks requiring complex mental operations were interfered with by intrusive thoughts or behaviors in this syndrome. Another study has been completed in adults with obsessive-compulsive disorder with a similar pattern of results. A study of the effects of attention deficit disorders in children on auditory processing and language was completed this year confirming the relationship of attention deficits with auditory processing deficits, and the independence of both from language processing skills. The final stages of subject testing are being completed in a study of the effects of d-amphetamine on the auditory processing and communi-cative language skills of language delayed children with attention deficit disorder. The purpose is to determine whether stimulants can be benefical in the treatment of language and auditory processing deficits. With completion of this study, this project will be integrated into Z01 NS 02563-02.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02185-10 MNB

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 charecters or less. Title must fit on one line between the borders.)*
Patterns of Speech Breakdown in Neurological Disease

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	Christy Ludlow, Ph.D.	Speech Pathologist Chief	SPU HMCS MNB	IRP NINCDS
Others:	Nadine Connor, M.A.	Speech Pathologist	SPU HMCS MNB	IRP NINCDS
	Celia Bassich, M.A.	Speech Pathologist	SPU HMCS MNB	IRP NINCDS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Medical Neurology Branch, Intramural Research Program

SECTION
Speech Pathology Unit, Human Motor Control Section, (HMCS, MNB)

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.60	.30	.30

CHECK APPROPRIATE BOX(ES)

☑ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standerd unreduced type. Do not exceed the space provided.)*

The project objectives are: 1) to develop quantitative measures of speech production in normal adults and patients with neurological disorders; 2) to identify measures sensitive to changes in speech associated with neurological disorders; and 3) to identify separate aspects of speech production independently affected in neurological disorders as an indication of the components of the speech production system. Three studies have been completed this year. A study of normal speech production demonstrated adverse effects of increased age (between 40 and 80 years) on: speaking rates; phonatory reaction times; maximum and range in intensity; and use of phonatory frequency to differentiate stress. Male speakers had better performance than females in phonation time; speaking rate; maximum rate change; rate of laryngeal adduction-abduction; and mean, range and maximum intensity levels. Timing for speech articulation in normals was slower on tasks coordinating tongue with laryngeal movements than those with the larynx alone or the larynx, lips and jaw combined. Patients with involvement limited to the autonomic system (idiopathic orthostatic hypotension) were without speech production deficits in comparison with normal. Patients in the early stages of multiple systems atrophy (Shy-Drager Syndrome) were significantly impaired in comparison with normal on all measures of laryngeal movement for speech, suggesting a selective effect on vocal fold function in the early stages of the disease.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02557-03 MNB

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Location and Size of Brain Lesions Associated with Speech Deficits

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	Christy Ludlow, Ph.D.	Speech Pathologist Chief	SPU, HMCS, MNB IRP NINCDS	
Others:	Andres Salazar, M.D.	Neurologist	VHIS	WRAMC
	Grace Yeni-Komshian, Ph.D.	Guest Researcher	SPU, HMCS, MNB IRP NINCDS	
	Jeannette Rosenberg, M.S.	Speech Pathologist	VHIS	WRAMC

COOPERATING UNITS *(if any)*
Vietnam Head Injury Study, Walter Reed Army Medical Center

LAB/BRANCH
Medical Neurology Branch, Intramural Research Program

SECTION
Speech Pathology Unit, Human Motor Control Section, (HMCS, MNB)

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.55	.25	.30

CHECK APPROPRIATE BOX(ES)
☑ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The aim is to improve understanding of the neurological organization of the speech and language system by determining the correspondence between long term sequelae of penetrating head injuries and the location and size of brain lesions. Neuroradiological coding of brain structures from CT scans and measurement of lesion volume within brain regions is conducted independent of experimental speech production, speech perception and language processing studies. In a study completed this year, patients with residual expressive syntactic deficits were identified and compared with patients who recovered from Broca's aphasia within 5 years following head injury. The language test results demonstrated a specific syntactic residual in language expression, comprehension and reading and writing in addition to non-fluent speech in the non-recovered group. The only residual deficit in the recovered group was in written syntax. CT scan results of the two groups demonstrated that Broca's area was involved in both groups. Only the non-recovered patients with syntactic deficits and non-fluent speech had the lesions which extended posteriorly to involve Wernicke's area, the underlying white matter and the caudate. Subject testing for the entire project will be completed in September 1984, including over 500 head-injured cases and 100 non-head injured veterans. Data analysis will continue for a full year examining groups with specific speech and language residuals.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02247-08 MNB

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 charecters or less. Title must fit on one line between the borders.)*
Speech and Language Abnormalities in Tourette Syndrome

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	Christy Ludlow, Ph.D.	Speech Pathologist Chief	SPU HMCS MNB	IRP NINCDS
Others:	Roswell Eldridge, M.D.	Medical Geneticist	NEB	IRP NINCDS

COOPERATING UNITS *(if any)*
NEB, IRP, NINCDS

LAB/BRANCH
Medical Neurology Branch, Intramural Research Program

SECTION
Speech Pathology Unit, Human Motor Control Section, HMCS, MNB

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.25	.15	.10

CHECK APPROPRIATE BOX(ES)
- ☑ (a) Human subjects
  - ☑ (a1) Minors
  - ☐ (a2) Interviews
- ☐ (b) Human tissues
- ☐ (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Subject testing for the final study of the project has been completed. This study examined the effects of haloperidol on facial, oral and vocal tics and language processing deficits in monozygotic twins with Tourette Syndrome and attention deficit disorder. The purpose was to determine: 1) whether the response to haloperiodol was beneficial in these patients with Tourette Syndrome with attention deficit disorder: 2) whether haloperiodol benefitted the language processing and attentional deficits in these patients; and 3) whether a similar type and degree of response to haloperidol was found in both twins. The final stages of analysis of video tapes, language tests and auditory vigilance tests are close to completion. With the completion of this study, this project will be integrated into Z01 NS 02563-02.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02564-02 MNB

PERIOD COVERED: October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Relationships Between Language and Speech Deficits in Neuropathologies

PRINCIPAL INVESTIGATOR *(List other professionel personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	Christy Ludlow, Ph.D.	Speech Pathologist SPU, HMCS, MNB IRP NINCDS Chief
Others:	Celia Bassich, M.A.	Speech Pathologist SPU, HMCS, MNB IRP NINCDS
	Nadine Connor, M.A.	Speech Pathologist SPU, HMCS, MNB IRP NINCDS
	Grace Yeni-Komshian, Ph.D.	Guest Researcher SPU, HMCS, MNB IRP NINCDS

COOPERATING UNITS *(if any)*:

LAB/BRANCH: Medical Neurology Branch, Intramural Research Program

SECTION: Speech Pathology Unit, Human Motor Control Section (HMCS, MNB)

INSTITUTE AND LOCATION: NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.50	.20	.30

CHECK APPROPRIATE BOX(ES)

- ☑ (a) Human subjects
  - ☐ (a1) Minors
  - ☐ (a2) Interviews
- ☐ (b) Human tissues
- ☐ (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose is to determine the relationship between impairments in speech production and language in patients with different neurological diseases. The degree of language impairment and the relationship between deficits in speech production, language processing, and speech perception in various forms of neurological diseases is unclear. The speech problems of patients with dysarthria associated with Parkinson's disease have previously been thought to be neuromotor. Recent results have indicated that these patients may also have language encoding and decoding difficulties which could further confound their neuromotor difficulties. Three studies are ongoing. The results of testing language encoding and decoding in patients with Parkinson's disease are being examined for relation-ship with several other characteristics such as duration of disease, side of involvement, drug treatment and speech impairment. Auditory processing and speech perception studies are ongoing in patients with Huntington's disease. Finally, a unique case of auditory agnosia was studied and found to exhibit normal peripheral hearing with no volitional response to sound and intact phonological coding. This case demonstrated the independence of phonological decoding from auditory perception.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02563-02 MNB

PERIOD COVERED: October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Rate Manipulation and Neuropharmacological Effects on Speech

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation)*

P.I.:	Christy Ludlow, Ph.D.	Speech Pathologist Chief	SPU, HMCS, MNB	IRP NINCDS
Other:	Nadine Connor, M.A.	Speech Pathologist	SPU, HMCS, MNB	IRP NINCDS

COOPERATING UNITS *(if any)*

LAB/BRANCH: Medical Neurology Branch, Intramural Research Program

SECTION: Speech Pathology Unit, Human Motor Control Section (HMCS, MNB)

INSTITUTE AND LOCATION: NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.50	.20	.30

CHECK APPROPRIATE BOX(ES)

☑ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose is to determine to what extent speech production can be altered in neurological disease by either behavioral manipulation or neuropharmacological treatment. One study was completed this year. The aim was to examine the relationship between speech rate, repetition rates and reaction times for the various articu-lators. Since reaction time tasks assess the initiation of a response by the articulators, the purpose was to determine whether speech rate was related to such reaction times in two different patient groups. If speech rate is related to articulator reaction times, then patients' speech rates may not be easily manipulated. Similarly, if the maximum rate of syllable offset is related with speech rate, impaired speeds of movement offset could be a limiting factor on patients' abilities to change their speech rate. Patients with Parkinson's disease and Huntington's disease were studied. Both groups demonstrated a relationship between syllable offset time and speech rate indicating that movement offset time may be a limiting factor. Reaction time and speech rate were not related in either group. The Huntington's patients had slower reaction times than normal for speech movements requiring laryngeal and lip coordination and were excessively slow on all repetitive and speech rate measures. The Parkinson's patients were only affected on movements requiring rapid changes between laryngeal adduction and abduction. These results suggest that speech rate might be more easily manipulated in Parkinson's disease patients than in Huntington's disease patients.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02562-02 MNB

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Characteristics of Voice Disorders of Unknown Etiology

PRINCIPAL INVESTIGATOR *(List other professionel personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	Christy Ludlow, Ph.D.	Speech Pathologist Chief	SPU, HMCS, MNB	IRP NINCDS
Others:	Ralph Naunton, M.D.	Otolaryngologist Director	CDP	NINCDS
	Nadine Connor, M.A.	Speech Pathologist	SPU, HMCS, MNB	IRP NINCDS
	Daniel Weinberger, M.D.	Neurologist	ETB	NINCDS

COOPERATING UNITS *(if any)*

Communicative Disorders Program
ETB, IRP, NINCDS

LAB/BRANCH

Medical Neurology Branch, Intramural Research Program

SECTION

Speech Pathology Unit, Human Motor Control Section (HMCS, MNB)

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.55	.25	.30

CHECK APPROPRIATE BOX(ES)

☑ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

An examination of the phonatory and speech motor control characteristics of patients with spasmodic dysphonia is being conducted to develop a model of the neurological processes involved in this disorder. Three studies have been completed. Patient characteristics predictive of benefit from unilateral surgical resection of the recurrent laryngeal nerve (RLN), were identified. The results suggested two subtypes of this syndrome with only patients with one subtype considered as good candidates for surgical section. In another study, experimental tasks assessing control over phonatory gestures controlled by the RLN, and others controlled by the superior laryngeal nerve were administered to 8 patients. Only tasks involving the RLN were impaired in comparison with normal. Laryngographic signals demonstrated excessive prephonatory changes in laryngeal height and vocal fold movements prior to phonation in patients. The third study compared laryngeal control pre- and post RLN resection. Abnormal prephonatory laryngeal movements persisted post surgical section suggesting that the movement disorder was unaltered. An ongoing study of timing and coordination of respiratory movements prior to the onset of phonation is aimed at determining whether this is an upper motor neuron disorder affecting the coordination and timing of both laryngeal and respiratory movements. A double blind crossover study of Buspirone for the treatment of this disorder is ongoing.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02115-11 MNB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biochemical Indices of Adrenergic Function in Humans

PRINCIPAL INVESTIGATOR *(List other professionel personnel below the Principal Investigetor.) (Name, title, laboratory, and institute affiliation)*

Ronald J. Polinsky, Chief, CNS, MNB, NINCDS
Robert T. Brown, CNS, MNB, NINCDS
Lillian Recant, Division of Endocrinology, VA Hospital, Washington, D.C.
Richard S. Burns, ODIR, NINCDS
David S. Goldstein, Hypertension-Endocrine Branch, NHLBI

COOPERATING UNITS *(if any)*

Division of Endocrinology, VA Hospital, Washington, D.C.
Hypertension-Endocrine Branch, NHLBI

LAB/BRANCH

Medical Neurology Branch, IRP, NINCDS

SECTION

Clinical Neuropharmacology Section (CNS), MNB

INSTITUTE AND LOCATION

NINCDS, NIH Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Autonomic nervous system activity is essential for maintaining circulatory and metabolic homeostasis. In order to study sympathetic nervous system function and its relationship to other neuroendocrine systems, it is necessary to measure neurotransmitter, hormonal, and peptide levels in response to various stimuli. The levels of norepinephrine, epinephrine, and dopamine and their metabolites in various body fluids reflect the activity of the neurones from which these neurotransmitters are released. Although plasma levels of norepinephrine reflect the responses of the peripheral sympathetic nervous system it is necessary to consider removal rates of the catecholamine. Measurement of urinary catecholamine metabolites and their stereospecific labelling pattern following administration of radiolabelled isomers of norepinephrine provides a means for investigating intraneuronal norepinephrine metabolism. Cerebrospinal fluid levels of monoamine metabolites can be used to assess central nervous system neurotransmitter metabolism. It is necessary to consider the origin of these metabolites to make appropriate corrections for valid interpretations of the data. These strategies have been used to study patients with neurogenic orthostatic hypotension and in other clinical situations in which adrenergic function is abnormal. Investigation of the effects of aging on autonomic nervous system function is in progress. A more thorough understanding of neurotransmitter metabolism in these clinical situations leads to more rational approaches to therapy.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02630-01 MNB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Clinical, Genetic, and Biochemical Studies of Familial Alzheimer Disease

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Ronald J. Polinsky, Chief, CNS, MNB, NINCDS
Linda E. Nee, LCS, NIMH; Jay Robbins, Dermatology Branch, NCI
Robert T. Brown, CNS, MNB, NINCDS
James Gusella, Genetics Unit, Dept. of Neurology, Mass. General Hospital,Boston,MA
Michael Conneally, Department of Genetics, Indiana University
Luigi Amaducci, Department of Neurology, Univ. of Florence, Italy
Jean-Francois Foncin, Laboratory of Histopathology, Le Salpetriere, Paris, France
Herbert Weingartner, Laboratory of Neuropsychology, NIMH

COOPERATING UNITS *(if any)* Laboratory of Histopathology, Le Salpetriere, Paris, France
Laboratory of Clinical Science, NIMH; Laboratory of Neuropsychology, NIMH
Genetics Unit, Dept. of Neurology, Mass. General Hospital, Boston, MA
Department of Genetics, Indiana University; Dept. of Neurology, Univ. of France

LAB/BRANCH

Medical Neurology Branch, IRP, NINCDS

SECTION

Clinical Neuropharmacology Section (CNS), MNB

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.0	3.0	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Alzheimer disease is the most common cause of irreversible, chronic dementia. One factor which complicates the interpretation of many clinical research studies is that 20% or more of clinically diagnosed cases do not have Alzheimer disease at autopsy. Although Alzheimer disease may be inherited in less than 10-20% of all cases, the main justification for studying familial cases lies in the accuracy of diagnosis which may be inferred through post-mortem examination of other affected family members.

Previous genetic studies have not clarified the role of inheritance. Recent advances in the field of molecular biology have resulted in the development of recombinant DNA technology. Other molecular approaches that are being used to study degenerative neurological disorders include investigations of DNA repair, immunological function and abnormal protein production. In this project skin fibroblast and peripheral blood lymphoblast cultures will be established from members of large kindred with familial Alzheimer disease. These cultures will serve as a renewable source of DNA and cell lines which can be used for genetic linkage, viability, and biochemical studies.

Alzheimer disease may result from a form of primary neuronal degeneration. Neurotransmitter studies suggest that there is a central nervous system degeneration of cholinergic neurons. However, there is substantial evidence which shows that the locus ceruleus, an important noradrenergic nucleus, is also involved as well as other neurotransmitter and peptide systems. In order to define the natural history, temporal progression, and biochemical abnormalities in Alzheimer disease, this project will include a longitudinal study of affected and at-risk subjects from large kindreds with familial Alzheimer disease. Detailed neuropsychological testing, PET scanning, NMR imaging, neurotransmitter studies, and pharmacological investigations are planned. Neuropathological and neurochemical studies of post-mortem specimens from these families will also be conducted.

TAB 19 -- NEUROEPIDEMIOLOGY BRANCH -- (NEB)

ANNUAL REPORT

October 1, 1983 through September 30, 1984

Neuroepidemiology Branch
National Institute of Neurological and Communicative Disorders and Stroke

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Annual Report
for Period October 1, 1983 through September 30, 1984
Neuroepidemiology Branch
Intramural Research Program
National Institute of Neurological and Communicative Disorders and Stroke

Bruce S. Schoenberg, M.D., Dr.P.H., Chief

The Neuroepidemiology Branch is responsible for the development and implementation of epidemiologic and genetic programs to investigate the cause, prevention, and treatment of neurologic disorders in human populations. Emphasis has been placed on major neurologic diseases in which the diagnoses can be clinically verified to the satisfaction of skilled neurologists. The Branch is unique in being the only unit devoted exclusively to research in the epidemiology of diseases of the nervous system. Neuroepidemiologic research studies require collaboration of many individuals. However, since there is a severe shortage of available manpower in neuroepidemiology, the Branch developed an active teaching program for current and future collaborative investigators. A series of six videotapes produced by the Branch are distributed on a loan basis without charge. A textbook, entitled NEUROLOGICAL EPIDEMIOLOGY: PRINCIPLES AND CLINICAL APPLICATIONS, has been prepared, and a scientific quarterly journal entitled NEUROEPIDEMIOLOGY has been in publication since 1982. This journal received an Award of Merit from the Society for Technical Communication. In co-operation with the World Health Organization and the World Federation of Neurology Research Committee on Neuroepidemiology, formal courses were conducted in Caracas, Venezuela, and Shanghai, the People's Republic of China. Additional courses will be held in Nijmegen, the Netherlands; Bombay, India; and Jerusalem, Israel. A meeting of the Research Committee on Neuroepidemiology of the World Federation of Neurology was organized by our branch in Boston. Representatives from Colombia, Ecuador, Italy, the People's Republic of China, Switzerland, the U.S., and Venezuela attended the session and presented data based on a uniform protocol. A workshop was held in Bethesda to plan research strategy to investigate the problem of spastic paraparesis in different parts of the world. This is a significant problem in Colombia, India, the Seychelles Islands, and the West Indies. Further studies are

planned in these countries. A large study of mental retardation is being planned using a uniform protocol for implementation in Ecuador, India, and the People's Republic of China. Neuroepidemiology has been selected as one of the four main themes for the next World Congress of Neurology to be held in Hamburg, W. Germany in 1985. These sessions serve as a stimulus for neuroepidemiologic research on a worldwide basis. We are also providing opportunities for fellows to spend from six months to one year working with members of the Branch in order to learn the techniques of neuroepidemiology. During the past year we have had physicians from Ecuador, Kenya, Nigeria, Mexico, Turkey, India, Spain, Italy, the People's Republic of China, Costa Rica, and Peru, and have received inquiries from Tunisia, and Israel for future opportunities. There is considerable neuroepidemiologic interest among senior neurologists (one of the physicians working in the Branch is a professor and chairman of his own unit abroad). Finally, current individual and institutional research training grant programs have been expanded to include neuroepidemiology. Institutional grants for training in neuroepidemiology have been awarded to Columbia University, New York, the University of California at Los Angeles, and Temple University, Philadelphia. In addition to an educational program, the Branch has focused on research investigations.

Epidemiologic studies have two basic requirements: uniformity and accuracy of data collection. This necessitates the use of a standardized, internationally accepted classification and coding system. The most recent scheme generated by the World Health Organization is seriously deficient with regard to neurologic disorders. The Branch is therefore collaborating with the World Health Organization Neurosciences Program, the World Federation of Neurology, and the American Academy of Neurology to revise this system of classification and improve its usefulness for neuroepidemiologic research. Two members of the Branch were selected to serve on the advisory committee to the World Health Organization to make recommendations for changes in this classification. The first meeting of this committee was held in Geneva, Switzerland in April 1984. During this session the basic structure of the revised classification was established. A second meeting is planned for September 1984.

Another important problem for the neuroepidemiologist is the enormous cost of maintaining neurologic surveillance on a large number of patients. Therefore, we have attempted to utilize existing registries of neurologic disease, such as in a study of presenile dementia based on the Israeli National Neurologic Disease Registry. In addition, we have assisted British investigators in organizing information routinely collected through the British National Health Service on all neurologic inpatients in a section of London with a population of 3-1/2 million inhabitants. The utility and accuracy of

these data have been demonstrated in a study of the Guillain-Barré syndrome. A similar registry is being organized for the population of northeastern Italy. We also collaborate with the Mayo Clinic in Rochester and utilize their record-linkage system to study neurologic diseases in the population of Rochester, Minnesota.

There have been a number of neuroepidemiologic case-control studies which have suggested associations between a given factor and a particular disease, but the number of patients has been inadequate for meaningful conclusions. We are working in collaboration with a number of clinical units in Italy to conduct case-control studies of clinically diagnosed cases of Alzheimer's disease. Similar arrangements have been made to work in conjunction with the Alzheimer's Disease and Related Disorders Association. The first study in collaboration with this Association is currently in progress in Denver, Colorado. These several projects in support of research activities have been initiated in conjunction with a very active research program.

With regard to neurologic problems in children, the Branch documented the frequency of primary intracranial neoplasms in the pediatric population of Rochester, Minnesota, and the State of Connecticut. In addition, we investigated cerebrovascular disease in infants and children. The magnitude of this problem was documented for the first time. The study demonstrated that neonatal intracranial hemorrhage is relatively common (1.1 cases/1,000 live births), that it is strongly associated with prematurity and hyaline membrane disease, and that it is difficult to recognize clinically. For pediatric cerebrovascular disease unassociated with birth, trauma, or infection, the incidence rate was 2.5/100,000/year. These cases were further characterized by survival, residual disability, and cause (whenever possible). The clinical and angiographic features of children with moyamoya disease were examined in detail. This condition appears to be more common than suggested by early case reports.

The Branch is also investigating the epidemiology of cerebral palsy (CP). A study of temporal trends in the incidence rate of CP for Rochester, Minnesota, addressed the concern that advances in perinatal care, by rescuing the compromised neonate, are increasing the rate of neurologic handicap. All identified cases of CP born to Rochester residents during a 27-year period were studied. The overall incidence rate of CP declined from 2.3 to 1.6 cases per 1,000 neonatal survivors. Correlation of birthweight-specific rates of neonatal mortality and CP incidence showed that for the low birthweight neonate, coincident with a marked drop in mortality, the CP incidence rate remained unchanged. For the newborn with birthweight over 2500 g., the rates of CP incidence and neonatal mortality declined in parallel. In a

study of CP outcome, a decreased survival was limited to individuals who needed custodial or total nursing care. For the remainder of the case sample, all survived a minimum of 10 years, and in several of the cases there was resolution of the motor handicap.

Studies of neonatal mortality were initiated by the Branch because antecedents of pre- and perinatally incurred neurologic handicap and those of neonatal death overlap. While uniform and complete case identification in a large population over a long period of time is not available for CP, infant death/birth certificate linkage provides such case identification for neonatal death. Using the infant death/birth file of the State of Minnesota, the Branch is now completing two descriptive investigations of neonatal mortality: 1) delineation of neonatal mortality rates (NMR) by sex in gestational age/birthweight-specific subgroups for years 1970-1980, and 2) a study of sex- and birthweight-specific NMR trends for years 1967-1976. The future objective of both the Rochester CP incidence study and Minnesota NMR study is to conduct case-control studies in search of maternal, fetal, and obstetric risk factors of CP and of neonatal death.

The Branch has conducted extensive investigations of primary intracranial neoplasms. First, problems with nomenclature and disease definition were resolved. A number of descriptive studies were carried out, revealing two patterns of age-specific incidence. Analyses of most population-based data worldwide demonstrated a small childhood peak, followed by a later peak between ages 50 and 80. Data for Rochester, Minnesota, however, showed the childhood peak, followed by an increasing incidence rate with increasing age. Careful study of this discrepancy showed 1) that the greater percentage of cases first diagnosed at autopsy in Rochester accounted in large part for this difference, and 2) that a substantial number of brain tumors remain undiagnosed in the elderly during life. Studies have just been completed to evaluate the role of computerized tomography in the diagnosis of brain tumors and to explain the recent increase in the incidence of pituitary tumors among women of childbearing age. The introduction of computerized tomography has not resulted in any increase in the reported frequency of these tumors in the Rochester, Minnesota population, while the apparent rise in the incidence of pituitary tumors seems to be the result of more sophisticated neuroendocrine diagnostic procedures. A comprehensive study of U.S. and international mortality data for primary nervous system neoplasms over a 15-25 year period demonstrated an increasing death rate, especially among the elderly. This was thought to be due to improved diagnosis and case ascertainment. An exhaustive, critical review of a survey strategy to measure the national incidence and prevalence of intracranial neoplasms has been completed. In addition, racial differentials in the frequency of certain intracranial tumors

(meningiomas and pituitary adenomas) are being examined. Investigations of the relationship between intracranial neoplasms and extracranial tumors have been especially rewarding. An association was found between the occurrence of breast cancer and meningioma in women. This result raises interesting etiologic possibilities when considered with other evidence: 1) meningioma is the only common intracranial neoplasm with a higher incidence in females; 2) the abrupt clinical appearance or enlargement of this tumor during pregnancy has been described; and 3) the finding of estrogen receptor protein in meningioma has been reported.

The record-linkage system for Rochester, Minnesota, has also been used to identify all possible cases of complex partial seizures occurring in the years 1960-1980. A case-control study is being designed to identify risk factors associated with the occurrence of such seizures.

At the present time, there is little to suggest that improved medical management of the completed stroke will substantially affect the cerebrovascular disease problem. It would appear that greater benefit could be achieved by dealing with the precursors of stroke rather than delaying treatment until after the event has occurred. Therefore, a non-concurrent, prospective study of a cohort of 2,000 elderly individuals was undertaken to determine the role of heart disease and hypertension as risk factors for <u>both</u> transient ischemic attacks (TIA) and completed stroke. When the case-control approach was applied to these data, different patterns of risk factors were demonstrated for transient ischemic attacks and completed ischemic stroke. While hypertension, diabetes mellitus, definite hypertensive heart disease, and valvular heart disease are important risk factors for completed ischemic stroke, these disorders do not have a substantial effect on the subsequent risk of TIA. When these data were analyzed in the format of a prospective study, it was possible to calculate the absolute risk of stroke as a function of the presence or absence of specific forms of cardiovascular disease. The following types of cardiovascular disease yielded the highest completed ischemic stroke incidence rates (cases/1,000/year): myocardial infarction (15.5); congestive heart failure (20.5); and TIA (42.0). In considering risk factors for TIA, both angina/coronary insufficiency and congestive heart failure yielded the highest rates (10.4 and 10.9, respectively). Once etiologic precursors of stroke have been identified, medical intervention before the occurrence of long-lasting disability requires that there be an interval of time between the onset of the risk factor and the development of completed stroke. Analysis of data from this non-concurrent prospective study revealed that those developing borderline hypertension, valvular heart disease, or ischemic heart disease remained stroke-free for the initial one and one-half years after the first occurrence of each specific form of

cardiovascular disease. This finding implies that there is an interval of time following the onset of these conditions when it may be possible to intervene medically to reduce the risk of stroke.

Previous studies of stroke incidence have generally utilized one of two techniques: a) survey of an entire community to identify all cases of stroke or b) survey of all community residents hospitalized for stroke in medical institutions serving that population. Rates derived from community surveys are usually higher than those obtained from hospital statistics. To quantify the size of the error inherent in using hospitalized cases, we applied both methodologies to the same population. Cases of completed stroke occurring among residents of Rochester, MN, during 1955-1969 were verified by neurologic review of data from a records-linkage resource. In this community, patients are hospitalized following stroke on the basis of medical necessity. Records for all 993 patients were reviewed to determine whether the patient was admitted to an acute care hospital for the stroke. Overall, 69% of stroke cases were admitted to an acute-care facility. This study suggests that incidence rates derived from hospital data underestimate the frequency of new strokes by 25-30%; this discrepancy is most marked in the elderly. Another investigation based in this same community studied stroke in patients already hospitalized for other conditions. Sixty-five individuals suffered a first completed stroke while in a short-stay hospital for either a medical problem or surgical procedure. This represents 6.5% of all first strokes in the Rochester population. The percentage of all first completed strokes occurring during a short-stay hospitalization was slightly higher for women (8%) than for men (5%). In 74%, the stroke was directly related to medical conditions or surgical procedures. Etiologic factors preceding stroke, in order of frequency, were acute heart disease (21), major surgical procedures (10), fractures (8), leukemia or blood dyscrasias (5), acute gastrointestinal bleeding (3), and cerebral angiography (1). In the remaining 17 patients without an obvious event or clearly attributable etiologic factor leading to the stroke, all but 5 had either diabetes mellitus, chronic heart disease, or hypertension. There were 99 additional Rochester residents suffering a first completed stroke while in a nursing home or chronic care facility, raising the total strokes in residents of hospitals or nursing homes to 11.5% of all first strokes in the community.

Other investigations in the area of stroke involve a careful analysis of unusual patterns of cerebrovascular disease (e.g., more than 20 TIA's/day).

Alzheimer's disease/senile dementia, despite its high apparent clinical frequency among the elderly, has not been well studied in a U.S. population. Thus a major effort is

being made by the Branch to study dementia in general and Alzheimer's disease in particular. Three descriptive studies based on well-defined populations have been conducted. One is derived from a review of detailed clinical records utilizing a population-based, records-linkage system. A neurologist using fixed diagnostic criteria reviewed records from all medical facilities serving the residents of Rochester, Minnesota. This made it possible for the first time to determine the incidence of dementia coming to medical attention in a well-defined U.S. population. For those age 30 plus, the incidence rate was 110 new cases/100,000 population/year. The rates increase with age, and the age-specific rates were higher in women. To confirm the reduced survival of demented patients reported on the basis of individuals hospitalized at specific medical centers, we examined the survival of all demented individuals identified through our records-linkage study. Dealing with an entire population minimizes any possible selection bias that may be present for a series of patients seen at a particular medical institution. The survival rates generated for all demented patients in the defined population were significantly reduced compared to age- and sex-matched survival statistics derived from life-tables for residents of the northwest central region of the U.S., thereby documenting in a community study previous observations based on hospitalized patients.

The second investigation, a two-stage survey, permitted us to estimate the prevalence of dementia in a biracial community. For each race, prevalence ratios were higher for females. For each race and sex, the prevalence figures rise dramatically with age. This morbidity study indicates that dementia represents a major health problem for both racial groups.

A third population-based study was conducted in Israel. There has been some debate as to whether Alzheimer's disease is a single disease entity regardless of its age at presentation. Since the frequency of Alzheimer's disease is relatively low before age 60, an enormous population is required for surveillance in order to obtain an adequate number of patients for study. We have therefore utilized the resources available through the Israeli National Neurologic Disease Registry to identify all potential cases among the population of Israel. These cases were intensively reviewed to determine the accuracy of diagnosis and to explore a number of epidemiologic studies of the distribution and risk factors for this disease. A similar pattern has emerged for those age 60 and under as has been described in previous studies for older individuals. The incidence rates increase with age, and the disease is slightly more common in women. Of particular interest is the finding that the risk of early-onset Alzheimer's disease (age 60 years and earlier) is significantly higher among Jews of European-American origin compared to those born in Africa or Asia.

In addition, three case-control studies have been planned. The first utilizes cases and controls selected from the Rochester, Minnesota population. Past medical records will be utilized to obtain information concerning possible associations between Alzheimer's disease and either medical conditions or surgical procedures. Two case-control studies of Alzheimer's disease utilizing interview data are being carried out in conjunction with a) the Alzheimer's Disease and Related Disorders Association, and b) the Italian National Research Council. The latter two studies are utilizing a similar protocol. Patients affected by Alzheimer's diseases or senile dementia of the Alzheimer's type have been identified by means of a specific protocol employing a defined algorithm. Since most of the patients are unable to give adequate information at the interview because of the mental impairment, a questionnaire for a next-of-kin interview has been prepared. The questionnaire attempts to obtain information on various risk factors.

Investigations are in progress to identify familial cases of Alzheimer's disease in the Italian population. These familial cases will be studied in great detail utilizing the latest technology available such gene mapping. Methodologies for sample collection and transportation have been discussed with the Camden cell-line depository.

Yet another approach will utilize information obtained from clinical examination and combine it with autopsy data, thereby establishing a more definitive diagnosis of Alzheimer's disease. The objective of this study is to highlight the clinical characteristics which are most closely associated with pathologically proven Alzheimer's disease. This should help improve clinical diagnosis.

In addition a careful review of the literature on dementia since 1907 has been done. Special attention has been given to the cases of dementia originally described in Alzheimer's laboratory in Munich (Germany). Using the United Nations population projections for the 20-year period 1980-2000, the possible effect of demographic trends on senile dementia prevalence in several "developed countries" (United Nations definition) has been studied.

The Branch is also interested in accurately documenting possible racial differentials in the prevalence of major neurologic disorders. A number of early investigations suggested possible differences by race, but were based on hospital or clinic experience and could not identify a well-defined population from which cases were derived. Population-based studies followed, but questions concerning the results centered on possible racial differentials in access to expertise in neurologic diagnosis and treatment. We reinvestigated (in conjunction with the Surveys and Demographic

Studies Branch, BFSB, IRP, NINCDS) this problem of possible racial differentials in the prevalence of major neurologic disorders by surveying a well-defined population (approximately 25,000, almost equally divided between blacks and whites). We developed a strategy which eliminated the requirement that persons must have entered the health-care system for detection of disease. The disorders investigated included cerebral palsy, dementia, psychomotor delay, epilepsy, Parkinson's disease, essential tremor, and cerebrovascular disease (both transient ischemic attacks and completed stroke). The basis of the investigation was a door-to-door survey which utilized a detailed questionnaire inquiring not only about diagnoses, but also about signs and symptoms suggestive of neurologic dysfunction. Over 97% of the households agreed to the interview. Those household members suspected of having one of the disorders of interest were then asked to have a neurologic examination conducted by a senior, board-certified neurologist. The interviews and examinations have been completed, and the data are being edited and analyzed. Data currently available for Parkinson's disease indicate that in the population studied, the disorder is more common in whites but the difference between races is not as great as suggested by earlier studies. The same survey yielded information on essential tremor, thereby providing the first data on the prevalence of this condition in a defined U.S. population. For either race, the prevalence ratios were slightly greater in women, and for either sex, the figures were slightly higher for whites. In this same population, it was also possible to measure the prevalence of cerebral palsy. Prevalence ratios of cerebral palsy were higher in males than in females, and greater in blacks than in whites.

Similar strategies are being developed for application in developing countries (e.g., Nigeria, Ecuador, India, the People's Republic of China, Peru, Ecuador, Chile, Tunisia, Senegal, and Venezuela), in collaboration with the World Health Organization. Preliminary results from pilot studies in Nigeria and the People's Republic of China have already revealed interesting findings. For example, migraine is as common among a rural black African population as among urban populations of Western Europe. Furthermore, epilepsy is a major problem in Nigeria, with a prevalence considerably higher than reported in developed countries. In areas of Beijing and Harbin, northern cities of the People's Republic of China, the incidence and prevalence of cerebrovascular disease is higher than anywhere else in the world where this problem has been studied. In addition, stroke follows a definite geographic pattern in China with the lowest rates occurring in southern China. A protocol to study the problem of mental retardation is being developed. This protocol will be applied in Ecuador, India, and the People's Republic of China.

We currently have very little information on the patterns of medical care received by all individuals with neurologic disease in a given community. The Branch is, therefore, studying this problem in Rochester, Minnesota. Although the findings of this investigation will not necessarily be applicable to other regions of the U.S., the City of Rochester does offer particular advantages. Cases of neurologic disease among residents have already been identified through previous studies. Medical encounters are easily documented through a records-linkage resource. In addition, Rochester residents have access to high-quality medical care, and physicians with neurologic expertise are available within the community. Thus, the Rochester experience may provide some estimate of the pattern of medical care in the ideal situation in which the population has ready access to neurologic expertise, and in which there is little financial restraint to such care. The study for patients with brain tumor is being prepared for publication, and similar data are being analyzed for completed stroke.

Although death certificate data are limited by possible misdiagnosis, incomplete case ascertainment, errors in coding, etc., detailed morbidity information on neurologic diseases for the entire U.S. and for other countries is not available. The Branch has analyzed mortality data for selected neurologic disorders by country and by county in the U.S. The overall patterns which emerge may be useful in evaluating trends over time and in formulating etiologic hypotheses. Among the most interesting findings is that the mortality from cerebrovascular disease has decreased in most developed countries over a 20-year period. This trend is not universal, however. For multiple sclerosis, countries initially reporting high mortality rates have generally reported declines, so that more recent mortality data for multiple sclerosis by country show less of a differential than previously reported. United States mortality rates for motor neuron disease and anencephaly were analyzed by county. For anencephaly, counties in the Mississippi River region and in the Appalachian Region had the highest rates. With regards to motor neuron disease, counties in the west (especially the northwest) had the highest rates and there was a positive association with rural farming. These leads will be pursued in more definitive studies.

Many neurologic disorders (such as epilepsy) are important causes of morbidity during life and may contribute to mortality indirectly. The potential for neurologic diseases to indirectly lead to death has been studied by analyzing national mortality data for the U.S. for the years 1971 and 1973 through 1978. Marked differences were found in the mortality rates for deaths due to and related to 20 categories of neurologic diseases studied except anencephaly. For example, the mortality rates for deaths related to epilepsy are more than double the rates for deaths due to epilepsy. This suggests

that mortality data for epilepsy based on underlying cause considerably underestimates the magnitude of the problem.

Diseases occurring together may provide important information in the search for etiology. Association of diseases occurring at the time of death was also studied for all deaths occurring in the U.S. from 1971, and 1973 through 1978. Case-control studies for associated conditions at the time of death for patients dying due to motor neuron disease, epilepsy, nervous system tumor, and cerebrovascular disease without hypertension have been conducted. Results have provided important new information; for example, the frequent association of infections with motor neuron disease suggests that aggressive management of infections may prolong longevity in these patients.

A number of other collaborative projects include the investigation of space/time clusters of neurologic disease (with the Centers for Disease Control and the Government of Colombia), the development of survey strategies (with the World Health Organization and the Section on Disease Statistics Surveys), a study of myasthenia gravis and multiple sclerosis in the same patient (with the Mayo Clinic), an investigation of neurologic disorders during pregnancy and the postpartum period (with the Mayo Clinic), a study of the epidemiology of eye tumors (with the Connecticut State Department of Health), the effect of weather on the incidence of stroke (with the Mayo Clinic), and international comparisons in the incidence of brain tumors. Finally, extensive reviews have been prepared on the epidemiologic aspects of Huntington's disease, otitis media, Alzheimer's disease, cerebrovascular disease, primary intracranial tumors, Tourette's syndrome, peripheral neuropathy, neurologic diseases in the elderly, controlled therapeutic trials of motor neuron disease, epilepsy, descriptive, analytic, and experimental methods in neuroepidemiology, statistical methods for calculating confidence intervals, and procedures for neuroepidemiologic investigations in developing countries.

The clinical neurogenetics component of the program involves three areas: 1) genetic-epidemiologic studies of movement disorders (e.g., the dystonias); 2) genetic-epidemiologic studies of multifactorial neurologic disorders (e.g., Parkinson's disease, Alzheimer's disease, and multiple sclerosis); and 3) genetic and biochemical studies of hereditary nervous system tumors.

Collaborative studies are planned with personnel in LCS, DCBR, NIMH to explain our observations of altered dopamine beta hydroxylase and norepinephrine levels in blood and biopterin in cerebrospinal fluid (CSF) in genetic subsets of dystonia patients. Based on low CSF biopterin in a form of familial dystonia, biopterin was administered intravenously leading to brief improvement in several members of this family.

Genetic study of 41 monozygotic twin pairs and 19 dizygotic twin pairs, selected because at least one member had Parkinson's disease, revealed only one monozygotic twin pair and none of the dizygotic pairs definitely concordant for the disease. Although the unaffected co-twin in each case remains at risk, this very low concordance suggests that neither typical environmental nor genetic factors are critical determinants. Data on smoking from three of our studies support an earlier impression that there is a decreased risk for Parkinson's disease in smokers. Analysis of clinical and psychological observation and interview data on 21 MZ twin pairs discordant for Parkinson's disease indicates life-long differences in personality are present in affected versus unaffected twins, as our preliminary study suggested.

The existence of a protective factor present in limited amount, supplied unequally to the twins in utero so that one twin is at less risk and the other at greater risk for Parkinson's disease, could explain these observations.

An hereditary leukoencephalopathy simulating MS, with onset at about age 35, is under study in a kindred with over 20 affected individuals. Derangement of the autonomic nervous system is often seen early in the course and when recognized, serves to distinguish this single gene disorder clinically from multiple sclerosis of the chronic progressive type. Computerized tomographic scan changes are highly characteristic.

Our studies have led to the recognition of at least two distinct genetic forms of neurofibromatosis: 1) the classical form as described by von Recklinghausen, and 2) a form in which bilateral acoustic neuromas are the hallmark. We have focused on neurofibromatosis with bilateral acoustic neuroma. Efforts have been directed at improving and simplifying screening high-risk individuals, confirming diagnosis, and establishing criteria for intervention. Audiologic studies, including evaluation of auditory-evoked response and acoustic reflex decay, are a useful, non-invasive means for early detection of acoustic neuroma and for following their growth.

In our first major study involving neurofibromatosis of the von Recklinghausen type, a multidisciplinary program is being prepared to evaluate specific neurologic and cognitive status in patients and their first-degree relatives.

Reviews are in preparation regarding the genetic epidemiology of movement disorders and of neurofibromatosis.

Awards to Branch personnel:

Dr. Schoenberg's contribution to the science of neuroepidemiology was recently recognized by his being awarded the NIH Commendation Medal for furthering understanding of the magnitude, distribution, and risk factors for cerebrovascular disease in the United States and thereby providing opportunities for prevention.

The journal Neuroepidemiology, of which Dr. Schoenberg is the Editor-in-Chief, recieved an Award of Merit from the Society for Technical Communication.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 01924-14 NEB

PERICD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Clinical, Genetic, Pathophysiologic Study of Hereditary Movement Disorders

PRINCIPAL INVESTIGATOR *(List other professionel personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Roswell Eldridge Medical Geneticist, NEB, IRP, NINCDS

COOPERATING UNITS *(if any)*
ET, IRP, NINCDS; HE, NHLBI; LCS, DCBR, NIMH

LAB/BRANCH
Neuroepidemiology Branch, Intramural Research Program

SECTION

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.75	0.25	0.5

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In this project, we seek to 1) clarify and expand the nosology of the hereditary movement disorders; 2) contribute to the understanding of the underlying biochemical basis; 3) determine the most effective treatment for each disorder; and 4) suggest guidelines for counseling individuals at risk. General syndromes under study include the dystonias, tic disorders, blepharospasm, and myoclonus. Approaches include standard epidemiologic and clinical genetic studies together with collaborative efforts in evaluating the role of neurotransmitters such as dopamine, their precursors, and metabolites, and their necessary cofactors.

Collaborative studies are underway with personnel in LCS, DCBR, MIMH to explain our earlier observations of altered dopamine beta hydroxylase and norepinephrine levels in blood and biopterin in CSF in a genetic subset of dystonia patients. Members of selected families are being brought to the Clinical Center, NIH, for trial of several new pharmacological agents.

Biopterin administered intravenously has led to acute benefit in one form of generalized dystonia.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 01927-14 NEB

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Clinical, Genetic, Pathophysiologic Study of Hereditary Nervous System Tumors

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Roswell Eldridge Medical Geneticist, NEB, IRP, NINCDS

COOPERATING UNITS *(if any)* OP, CC: SN, IRP, NINCDS; Division of Medical Genetics, Dept. of Pediatrics, Children's Hospital National Medical Center; Dept. of Neurosurgery, Massachusetts General Hospital, Boston, MA

LAB/BRANCH

Neuroepidemiology Branch, Intramural Research Program

SECTION

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.00	0.75	0.25

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In this project we seek to define and classify hereditary tumors of the nervous system; to add to the clinical description and natural history of these diseases; to suggest methods for early diagnosis; to evaluate present modes of treatment: and to develop methods for preclinical detection and screening.

Our studies have led to the recognition of at least two distinct genetic forms of neurofibromatosis: 1) the classical form as described by von Recklinghausen, and 2) a form in which bilateral acoustic neuromas are the hallmark. We have focused on neurofibromatosis with bilateral acoustic neuroma. Efforts have been directed at improving and simplifying screening of high-risk individuals, confirming diagnosis and establishing criteria for intervention. Audiologic studies, including evaluation of auditory-evoked response and acoustic reflex decay, are useful means for early documentation and monitoring of acoustic neuroma.

In our first major study involving neurofibromatosis of the von Recklinghausen type, a multidisciplinary program is in progress to evaluate neurologic and cognitive status in these patients compared to their unaffected sibs. Initiation of gene linkage studies, so successful in Huntington disease, awaits availability of modest funds, primarily for travel.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02167-10 NEB

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Genetic Epidemiology Studies in MS and Other Multifactorial Neurologic Disorders

PRINCIPAL INVESTIGATOR *(List other professionel personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Roswell Eldridge Medical Geneticist, NEB, IRP, NINCDS

COOPERATING UNITS *(if any)*

NI, IRP and OBFS, OD, NINCDS; M CN NIMH; Department of Neurology, Monmouth Medical Center, Monmouth, NJ

LAB/BRANCH

Neuroepidemiology Branch, Intramural Research Program

SECTION

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL.	OTHER:
2.5	0.5	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In this project we are coupling genetic and environmental studies in selected families and twin pairs with disorders such as multiple sclerosis, Parkinson's disease, and Alzheimer's disease, in an effort to distinguish specific contributing factors.

A multi-disciplinary study of 41 monozygotic twin pairs and 19 dizygotic twin pairs, selected on the basis of at least one member being diagnosed as having Parkinson's disease has led to the novel hypothesis that at least some cases are due to a reduced number of critical neurons in the substantia nigra and related structures very early in life.

An hereditary leukoencephalopathy simulating MS with onset at about age 35 is under study in kindred with over 20 affected. Derangement of the autonomic nervous system is often seen early in the course and when recognized, serves to distinguish this single gene disorder from multiple sclerosis clinically. Computerized tomographic scan changes of the brain are dramatic.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02240-08 NEB

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Epidemiology of Dementia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Bruce S. Schoenberg Chief, NEB, IRP, NINCDS

COOPERATING UNITS *(if any)* Epidemiology, Demography, and Biometry, NIA; W. Massey, M.D., Duke Univ.; E. Kokman, M.D. and J.P. Whisnant, M.D., Mayo Clinic; B. Jordan, Harvard Medical School; M. Alter, Temple Univ.; E. Kahanah, Hadassah Hospital, Jerusalem, Israel; R. Katzman, Albert Einstein College of Medicine, New York

LAB/BRANCH

Neuroepidemiology Branch, Intramural Research Program

SECTION

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL.	OTHER:
3.0	3.0	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A number of different approaches are being utilized to estimate the mortality and morbidity of Alzheimer's disease/senile dementia in several population groups in the U.S. and to measure the distribution of this disease in segments of the population.

To study international variation in the epidemiology of Alzheimer's disease, a uniform protocol for definition of disease and methodology have been developed. This is now being applied in the U.S. in Denver, Colorado, and in a multicenter study in Italy. There have been many requests to apply this protocol in many different parts of the world.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02241-08 NEB

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Epidemiology of Cerebrovascular Disease in Adults

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Bruce S. Schoenberg Chief, NEB, IRP, NINCDS

COOPERATING UNITS *(if any)*
J.P. Whisnant, M.D., Mayo Clinic; D.G. Schoenberg, M.S., Bethesda, Maryland, A. Lilienfeld, M.D., Johns Hopkins University

LAB/BRANCH
Neuroepidemiology Branch, Intramural Research Program

SECTION

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.8	2.8	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This investigation is aimed 1) at evaluating the effect of heart disease and hypertension as potentially treatable precursors or completed stroke and transient ischemic attacks; 2) at documenting unusual patterns of cerebrovascular disease; 3) at determining the autopsy patterns for patients dying with cerebrovascular disease in defined community; and 4) at examining if weather parameters have any effect on stroke incidence.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02243-08 NEB

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Pediatric Neuroepidemiology

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Bruce S. Schoenberg, Chief, NEB, IRP, NINCDS

COOPERATING UNITS *(if any)* D. Schoenberg, M.S., Research Epidemiologist, Bethesda, Maryland; J.F. Mellinger, M.D., M.R. Gomez, M.D., L.T. Kurland, M.D., Dr.,P.H., and R.V. Groover, M.D., Dept. of Neurology, MAYO Clinic; L.L. Salkowicz, P. Gunderson, Ph.D., Minnesota Department of Health

LAB/BRANCH

Neuroepidemiology Branch, Intramural Research Program

SECTION

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	3.5	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The project documented the frequency of primary intracranial neoplasms in the pediatric populations of Rochester, Minnesota, and the state of Connecticut. In addition, using the records-linkage system available for residents of Rochester, Minnesota, we investigated the magnitude and risk factors for cerebrovascular disease in infants and children.

The same Rochester, Minnesota records-linkage system was used to determine temporal trends in the incidence rates of cerebral palsy as well as the distribution of clinical subtypes and survival by clinical subtype, for the years 1950-1976. For the state of Minnesota, sex-specific neonatal mortality rates (NMR) in gestational age/birthweight risk subgroups were delineated for the years 1970-1976, and sex- and birthweight-specific NMR trends were determined for the years 1967-1976.

The same record linkage system has been used to identify all possible cases of complex partial seizures occurring in the years 1960-1980. A case-control study is being designed to identify risk factors associated with the occurrence of such seizures.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02297-08 NEB

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Mortality from Neurologic Disorders: National and International Comparisons

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Bruce S. Schoenberg Chief, NEB, IRP, NINCDS

COOPERATING UNITS *(if any)*
W. Massey, M.D., Duke University; D.G. Schoenberg, M.S., Bethesda, Maryland

LAB/BRANCH
Neuroepidemiology Branch, Intramural Research Program

SECTION

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6.0	6.0	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Although death certificate data are limited by possible misdiagnosis, incomplete case ascertainment, errors in coding, etc., detailed morbidity information on neurologic diseases for the entire U.S. and for other countries is not available. The Section has analyzed mortality data for selected neurologic disorders by country and by county in the U.S. The overall patterns which emerge may be useful in evaluating trends over time and in formulating etiologic hypotheses.

Some neurologic disease may contribute to death indirectly. Since there are no uniform criteria for what constitutes the underlying cause of death in patients, it is important to examine all deaths in which a disease is listed as an underlying, immediate, associated or contributory cause of death to get more complete information about the relationship between the disease and death. Mortality data for the U.S. for deaths due to and related to twenty neurologic diseases were studied.

Diseases occurring together may provide important information in the search for etiology of diseases. Association of diseases occurring at the time of death was also studied for all deaths occurring in the U.S. from 1971 and 1973 through 1978.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02299-08 NEB

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Reviews of Epidemiologic Aspects of Neurologic Disease

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Bruce S. Schoenberg Chief, NEB, IRP, NINCDS

COOPERATING UNITS *(if any)*
W. Massey, M.D., Duke University; D. Schoenberg, M.S., Bethesda, Maryland

LAB/BRANCH
Neuroepidemiology Branch, Intramural Research Program

SECTION

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	3.5	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Development of new neurologic studies requires thorough historic and methodologic reviews of prior investigations. These yield important unexplored etiologic clues that may be investigated using current technology. Major emphasis has been to cerebrovascular disease, otitis media, inherited ataxias, Huntington's disease, febrile seizures, Tourette's syndrome, peripheral neuropathy, neurologic disease in the elderly, controlled therapeutic trials of motor neuron disease, epilepsy, descriptive, analytic, and experimental methods in neuroepidemiology, statistical methods for calculating confidence intervals, procedures for neuroepidemiologic investigations in developing countries, and epidemiologic studies of Primary Degenerative Dementia.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02300-08 NEB

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 charecters or less. Title must fit on one line between the borders.)*

Clinical Course and Medical Care for Neurologic Disorders

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Bruce S. Schoenberg Chief, NEB, IRP, NINCDS

COOPERATING UNITS *(if any)*

J.P. Whisnant, M.D., Department of Neurology, Mayo Clinic, Rochester, MN

LAB/BRANCH

Neuroepidemiology Branch, Intramural Research Program

SECTION

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.2	2.2	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The study uses a review and abstraction of data from records for a selected group of neurological disorders. It obtains the items of data necessary to determine onset of the disorder, duration, data and cause of death, or current status. These data will be used to construct modified life tables to estimate the expectation of life after diagnosis, the survival curve and morbidity and severity estimates. It will also include analysis of type and duration of medical care received by patients with neurologic disorders derived from a well-defined population.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02301-08 NEB

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Collaborative Studies of Less Common or Less Debilitating Neurologic Disorders

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Bruce S. Schoenberg Chief, NEB, IRP, NINCDS

COOPERATING UNITS *(if any)*

M. Zack, M.D., Atlanta, Georgia; Neurosciences Program, WHO, Geneva, Switzerland; D. Duane, M.D., B. Sandok, M.D., Mayo Clinic; G. Roman, Bogota, Colombia; P.S. Spencer, Albert Einstein College of Medicine, New York

LAB/BRANCH

Neuroepidemiology Branch, Intramural Research Program

SECTION

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.5	3.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A number of collaborative efforts involve the investigation of the characteristics of unusual or less debilitating (e.g., headache) neurologic disease phenomena. Unusual associations or space/time clusters of neurologic disorders may provide leads to etiology or therapy. These may be tested through more formal approaches.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02305-08 NEB

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Epidemiology of Intracranial Neoplasms

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Bruce S. Schoenberg Chief, NEB, IRP, NINCDS

COOPERATING UNITS *(if any)* B.W. Christine, M.D., M.P.H., Connecticut State Department of Health; J.P. Whisnant, M.D., and R.J. Campbell, M.D., Mayo Clinic; L. Mahalak, M.D., Jackson, MS; A. Heck, M.D., Univ. of TN; R. Simon, M.D., Berkeley, CA; B. Jordan, B.A., Harvard Medical School

LAB/BRANCH
Neuroepidemiology Branch, Intramural Research Program

SECTION

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Section has conducted extensive investigations on the descriptive epidemiology of primary intracranial neoplasms using data from population-based registries worldwide. Analytic studies were carried out to investigate the relationship between intracranial neoplasms and tumors occurring at other sites. These studies included careful review of tumor nomenclature, disease definitions, and survey strategies.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02307-08 NEB

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Educational Resources in Neurological Epidemiology

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Bruce S. Schoenberg Chief, NEB, IRP, NINCDS

COOPERATING UNITS *(if any)*

D. Schoenberg, M.S., Research Epidemiologist, Bethesda, Maryland

LAB/BRANCH
Neuroepidemiology Branch, Intramural Research Program

SECTION

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	3.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Because there is severe shortage of available manpower in neuroepidemiology, the Section developed an active teaching program for current and future collaborative investigators. A series of six video tapes produced by the Section are distributed on a loan basis without charge. A textbook, entitled NEUROLOGICAL EPIDEMIOLOGY: PRINCIPLES AND CLINICAL APPLICATIONS, has been prepared, and a scientific quarterly journal entitled NEUROEPIDEMIOLOGY has been in publication since 1982. This journal received an Award of Merit from the Society for Technical Communication. In cooperation with the World Health Organization and the World Federation of Neurology Research Committee on Neuroepidemiology, formal courses were conducted in Caracas, Venezuela, and Shanghai, the People's Republic of China. Additional courses will be held in Nijmegen, the Netherlands: Bombay, India; and Jerusalem, Israel.

A set of video tapes have been produced for training interviewers in the methodology of interviewing for case-control studies. This has been done in both Italian and in English.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02370-06 NEB

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

*Racial and Geographic Differences in Occurrence of Neurologic Disease

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Bruce S. Schoenberg Chief, NEB, IRP, NINCDS

COOPERATING UNITS *(if any)* OBFS, OD, NINCDS; A. Haerer, M.D., Univ. of Mississippi; U.S. Bureau of the Census; C.L. Bolis, M.D.,(WHO); B.O. Osuntokun, M.D.(Nigeria); F. Garcia-Pedroza, M.D.(Mexico); Wang Chung-cheng, M.D.(People's Rep. of China); E. Bharucha, M.D. (India); M.C. Gutiérrez del Olmo, M.D., & A. Portera-Sanchez, M.D.(Spain); J. Cabrera, M.D.(Peru); P. Ponce, M.D.(Venezuela), & Dr. M. Cruz(Ecuador)

LAB/BRANCH

Neuroepidemiology Branch, Intramural Research Program

SECTION

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
11.0	8.0	3.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this study is to accurately document possible racial differentials in the prevalence of major neurologic disorders by surveying an entire county, with a biracial population of approximately 25,000. The disorders investigated include cerebral palsy, dementia, psychomotor delay, epilepsy, Parkinson's disease, essential tremor, and cerebrovascular disease.

Variation in mortality rates by race and sex for the entire U.S. for the years 1971 and 1973 through 1978 were also studied for 20 categories of neurologic diseases.

In addition, research protocols for neuroepidemiologic studies in developing countries have been prepared for Ecuador, Mexico, Nigeria, Peru, the People's Republic of China, Spain, and Venezuela. Pilot investigations have been successfully carried out in Ecuador, Mexico, Nigeria, Peru, and the People's Republic of China.

*[Former title: Racial Differentials in the Prevalence of Major Neurologic Disorders and Surveys in Developing Countries].

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02423-05 NEB

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Development of Data Resources for Neuroepidemiology

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Bruce S. Schoenberg Chief, NEB, IRP, NINCDS

COOPERATING UNITS *(if any)* F. Clifford Rose, M.B., F.R.C.P., B. Benjamin, Ph.D., S. Haberman, M.A., F.I.A., and R. Capildeo, M.B., B.S., Charing Cross Neuroepidemiology Unit, London, England; W. Sibley, M.D., Univ. of Arizona, Tucson, Arizona; E. Kahanah, M.D., Neurology Unit, Ashkelon, Israel; Y. Leibowitz, Neuroepidemiology Unit, Jerusalem, Israel

LAB/BRANCH
Neuroepidemiology Branch, Intramural Research Program

SECTION

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.1	1.1	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

To develop 1) a registry of hospitalized patients with neurologic disease in a well-defined population of 3.5 million people, and 2) resources for case-control studies of neurologic diseases using uniform methods of data collection.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02424-05 NEB

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Standardized Nomenclature and Coding of Neurologic Diseases

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Bruce S. Schoenberg Chief, NEB, IRP, NINCDS

COOPERATING UNITS *(if any)* L. Kurland, M.D., Mayo Clinic, Rochester, MN; J.F. Kurtzke, M.D., Georgetown Univ., Washington, D.C.; F. Clifford Rose, M.B., F.R.C.P., B. Benjamin, Ph.D., S. Haberman, M.A., F.I.A., and R. Capildeo, M.B., B.S., Charing Cross Neuroepidemiology Unit, London, England; L. Schut, M.D., Minneapolis, MN; and K. Kondo, M.D., Tokyo, Japan

LAB/BRANCH

Neuroepidemiology Branch, Intramural Research Program

SECTION

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.1	2.1	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

To develop an internationally acceptable standard of nomenclature, classification, and coding of neurologic disorders.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02570-02 NEB

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Natural History of ALS-PD in Guam

PRINCIPAL INVESTIGATOR *(List other professionel personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Bruce Schoenberg, M.D., Chief, Neuroepidemiology Branch, IRP, NINCDS

COOPERATING UNITS *(if any)*

NONE

LAB/BRANCH

Neuroepidemiology Branch, Intramural Research Program

SECTION

Guam Research Section

INSTITUTE AND LOCATION

NINCDS, Tamuning, Guam 96911 and NINCDS, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
7.6	1.6	6.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

As a continuation of previous projects on clinical, pathological, and epidemiologic surveillance of Guamanian amyotrophic lateral sclerosis (ALS) and Parkinsonism-dementia (PD) in the Mariana Islands, a total of 112 cases, including suspects registered as of January 1, 1983, are to be followed at intervals of six months for detailed clinical descriptions of patterns of progression by a qualified neurologist until all of the patients expire. It has been learned that the average duration of ALS is 4.0 to 4.5 years after onset with a range of 2.0 to 25 years. The study of those long surviving cases (over ten years) has been completed. Clinically they showed three patterns: (1) onset with slowly but steady progression at the same pace throughout the course; (2) rapid progression to complete paralysis of the limbs within 1.5 to 3 years and then remaining pracitically stable for the next 5 to 10 years; (3) onset with minimal atrophy and weakness for the first 5 to 6 years and then rapid step-wise progression to death. A study of the neuropathology of these long-surviving cases by a guest neuropathologist from Japan showed a burned-out picture: few active areas of neuronal, axonal, or myelin destruction with the remaining neurons appearing surprisingly healthy.

A significant number of PD cases were found to show not only lower motor neuron involvement but also severe pelvicrural flexion contractures in the advanced stage of the disease. This observation presents an important question of: (1) motor neuron involvement as a part of the natural history of chronic diseases of the CNS, or (2) a process identical to ALS which occurs in the same patient. If the latter is true, these cases may represent a continuum of ALS and PD, and thus indicate a single etiology of these two diseases.

TAB 20 -- NEUROIMMUNOLOGY BRANCH -- (NIB)

ANNUAL REPORT

October 1, 1983 through September 30, 1984

National Institute of Neurological and Communicative Disorders and Stroke

Neuroimmunology Branch

Table of Contents

Annual Report
October 1, 1983 to September 30, 1984
Neuroimmunology Branch
National Institute of Neurological and
Communicative Disorders and Stroke

Dale E. McFarlin, M.D., Chief

The research in the Neuroimmunology Branch (NIB) is concerned with the study of fundamental immunological mechanisms and disorders of immune function which contribute to the development of neurological diseases. There are investigations of both experimental diseases and human diseases; the latter includes clinical trials of procedures and pharmacological agents which modify immune reactivity. Throughout the past year, the NIB has occupied new laboratories in Building 10. This has had an exceedingly positive impact on the overall branch activities. Interaction with immunologists in other institutes has been facilitated, and clinical investigations have become more efficient. The latter have been expanded.

During the past year a major administrative change occurred in the branch when Dr. William Biddison was converted to a permanent position. He is the first permanent senior investigator added to the staff since the branch was established approximately 9 years ago and is internationally known for his contributions to the understanding of human cellular immunology. Dr. Biddison has initiated a program designed to investigate molecular mechanisms of lymphoid cell - cell interactions and contributes broadly to the overall mission of the branch.

Although the various research projects are closely interrelated, the branch is organized into three distinct administrative groups: the Office of the Chief, Cellular Immunology Section and the Neurological Diseases Section. In addition to administrative activities, the Office of the Chief is concerned with clinical investigation. Significant effort has been spent in identifying appropriate patients for immunological studies, acquiring candidates for therapeutic protocols, and for longitudinal evaluation of concordance of multiple sclerosis in monozygotic and dizygotic twins of like sex. Previously, our group reported a higher concordance in monozygotic than dizygotic twins, but because many of these individuals were still within the age of risk and because a high frequency of spinal fluid abnormalities was detected in clinically normal twins it became apparent that longitudinal assessment was indicated. Over the past year the twins were reevaluated and concordance continues to be considerably higher in monozygotic twins. The series now consists of 27 twin pairs which can be clinically classified. Two of 12 dizygotic and 12 of 15 monozygotic twins are concordant. Although some degree of ascertainment bias probably exists

because many of the twins were acquired through advertisement, these observations are consistent with the concept that genetic factors, in addition to environmental factors, contribute significantly to the pathogenesis of multiple sclerosis.

Three therapeutic trials in multiple sclerosis are either in progress or have been completed. Because recent observations suggest that abnormalities of lymphocyte function exist in the disease, two of the therapeutic approaches have attempted to modify lymphocyte populations. First, a pilot study of lymphocyte depletion has been completed in seven patients. Although significant lymphopenia was induced by repeated lymphocytophoresis, this did not alter the course of rapidly progressive disease.

The reports that patients with multiple sclerosis have reduced suppressor T-lymphocytes provide a rational for the second therapeutic approach. In highly selected pairs of monozygotic discordant twins, lymphocytes are being removed from the normal twin and infused into the affected individual. Both clinical and laboratory abnormalities are being assessed longitudinally in the recipient.

A third therapeutic trial in progress involves the administration of Poly ICLC to patients with multiple sclerosis. Poly ICLC induces the in vivo production of alpha interferon and alters the migration of leukocytes. Eleven patients have entered this protocol, and although preliminary evaluation has suggested a stabilization of most patients, the documentation of a possible therapeutic effect of this agent will require detailed evaluation over the next 18 months. In addition to establishing safety, the goal of this preliminary study is to determine if there is sufficient evidence to warrant a more extensive trial.

One reason for trying Poly ICLC in the treatment of multiple sclerosis is that some patients with chronic demyelinating peripheral neuropathy responded rather dramatically to this agent. One of these patients is still being treated about once every six weeks. Immune studies in this patient have shown that the administration of Poly ICLC is followed by increased production of steroids and alpha interferon; in addition, shifts in circulating lymphocytes occur. Studies of the use of interferon in this patient and other individuals with demyelinating neuropathy are planned.

Other forms of peripheral neuropathy are being investigated in the Neuromuscular Diseases Unit. This group has recently completed the first quantitative histological and detailed physiological study of neuropathy in mitochondrial disease due to reduced cytochrome c oxidase. An axonal neuropathy localized to fibers 7um in diameter has been documented. Patients with neuropathy in association with systemic vasculitis, the hypereosinophilic syndrome, abetalipoproteinemia, Fabry's Disease and the Chediak-Higashi syndrome have been investigated by detailed electrophysiological studies and nerve biopsy. Evidence of parasympathetic neuropathy was assessed in 100 patients with neuropathies of a variety of

etiologies. Approximately one half of all these patients including most individuals with diabetic neuropathy had signs of parasympathetic dysfunction. The Neuromuscular Diseases Unit under the direction of Professor Fritz Buchthal continued to contribute significantly to the diagnosis and care of patients with neuromuscular diseases through consultations with physicians in other institutes and the metropolitan area.

Recently magnetic resonance imaging (MRI) has been initiated in the radiology department, and a new protocol which uses this technology to assess demyelinating disease in a variety of patients is in progress. To date approximately 25 patients have been studied, and it is already apparent that MRI will have a major impact on the diagnosis and longitudinal assessment of multiple sclerosis. Completely unexpected results have emerged in some cases. For example, normal MRI scans have been observed in some patients with clinically definite multiple sclerosis while in other patients with relatively mild clinical disease the procedure has disclosed widely disseminated lesions. In addition lesions have been seen in an identical asymptomatic twin of a patient with multiple sclerosis. This finding is consistent with the existence of subclinical disease which was also suspected from evaluation of CSF immunoglobulins. Our future efforts will be associated undoubtedly with greater use of MRI as an adjunct to clinical investigations. An additional goal will be to define the appropriate clinical emphasis to place on results obtained with this procedure. Ethical and social issues related to NMR use will require broad consultation and discussion with investigators at other institutions.

Investigation of CSF proteins has been extended by collaborations with scientists in NIMH. Proteins are separated by two dimensional electrophoresis and visualized by silver staining; computerized methods have been developed which enable the reproducible identification of approximately 109 proteins by migration patterns; 77 of these proteins can be quantitated by the density of silver staining. In SSPE, sporadic cases of multiple sclerosis and syphilis, a number of abnormalities have been identified. These include increased numbers of immunoglobulin light chains and an as yet unidentified component. The activator of C-3 and several proteins of unknown identity were consistently reduced. These initial studies are being extended using CSF from patients with other diseases and from twins with multiple sclerosis.

Studies of cell mediated immunity in the CSF has traditionally been hampered by relatively low numbers of cells available for such investigations. The NIB has pioneered in establishing T-cell lines from CSF. This should facilitate the evaluation of the immune reactivity of CSF lymphocytes. The observations to date indicate the specificity of a cell line is related to the type of antigen employed in the culture procedure; apparently antigen influences the selection of cells which proliferate and survive. Thus far two cell lines which respond to myelin basic protein and a single cell line which responds to a component of measles virus have been derived from patients with multiple sclerosis.

In recent years a variety of changes in the leukocytes of patients with multiple sclerosis have been described, and over the past year our evaluation of leukocyte markers in patients with the disease has been completed. The findings show that in comparison to age and sex matched normal controls, both male and female patients with chronic progressive multiple sclerosis have significant elevations of lymphocytes bearing the T4 phenotype. Male patients with chronic progressive disease also had reduced numbers of the subset of lymphocytes which bear the T8 marker. When T4/T8 ratios are calculated the value was increased in the chronic progressive patients of both sex; however, this shift in ratio was clearly the consequence of changes in both $T4^+$ 8^- and $T4^-$ $T8^+$ subsets. Although the significance of these observations is not known, it seems unlikely that alterations in lymphocyte phenotypes are associated with changes in lymphocyte function. Only a small number of patients were studied during acute exacerbations and no shifts of lymphocyte phenotypes were noted in these individuals.

Much of the research in the cellular immunology section is closely linked to the above clinical research. The function of molecules on the outer surface of the T-lymphocyte membrane is being investigated. In order to identify events involved in T-cell recognition and triggering, a large panel of cytotoxic T-lymphocyte (CTL) clones directed at one group, secondary B-cell (SB), of class II major histocompatability (MHC) antigens have been developed. These CTL clones have been used to analyze the role of T3 and T4 surface molecules in T-cell recognition of SB antigens. Antibody blocking studies demonstrated a large degree of functional heterogeneity among the clones: the cytotoxic activity of some clones could be readily blocked by antibodies to the T4 molecule, while other clones were quite resistant to such antibody blocking. These results indicate that the function of the T4 molecule may be to facilitate T-cell recognition of class II molecules by reacting with a nonpolymorphic region of the molecule and thereby increasing the tightness of T-cell binding to target cells. This hypothesis was tested by an assay which quantitates the capacity of target cells to induce dissociation of radiolabelled target cells bound to T-cells. In these experiments the CTL clones which were the most susceptible to blocking by anti-T4 antibody were also the weakest target cell binders. Based on these results, a model for T-cell recognition was proposed in which the antigen-specific receptor determines the fine specificity as well as affinity of antigen recognition and the T4 molecule provides the ancillary function of increasing the overall avidity of T-cell interactions with cells bearing class II MHC molecules.

Similar studies have been conducted on the functional role of the T8 molecule. This is predominantly expressed on T-cells that have specificity for class I MHC molecules. Some monoclonal antibodies to the T8 molecule block the cytotoxic function of T8 positive CTL which are restricted by class I MHC molecules. The possible contributions of particular sites on the T8 molecule to CTL function have been analyzed with a panel of

monoclonal antibodies directed to distinct non-overlapping epitopes. The results indicate that specific epitopes on the T8 molecule are involved in CTL function; these are believed to interact with class I MHC molecules. Evidance has also been obtained that more than one site on the T8 molecule may function in this manner.

Studies on the cellular immune response to viruses have focused on measles virus. There are two general goals of these investigations: first, to identify the parameters of normal cellular immune reactivity to measles virus and, second, to examine the role of regulatory mechanisms which operate in the immune response to this agent. Lymphocyte proliferation has been used in the past to assess the immune response to measles virus, and in a few of the twins with multiple sclerosis it was found that one twin member was a high responder and the other was a low responder. In every case the high responder was the individual with multiple sclerosis. Such studies have been conducted in 28 pairs of twins and in six of these a significantly higher response was found in the affected individuals.

A major aspect of the investigation of the cellular immune response to measles has been to establish appropriate means for examining the functional components of this response. Previous efforts to demonstrate specific T-cell MHC restricted killing of measles virus-infected targets were infrequently successful and only seen with lymphocytes from individuals who showed a very high response by lymphocyte proliferation. Recently, substantially different results have been obtained. Significant MHC restricted killing by the blood cells of most normal individuals can consistently be demonstrated by using measles-infected B cells transformed with Epstein-Barr Virus (EBV) as targets. Although EBV transformed B-cells express both class I and class II MHC antigens on the cell surface, an exciting new finding is that the cytotoxic activity is restricted by class II MHC molecules. This is in contrast to many other examples of T-cell specific killing which are restricted by class I MHC antigens.

In order to extend and confirm the above observations on cell-mediated responses to measles virus, a number of T-cell clones have been derived from one of the twins who shows a high response to measles virus. A significant number of the T-cell clones produce class II restricted killing of measles infected targets. These findings indicate that the T-cell response to measles virus and possibly other agents is unique. These concepts are currently being extended in the study of immunity to other viruses and are being incorporated into our investigations of immune function in patients with multiple sclerosis and with other diseases.

The details of the immune response to intact viruses are virtually unknown. For example, although measles virus consists of six polypeptides, it is not established if all of these react with the same components of the immune system. Consequently, considerable effort in the Neurological Diseases Section has been directed at preparing purified components of measles virus. These are being used in conjunction with the above populations of

high responder lymphocytes and lymphocyte clones to examine the specificity of the cellular immune reactions to measles virus. Lymphocytes from high responder twins showed a high response to each of the measles polypeptides. In contrast, lymphocytes from low responder twins and normal individuals did not respond substantially to any of the specific antigens. From these observations, it has been concluded that the low response generally found by proliferation is not due to the absence of a relevant polypeptide in the virus preparation. The specificities of T-cell clones are being determined in similar experiments, and a number of clones which react to single polypeptides have been identified. Experiments have been initiated to determine if the response to a single polypeptide activates helper T-lymphocytes and is primarily responsible for providing amplification of the immune response to the entire virus.

In the past, a panel of monoclonal antibodies produced against the HA protein of Edmonston Strain of measles virus have been used to purify the surface components of this virus and also to study the Hamster Neurotrophic (HNT) Strain of measles which produces chronic encephalitis in some strains of mice. Certain monoclonal antibodies prepared against the Edmonston HA protein do not react with the HNT HA protein, and recent data indicate that the HA protein of the HNT strain has a lower molecular weight (75K). Thus, certain epitopes appear to be missing from the HA protein of the HNT strain. These data are consistent with the concept that HNT may have evolved through a deletion mutation. These biochemical findings may relate to the biological property of neurovirulence.

Analysis of the mechanisms responsible for the production of experimental allergic encephalomyelitis (EAE) in mice has been continued. Previously our laboratory developed a highly efficient system for the adoptive transfer of the disease in syngeneic animals. The cells responsible for the transfer were identified as the Ly $1^{+}2^{-}$ subset of T-cells . These studies have been extended, and it has been established that the acute disease, which occurs after a single transfer of myelin basic protein (MBP) sensitized lymphoid cells or T-cells, is characterized by infiltration of inflammatory cells and significant primary demyelination. The latter was unexpected and raises important questions about the mechanisms responsible for the migration of lymphocytes from the blood into the nervous system and the production of primary demyelination. It has been postulated that an early initial event involves the recognition of antigen at the luminal surface of the vascular endothelial cell surface. It is believed that in order for this to occur the presence of both specific antigen (myelin basic protein) and Class II MHC would be required. Morphological evidence of this has been obtained and currently experiments are in progress to access functional interactions between sensitized lymphocytes and vascular endothelial cells from the brain.

A second unexpected finding in the study of adoptively transferred EAE was that after the acute episode most recipients of sensitized lymphocytes recover and subsequently develop a chronic relapsing form of the disease

with wide-spread lesions which show primary demyelination and in some cases remyelination. The mechanism for the relapsing demyelinating disease in the absence of an antigen depot is not known. One possibility is that myelin basic protein in antigen-presenting cells is transferred along with immune T-cells and stimulates immune reactivity. Our findings with radiolabelled myelin basic protein indicate that the amount of antigen transferred is less than fifty ng per animal. Further, T-cells depleted of antigen-presenting cells still produce the chronic disease. Another possibility is that the acute disease produced by transfer of T-lymphocytes causes release of myelin antigens. Myelin basic protein released with other myelin components during tissue damage may be more immunogenic than soluble basic protein and lead to the production of disease. Such antigen could either induce an immune response in the recipient or lead to expansion of transferred memory effector cells. It is also possible that the acute disease induces an immune response against components of myelin other than basic protein. Identification of the pathogenic mechanisms responsible for the chronic autoimmune disease are of considerable importance because similar disease processes could well occur in human demyelinating disorders.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02202-09 NI

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Immunological Studies in Patients with Multiple Sclerosis and other CNS Diseases

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

D. McFarlin, Chief, NI NINCDS
J. Rose, Med. Stf. Fel., NI NINCDS
R. Mandler, Vis. Assoc., NI NINCDS
A. Goodman, Med. Stf. Fel., NI NINCDS
W. Biddison, Sr. Stf. Fel., NI NINCDS
C. Bever, Sr. Stf. Fel., NI NINCDS
X-H Xu, Gst. Work., NI NINCDS
M. Harrington, Vst. Fel., LGCB NIMH
C. Merril, Sec. Hd., LGCB NIMH
F. Buchthal, Vst. Sci., NI NINCDS
J. Kurent, Med. Ofcr., NI NINCDS
C. Krarup, Vst. Sci., NI NINCDS
J. Richert, IPA, NI NINCDS

COOPERATING UNITS *(if any)*

ID, NINCDS

LAB/BRANCH

Neuroimmunology

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.0	3.0	2.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Investigation of patients with Neurological Dysfunction. The general aim of this project is to obtain more precise understanding of multiple factors possibly related either singly or in combination to the pathogenesis of a number of neurological disorders including multiple sclerosis, myasthenia gravis, polyneuropathy and other neuromuscular diseases. The studies of multiple sclerosis include a detailed evaluation of the histocompatibility makeup and the relationship between immunogenetic background and clinical disease as well as immunological function including the cellular response to various human viruses. Nuclear magnetic resonance imaging is being used to assess the extent and magnitude of lesions in the white matter. These studies are performed in patients with sporadic disease, patients with a family history of demyelinating disease as well as identical and nonidentical twins who are either concordant or discordant for MS. Cerebrospinal fluid immunoglobulin content and specificity are being evaluated by new highly sensitive techniques. Trials of experimental therapeutic approaches are being conducted in carefully selected patients with multiple sclerosis. One trial currently in progress involves the administration of Poly ICLC an interferon inducer. Studies of myasthenia gravis are directed at assessment of lymphocyte markers in the blood and thymus. The reactivity of thymocytes and blood lymphocytes to acetylcholine receptor is being evaluated and correlated with antibodies to acetylcholine receptor in the blood. In myasthenia gravis and a wide range of other neuromuscular disorders, detailed electrophysiological evaluation, histopathological studies of muscle and nerve are being conducted.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02203-09 NI

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Immune Response Against Membrane Antigens

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Dale E. McFarlin, Chief, NIB, NINCDS
Henry F. McFarland, Asst. Chief, NIB, NINCDS
W. Bellini, Special Expert, LMG, NINCDS
J. Rose, Med. Staff Fellow, NIB, NINCDS
E. Norrby, Professor, Karolinska Institute
J. Stominger, Professor, Harvard Medical School

COOPERATING UNITS *(if any)*
Dpt of Biochemistry and Molecular Biology, Harvard Medical School, Boston, MA.
Laboratory of Immunogenics, NIAID, NIH
Virology Department, Karolinska Institute, Stockholm, Sweden

LAB/BRANCH
Neuroimmunology

SECTION
Neurological Diseases Section

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	2.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The major goal of this project is to characterize virus antigens and other components expressed on the surface of infected cells which are the targets of the immune response. Monoclonal antibodies against the major surface component of measles virus, the hemagglutinin (HA), have been produced and used to characterize the biosynthesis, glycosylation, and assembly of this protein. The HA protein is being studied in strains of measles virus which differ in biological activity. Similar studies are being conducted with the fusion (F) protein. New methods for the isolation of measles virus polypeptides have been developed and are now being used to prepare pure antigens for assessment of the immune response.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02204 09 NI

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Immunologic Mechanisms Operative in Experimental Autoimmune Diseases of the Nervous System

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Dale E. McFarlin, Chief, NIB, NINCDS
F. Mokhtarian, Sr. Staff Fellow, NIB, NINCDS
D. McCarron, Sr. Staff Fellow, NIB, NINCDS
J. Richert, IPA, NIB, NINCDS
Maria Spatz, Section Head, LCNP, NINCDS
Oliver Kempski, Visiting Fellow, LCNP, NINCDS
C. Raine, Professor, Albert Einstein, N.Y.

COOPERATING UNITS *(if any)*

Departments of Pathology (Neuropathology) and Neuroscience, Albert Einstein College of Medicine, New York, N.Y.

LAB/BRANCH

Neuroimmunology

SECTION

Neurological Diseases Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	2.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The aim of this project is to identify the relative role of various mechanisms operative in the production of experimental allergic encephalomyelitis, a model of autoimmune disease which is manifested by demyelination. This disease is being studied in mice because this species is ideally suited for the analysis of immunologic and genetic factors which lead to disease. Three forms of the murine disease: 1) Acute Experimental Allergic Encephalomyelitis, 2) Chronic Relapsing Experimental Allergic Encephalomyelitis and 3) Adoptively Transferred Experimental Allergic Encephalomyelitis have been produced. Currently effort is being focused on the adoptively transferred form of the disease. The subpopulation of lymphocytes responsible for the tranferred disease has been identified and the mechanisms related to the migration of immune cells across the blood brain barrier into the nervous system are being assessed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02205-09 NI

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Interaction Between Viruses and the Host Immune System

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Henry F. McFarland, Asst. Chief, NIB, NINCDS
Dale E. McFarlin, Chief, NIB, NINCDS
S. Jacobson, Guest Worker, NIB, NINCDS
W. Biddison, Sr. Investigator, NIB, NINCDS
J. Rose, Med. Staff Fellow, NIB, NINCDS
J. Richert, IPA, NIB, NINCDS
A. Goodman, Med. Staff Fellow, NIB, NINCDS

COOPERATING UNITS *(if any)*

LMB, NINCDS
ID, NINCDS

LAB/BRANCH

Neuroimmunology

SECTION

Cellular Immunology Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.5	4.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this study is to examine the host immune response to viruses. The major goal is to examine the normal immune response to naturally occurring viruses in man and to extend these studies to patients in order to identify abnormalities of immune regulation which may be related to the pathogenesis of certain diseases of the nervous system. These studies involve a functional analysis of the cellular immune response to measles virus and other viruses of man. This includes studies of cytotoxic, helper and suppressor T-cell populations. The genetic influence on the generation and expression of these responses is being examined. In addition, T-cell lines and clones are also being used to examine cellular reactivity to these viruses.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02603-01 NI

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Mechanisms of Lymphoid cell-cell interactions

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigetor.) (Name, title, laboratory, and institute affiliation)*

William E. Biddison, Sr. Investigator, NI NINCDS
Elli Leontsini, Visiting Fellow, NI NINCDS
Stephen Shaw, Sr. Investigator, IB NCI

COOPERATING UNITS *(if any)*

Immunology Branch, NCI

LAB/BRANCH

Neuroimmunology

SECTION

Cellular Immunology

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.0	2.0	2.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The goal of this project is to define the mechanisms by which T-cell surface molecules function in the recognition of foreign cell surface molecules. A large panel of SB-specific cytotoxic T-cell (CTL) clones have been developed to facilitate the study of membrane molecules that are involved in T-cell recognition and triggering. These CTL clones have been used to analyze the roles of the T3 and T4 surface molecules in T-cell recognition of the class II MHC antigens. The results suggest that the role of the T4 molecule may be to facilitate T-cell recognition of class II molecules by binding to a nonpolymorphic region of the molecule and thereby increasing the overall tightness of binding of T-cells to target cells. This hypothesis is being tested by an assay that was developed to quantitatively measure the avidity of individual T-cell clones for target cells. A model for T-cell recognition is proposed in which the antigen-specific receptor determines the fine specificity and affinity of antigen recognition and the T4 molecule provides the ancillary function of increasing the overall avidity of T-cell interactions with cells bearing class II MHC molecules.

Studies are also conducted on the functional role of the T8 molecule. The T8 molecule is predominantly expressed on T-cells that have specificity for class I MHC molecules. Some monoclonal antibodies to the T8 molecule block CTL function of T8-positive cells. We are analyzing the role of particular epitopes of the T8 molecule in CTL function by utilizing a panel of anti-T8 monoclonal antibodies directed to distinct non-overlapping epitopes. The results indicated that specific epitopes on the T8 molecule are involved in CTL function, and that there could be more than one functional site on the molecule.

TAB 21 -- SURGICAL NEUROLOGY BRANCH -- (SNB)

ANNUAL REPORT
October 1, 1983 through September 30, 1984

Surgical Neurology Branch, IRP
National Institute of Neurological and Communicative Disorders and Stroke

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ANNUAL REPORT
October 1, 1983 through September 30, 1984
Surgical Neurology Branch, IRP
National Institute of Neurological and Communicative Disorders and Stroke

Paul L. Kornblith, M.D., Chief

I. Summary of Studies in the Surgical Neurology Branch

This annual report is the sixth of the Surgical Neurology Branch beginning October 1, 1983, under the leadership of Dr. Paul Kornblith. The Branch has continued to be increasingly productive in its mission of the conduct of basic and clinical research on brain tumors.

Reorganization of the Branch, including the reequipping and redesign of all laboratory facilities, is complete and the tissue culture, electron microscopy and quantitative image analysis, neuropathology, humoral immunology, cellular immunology, metabolism and neurochemistry, positron emission tomography, and differentiation/monoclonal antibody modules are all functioning.

Addition of scientific personnel to work in each of these areas has included:

Dr. Richard Youle - biochemistry (1984)
Dr. Elizabeth Grimm - cellular immunology (1984)
James D. Bona, R.Ph. - chemotherapy and tissue culture (1984)
Dr. Joseph Bressler - immunochemistry

Senior clinical personnel, in addition to Drs. Kornblith and Smith include:

Dr. Conrad Kufta
Dr. Edward Oldfield
Dr. Donald Wright
Dr. William Meyer
Dr. David Katz
Dr. Thomas Staunton

The primary areas of our research activities have included:

1. Biological, immunological and chemotherapeutic studies in human brain tumors.
2. Biological studies of human pituitary tumors.
3. Neurodiagnostic studies including the PET scan.

The Clinical Service now has 14 beds on both 5E and 5W as well as operating facilities in Building 10A. More than 120 major neurosurgical cases will be done this year. Clinical admissions are close to 200 per year with consultations for other Institutes at NIH numbering approximately 100/year. Two clinics are functional with more than 800 clinic visits per year. The SNB, through Dr. Katz, now provides a neuropathology service to the NIH. Ten clinical protocols for brain tumor patients are currently in effect. These are:

1. Evaluation of Biological, Immunological and Chemotherapeutic Parameters in Brain Tumor Patients.
   Project No. 79-N-89

2. Immunotherapy of Malignant Brain Tumors
   Project No. 79-N-133

3. Biological Studies of Human Pituitary Tumors
   Project No. 79-N-151

4. Evaluation of Biological, Immunological and Chemotherapeutic Parameters in Patients with Non-Astrocytic Central Nervous System Tumors
   Project No. 82-N-25

5. Selective Intra-arterial Chemotherapy in the Treatment of Recurrent Malignant Brain Tumors
   Project No. 82-N-41

6. ^{18}F-2-Fluoro-2-deoxy-D-glucose (FDG) Positron Emission Computed Tomography (PECT) in Typing of Cerebral Gliomas
   Project No. 80-N-36

7. Use of Argon Laser for Surgical Excision of Brain, Spinal Cord, and Pituitary Tumors
   Project No. 81-N-181

8. Use of the Alkaline Elution and Microtiter Assays for Selection of Chemotherapeutic Drugs for Individual Brain Tumor Patients on the Basis of Their Tumor Cell Drug Sensitivities
   Project No. 83-N-63

9. Selective Intra-arterial Chemotherapy in the Treatment of Recurrent Malignant Brain Tumors
   Project No. 84-N-41

10. Intracarotid cis-diaminedichloroplatinum (DDP) and Hemodialysis of Jugular Blood in the Treatment of Malignant Brain Tumors
    Project No. 84-N-78

The following clinical protocols for brain tumor patients are being pursued in collaboration with other members of NINCDS as well as with the National Cancer Institute:

1. Phase II Trial of AZQ in Patients with Malignant Glioma and Metastatic Brain Tumors

2. Phase I Trial and Pharmacokinetic Study of CBDCA (NSC 241240)

3. Phase I Trial and Pharmacokinetic Study of Spirohydantoin Mustard (NSC-172112) in Adults

4. Phase I Trial and Pharmacokinetic Study of Trimetrexate in Adults (NSC 352122)

5. I^{125} Interstitial Brachytherapy in the Treatment of Primary and Secondary Brain Tumors: A Pilot Study

6. Neurofibromatosis and Acoustic Neuroma - Evaluation of High Risk Individuals Project No. 84-N-123

7. Quantitative Sequential Determination of Regional Blood-Brain Transfer Constants in Patients with Malignant Brain Tumors Using 68-Gallium-EDTA Positron Computed Tomography (PET)

8. Localization of Activated Human Brain Regions by Stimulated-produced 18(F)-2-Fluoro-2-Deoxy-D-glucose (FDG) Positron Computed Tomography (PET) Project No. 84-N-77

9. Phase I Study of Bromodeoxyuridine (NSC-38297) Given by Peripheral Venous Infusion Project No. 80-C-143

10. Phase I and Pharmacokinetic Study of Tiazofurin (NSC 286193) in Adults

11. A Phase I Study of Iododeoxyuridine (NCI 39661) Given by Constant Infusion Project No. 83-C-135

The clinical neurosurgical service includes formal rounds twice a week, a yearly sequence of neuroscience, neuro-oncology, and neurochemistry courses for senior clinical staff as well as a weekly neurosurgical journal review, a weekly neuropathology conference and a biweekly neuroradiology conference. In addition, the SNB takes an active part in the weekly NINCDS Grand Rounds.

The sequence of protocols developed over the past four years covers each of the major present or potential treatment modalities for brain tumor. The argon laser and use of a stereotactic apparatus has been introduced in an attempt to improve surgical resection, and has been of value in selected cases. Rapid frozen section glial fibrillary acidic protein and fibronectin staining have been useful in providing more accurate intraoperative neuropathological diagnosis.

For diagnostic radiological improvements, Dr. DiChiro continues developments in computer tomography, positron emission tomography and nuclear magnetic resonance modalities.

For clinical chemotherapy, we are continuing the use of four relatively new drugs in the antiglioma armamentarium. AZQ (aziridinylbenzoquinone), "chocolate" or CBDCA platinum, trimetrexate, spirohydantoin mustard or spiromustine. AZQ is completing Phase II testing with some 60 patients having received the drug. CBDCA platinum is now in Phase II evaluation with the results of Phase I demonstrating CBDCA to be less toxic than its parent compound platinum. Spirohydantoin mustard is nearing a close to Phase I studies with some 15 patients being examined. Trimetrexate, a methotrexate analog, has been chemically developed to increase CNS penetration and is presently just beginning Phase I studies.

Selective intra-arterial BCNU and cis-platinum therapy has also been instituted. This method of drug delivery, most suitable for patients in whom the main vascular tumor supply is via the anterior or middle cerebral artery, permits delivery of up to five times the dose delivered by the intravenous route with no apparent increase in bone marrow toxicity of the BCNU. Such elevated BCNU levels should increase overall tumor response to nitrosourea since in vitro microcytotoxicity data indicate that most cell lines are resistant at normally-achieved intravenous levels but are sensitive at the higher exposures achieved by intra-arterial drug delivery. Dr. Oldfield has found that by using a hemoperfusion cartridge, the majority of BCNU delivered into the carotid artery can be removed. This allows the levels of drug reaching the bone marrow to be significantly decreased. It may allow higher drug levels at tumor cell level with decreased systemic toxicity. We have now demonstrated that the systemic exposure to BCNU can be reduced to 60-90% by using this drug-removing cartridge. This resulted in increases in exposure of the tumor to BCNU compared to the exposure of the remainder of the body by 21-55 fold. We have evaluated the capability of using a similar strategy using cisplatinum, another potent tumor chemotherapeutic agent. By using 2 hemodialyzers in series, 90% of the cisplatinum contained in whole blood can be removed at high rates of flow (300 ml/min). We are now evaluating this system in patients by continuous intracarotid delivery with extracorporeal drug removal from the jugular blood.

The procedures are now becoming safer and more rapid. Evaluation of therapeutic results suggests some impressive tumor regressions but more time will be required before a determination of efficacy can be made.

Assisting the clinical neurosurgical service is the Clinical Center Pharmacy Department. The Pharmacy has continued its operation of the Neurology Pharmacy Satellite on the 5 West Nursing Unit. The availability of a clinical pharmacist and pharmacy on the unit has provided improved patient care in the areas of extensive patient medication counseling, rapid filling of physician medication requests, anti-convulsant blood concentration monitoring, prompt preparation of short-lived chemotherapeutic agents, such as spirohydantoin mustard, and monitoring of drug interactions and overall ward medication use.

Tumors available for in vitro study now number well over 120 each year and include glial as well as other types of central nervous system tumors. Cooperating centers include Walter Reed Army Medical Center, George Washington University, Georgetown University, Children's Hospital (Washington, D.C.) and a variety of other centers scattered around the country.

Over one hundred and twenty surgical cases have been done in the past year, and have provided tumor tissue for chemotherapeutic, immunologic and biochemical studies. Major upgrading of the surgical facilities has been ongoing and has included the addition of an argon laser, stereotactic equipment and a cavitron. Metabolic studies of patients with brain tumors have continued. Studies in over 150 patients with positron emission tomographic scanner have shown a relationship between glucose uptake and degree of tumor growth with a clear indication that PET scanning can be helpful in the grading of tumor malignancy. In addition, the utilization of computed tomography, positron emission tomography, and nuclear magnetic resonance modalities have provided an exquisite capability for our Neuroradiology Section to aid in the diagnosis, location, and degree of malignancy of tumors in our patients.

II. BIOLOGICAL, IMMUNOLOGICAL AND CHEMOTHERAPEUTIC STUDIES IN HUMAN BRAIN TUMORS

A. Cellular Biology

It has been apparent that the biological factors influencing tumor growth are multifold and include tumor heterogeneity, vascular supply and the intrinsic tumor cell kinetics. Heterogeneity has been found not only for tumors of the same pathological grade, but also within the cell populations of a single tumor. While the biological origins of this diversity are not as yet clear, the therapeutic significance of such facts makes individualized glial tumor study critical to further clinical and basic research progress. The tissue culture of human brain tumor cells obtained at surgery offers the opportunity for both improved understanding of the cell biology of these tumor cells and the individualization and, thereby, optimization of brain tumor therapy.

Vascular factors such as the size and distribution of vessels, blood flow rates, and vascular permeability all play a role in the growth of glial tumors. Ultrastructural studies of these vascular factors have proven useful in the understanding of glioma biology.

Cell kinetics and chromosomal patterns have proven to be of import in predicting tumor growth and behavior. Cytofluorometric and flow cytometric techniques have been shown to define the phenotypically characteristic cell populations in specific tumors and have been helpful in understanding the effects of heterogeneity on glioma cell biology. Flowcytometric and cytogenetic analysis were applied to a series of cultured cell lines derived from high- and low-grade astrocytomas. Five human cell lines cultured from high- and low-grade astrocytomas in cerebral hemisphere have been analysed for DNA and protein distribution by flowcytometric (FCM) and correlated with cytogenetic profiles. Simultaneous calibration with chicken erythrocytes as a co-running

standard provided an estimate of chromosomal number of predominate stem cells of each cell line by the ratio of the DNA content of the major peak(G^1) to that of chicken erythrocyte (T/E ratio) of FCM. Various lines had different distributions of chromosomal number, ranging from near diploid to tetraploid. Each line had a stem-cell population and chromosomal markers indicative of clonal selection, but no common marker specific to astrocytomas. The histogram of DNA distribution obtained by FCM correlated well with the chromosomal distribution by cytogenetic analysis. In addition, simultaneous measurement of protein and DNA content in multidimensional FCM demonstrated a sigmoid configuration of the profiles, which indicated a gradual increase of protein content associated with an increase of chromosonal number or with progression of cell cycle. To avoid confusion of a bimodal chromosomal distribution with the G^2/M phase of the cell cycle, and to determine chromosomal numbers associated with a DNA histogram, simultaneous cytogenetic and FCM study are required. More rapid than cytogenetic analysis, the T/E ratio allows estimation of chromosomal number of the stem-cell population associated with DNA histograms of cultured glioma-derived cell lines.

There are a variety of morphological, cell biological and biochemical parameters which are relevant to the characterization of glial tumor cells. For example, from a morphological point of view, surface membrane, nuclear, and cytoplasmic features have long been felt to be useful in the evaluation of malignancy in tumor cells at both the light and electron microscopic levels. Evident from experience with any one characterization modality are the limitations of "static" evaluations. Morphologic features of extensive surface microvilli, dilated endoplasmic reticulum, and bizarre, multilobular nuclei are, in themselves, indicators of limited value in determining the dynamic response characteristics of any given malignant cell, just as static metabolic measurements of anaerobic or oxidative metabolism, cytogenetic analyses, or even cell kinetics may tell only a part of the tumor cell's biology. No one "static" approach to glial tumor cell characterization is likely to lead to significant advances in understanding malignant cell behavior. Needed are "dynamic" behavioral characteristics of tumor cells to which a multimodal analytical, biophysical and biochemical approach can be applied. Utilizing these approaches it has been possible to show that certain characteristics of cultured human glioma cells also provide the opportunity to add therapeutically relevant information to the planning of optimal therapy and the prediction of the way in which a tumor will grow in a given patient. This type of work has two major areas. First is the area of the prediction of the behavior of tumors which are known to be malignant. Here the major question is how malignant a given tumor will be. Also, in certain tumors, which by and large are benign or nonmalignant in their growth, there are occasional instances in which tumors do grow in a malignant fashion. In the second category, the question is how to pick out ahead of time those tumors which behave in a malignant or invasive fashion. These are the two primary goals of the program in the study of tumor biology. There are, in addition, several secondary goals. These include: studies of the basic biologic mechanisms of tumor growth and the similarities to and differences from this tumor growth to the growth of normal cells.

Another area of major importance involving cellular biology is our work to identify glial specific gene products which are deregulated during the multistep process of neoplastic transformation. Since the processes of differentiation and neoplasia are tightly bound, we believe a model system which would allow us to study differentiation of glial cells will also allow us to identify these gene products. For the purpose of these studies, we define differentiation as the augmentation or appearance of glial specific properties and the loss of mesenchyme properties.

Quantitative assays for two astroglial and two oligodendroglial properties are currently being used or developed. The oligodendroglial properties include glucocorticoid regulation of glycerol phosphate dehydrogenase (GPDH) and the presence of 2'3' cyclic nucleotide phosphohydrolase (CNPase), both of which are measured by enzyme assays. GPDH activity is measured by a standard spectrophotometric assay. CNPase activity is measured by a fluorometric assay that was modified by Drs. Craig Cummins and Tom Staunton. The two astroglial properties include S-100 levels and the rate of glial fibrous acidic protein (GFAP) synthesis. S-100 protein is measured by a solid phase radioimmunoassay that was developed by Drs. Alan Hirschfeld, Yoshio Moriya and Joseph Bressler. Though other assays capable of detecting picogram quantities of S-100 protein have been reported, this assay is novel because it is not necessary to manipulate the antibody or the antigen as it utilizes radiolabeled protein A. Finally, the rate of GFAP biosynthesis is measured by determining the amount of radiolabeled amino acid incorporated into the protein after GFAP is isolated by two dimensional electrophoresis.

Major Findings:

CNPase

Though CNPase has been demonstrated to be quantitatively specific in oligodendroglial cells in vivo, no work to our knowledge has demonstrated that this property is oligodendroglial, or glial specific in vitro. This is most important since high RNAase activity, a characteristic of many cells in culture, may interfere with some types of CNPase assays. Drs. Staunton and Bressler are presently surveying glial and non-glial lines for CNPase activity.

S-100

Drs. Alan Hirschfeld and Bressler have been screening some of our more established tissue culture cell lines for the S-100 protein. Of the lines examined, only one, B. Green, exhibited substantial levels of S-100 protein. The amounts found, approximately 300 ng/ml protein, are comparable to levels found in C_6 rat glioma cell lines.

Glial Fibrillary Acid Protein (GFAP)

We have not yet quantitatively measured GFAP levels in our cell lines. Using the technique of indirect immunofluorescence, we have identified one line from five which is positive for GFAP. This small percent of GFAP positive lines is not surprising since to our knowledge only three positive lines have been previously reported. Besides the normal controls, we have confirmed the staining to be filamentous due to the marked disruption in the filament structure after addition of colchicine.

Glycerol Phosphate Dehydrogenase (GPDH)

Five of our lines have been examined for the ability of glucocorticoids to elevate GPDH levels. None have been found. This might suggest that either in the human this enzyme is not under glucocorticoid control, or we have not provided the cells with the correct environment for GPDH elevation.

The effect of phorbol esters on glucocorticoid regulation of GPDH activity in the C_6 rat glioma cell has been examined. Some of the phorbol esters act as tumor promoters in a number of different tissues, and they also have been demonstrated to modify the expression of differentiation characteristics in a number of different biological systems. PMA inhibited GPDH induction in both logarithmic and stationary phase cells. This event is most likely mediated through the phorbol ester receptor since the reported Ki of various phorbol ester analogs to block the phorbol ester correlated with their ability to block the glucocorticoid mediated increase in GPDH levels. Additionally, like tumor promotion in vivo, the inhibition of GPDH induction is reversible. The PMA effect is not restricted to the C_6 cell line since PMA also inhibited GPDH inducibility in another rat glioma cell line.

The PMA mediated event has been partially characterized. PMA did not effect the overall rate of protein or RNA synthesis. It was ineffective in altering both the ligand-receptor interaction and the rate of GPDH degradation. Therefore, PMA is effective at either the transcriptional or at the translational level.

Non-phorbol ester tumor promoter was also examined. Mezerin, which is not as potent as PMA in binding to the phorbol ester receptor, but which has been demonstrated to be a powerful stage two promoter, was found to be more potent than PMA in blocking GPDH induction. RPA, another powerful stage two promoter, was also found to be more potent than PMA in blocking GPDH induction.

Many other biological modifiers have been used in order to reverse the PMA effect. Protease inhibitors (leupeptin , antipain) and retinoic acid, which reverse an effect that PMA has in skin epithelia, has no effect in our system. PMA has been reported to decrease cAMP and Ca levels, but we have not been able to reverse the effect of PMA by simply increasing the levels of these second messengers.

Transforming Growth Factor (TGF)

In a collaborative project with the Laboratory at NCI, Drs. Craig Clark, Richard Assoian, and Joseph Bressler found that human brain tumors and glioma cell lines exhibit marked levels of TGF. The levels found were comparable to those found in other cell lines and tissues.

Wound Repair in the Central Nervous System

A prominent problem in neurology is the inability of CNS tissue to regenerate. An insult to the CNS often will result in the formation of glial scars which lack the proper nerve cell infiltration (glial cells block nerve cell spreading). In a collaborative project with Drs. L.

Hjelmeland, Laboratory of Vision Research, NEI, G. Grotendorst, Laboratory of Developmental Biology and Anomalies, NIDR, and Joseph Bressler, Surgical Neurology Branch, NINCDS, chemotaxis was used as an in vitro model to study factors which control astroglial migration. Chemotactic activity for astrocytes was found for platelet-derived growth factor (PDGF) with a ED_{50} occurring at 1-2 ng/ml. Affinity purified fibronectin was also found to stimulate the migration of astroglia, with ED_{50} of approximately 1 ug/ml. Several other factors including laminin, nerve growth factor, epidermal growth factor and insulin were not active. Substrates which mediated astroglial attachment were also studied. Fibronectin was found to stimulate the attachment of astrocytes to types I, IV and V collagen.

Proposed Course

A model is currently being developed which would allow glial cells to differentiate in vitro. The external environment of glial cells will be modified in order to activate genes important in differentiation. The various environmental factors which will be pursued include, soluble factors (hormones, chemically defined media, etc.), insoluble factors (fibronectin, laminin, co-cultured with other cell lines) and reaggregating cultures. As the changes are applied to the environment of the cells, we will determine alterations in the various properties described above. Once a system has been defined, we will then try to determine, either by 2D-gel electrophoresis, or gene cloning, new proteins being synthesized in this model.

The differential effects of PMA and mezerin on the glucocorticoid increases in GPDH levels are being studied. The PMA receptor is the protein kinase C. The ontogeny of the receptor correlates with synaptogenesis and myelination, and the highest concentration of receptor is found in the CNS. In a collaborative project with Drs. Karen Leach and Peter Blumberg, we are characterizing the PMA receptor on glial cells. We would like to know if the PMA receptor on glial cells behaves differently than the receptor on other tissues so as to explain why mezerin is more effective than PMA. We are also determining whether PMA has a shorter biological half-life than mezerin. Mezerin might be more active simply because it is present in culture longer. Furthermore, mezerin might be more active specifically in glucocorticoid-mediated functions. Therefore the ability of PMA and mezerin to block other differentiated functions in C_6 cells is being pursued. Finally, proteins which are phosphorylated by stimulating cells with mezerin and PMA are being studied.

The natural substrates for the protein kinase C are the diacylglycerols. The enzyme converts them to phosphatidylserine. Some small peptide neurotransmitters, for example, substance P, will induce the hydrolysis of phosphatidyl ionsitol to an unsaturated diacylglycerol. Therefore, there may be a profound relationship between the substance P receptor and the protein kinase C. We have recently started a collaborative project with Drs. C. Shults and T. O'Donohue (ET/IRP, NINCDS) investigating whether substance P receptors are present on astroglial cell cultures. Further work is directed to characterizing the receptor and identifying a cell line which exhibits the receptor. With the use of mutants, we hope to delineate the relationship between the kinase C and the substance P receptor.

Since these preliminary studies began on Substance P receptors on astrocytes, it has come to our attention that glial cells may have other types of neurotransmitter receptors. For example, at the recent Neurochemistry meetings, one of our glioma cell lines was reported (Abs. #298, DA uptake in amphibian and mammalian glia. 15th Annual Meeting, Society for Neurochemistry, 1984) to exhibit dopamine uptake. Though it has been well established that glial cells have beta receptors, other types of neurotransmitter receptors have not, to our knowledge, been reported. Therefore, future work will be directed to identifying cell lines that have receptors. It would be most difficult to establish assays for all known neurotransmitters. Therefore, we will use an indirect approach,which will be the ability of neurotransmitters to influence cAMP levels.

The relationship between the 95,000 cAMP inducible cell surface protein and differentiation will be studied. Mutant cell lines will be selected that do not respond to Bt2 cAMP. We will select for cells which proliferate in the presence of Bt2. Previous work from our laboratory demonstrated that glial cell proliferation is inhibited in the presence of Bt2 cAMP. These mutants will be analyzed for their ability to synthesize the 95,000 MW protein after Bt2 cAMP treatment as well as the ability to increase the levels of glial specific proteins. Furthermore, antisera and/or monoclonal antibodies will be produced against this protein for in vivo studies. We are particularly interested in the developmental regulation and localization of this protein.

Neurotransmitters, Chemoattractants and Ricin

In many biological systems, cyclic AMP has been shown to play a role in differentiation. In the rat nervous system, agents which increase cAMP levels have been shown to increase CNS specific properties such as galactocerebroside, CNPase, S-100 and GFAP, to name just a few. In addition, our laboratory has previously demonstrated that Bt2cAMP increases the sensitivity of glioma cell lines to human antibody mediated cytotoxicity. Therefore, we have asked whether there are cell surface changes in glioma cells after Bt2 cAMP treatment. Various glioma cell lines were treated for six days with Bt2 cAMP and labeled with 35 S-methionine. Cell membrane fractions were prepared and analyzed by gel electrophoresis under denaturing conditions. A protein with the approximate molecular weight of 95,000 was found to be induced after treatment with Bt2 cAMP. The protein was induced by elevated cAMP levels and not by the butyrate since theophylline was also active. An osteosarcoma, rhabydomyosarcoma, and colon carcinoma were not induced, thereby demonstrating that the protein was specific for glial cells in culture.

B. Chemotherapy

In order to develop approaches to improving chemotherapy, we have noted that glial and other central nervous system tumor cells vary in their sensitivity to the nitrosourea BCNU as well as aziridinylbenzoquinone (AZQ) and cis-platinum. This cellular response phenomenology is suitable for dynamic analysis as described above. In other words,, the response of glial tumor cells to given chemotherapeutic (cytotoxic) agents as well as biological growth regulatory agents provides both meaningful and easily accessible sets of tumor cell properties on which to base a new dynamic characterization of glial tumor cells. Thus, the clinical chemotherapy agents become biological

probes in the characterization process as well as objects of sensitivity/ resistance testing. On the basis of these approaches, we now are in the process of a prospective clinical trial of chemotherapy agent pre-selection.

At the heart of this approach to glial tumor cells is the aqueous micro-cytotoxicity assay. This simple assay has provided a quick, reliable determination of chemotherapeutic agent sensitivity or resistance applicable to almost all human glioma lines available from the operating room. In addition,as shown in a retrospective clinical study of fourteen patients, it appears to have clinical predictive value, most reliable for resistance.

The basis for the clinical protocol progress in chemotherapy that has been achieved in the SNB has been the application of in vitro microcytotoxicity testing, i.e., the testing of individual patient tumor lines with a series of chemotherapy agents to determine which may be most effective for a given tumor. Such studies, together with other characterization efforts, have indicated diversity of properties of malignant glial tumors. Given the same pathological diagnosis for a group of these tumors, a wide range of biological properties and, consequently, therapeutic sensitivities are found.

Utilizing the aqueous in vitro chemotherapy sensitivity assay developed by Dr. Kornblith, populations of glial tumor cells either sensitive or resistant to the nitrosourea, BCNU, and several other anticancer drugs including AZQ, cis-platinum, CBDCA, Henkel compound, rapamycin and spirohydantoin have been determined. The basis of resistance to BCNU of glial tumor cells, based on collaborative studies with Drs. Kurt Kohn and Len Erikson of National Cancer Institute, is the ability of the tumor cell to repair DNA damage resulting from drug-induced interstrand cross-links and strand breaks. In addition, we have determined that different cell membrane and microsomal protein properties (i.e., p 450) in sensitive and resistant cell populations also play a role in BCNU's effectiveness in tumor cell killing. The knowledge of such differences as they relate to the mechanisms of actions of various drugs has thus led not only to an appreciation of the importance of individualized glioma patient chemotherapy but also directly to the clinical protocols described above. In addition, the microcytotoxicity assay-derived sensitivity and mechanism data are suggesting ways to modify or begin to attempt to convert resistant cells into drug-sensitive cells.

The in vitro assays utilized have, for example, suggested the usefulness of both AZQ and cis-platinum (or derivatives thereof) for malignant glioma therapy. AZQ has been of particular interest because of:

a) Its demonstrated effectiveness in our in vitro microcytotoxicity assay,

b) Its high central nervous system penetration,

c) Its apparent tenfold concentration in glial tumors as opposed to plasma (as determined in our clinical studies),

d) Its selective mitochondrial destruction as well as nuclear DNA interstrand cross-linking,

e) Its relatively minimal side effects as seen in our Phase I studies.

Although BCNU, AZQ and cis-platinum all attack DNA, we have determined that they are not limited by the same mechanisms of resistance. Thus, AZQ and cis-platinum are rationally-based therapeutic alternatives to BCNU.

Based on SNB studies of AZQ in 40 patients (with recurrent malignant gliomas and failure to respond to radiation therapy and other chemotherapy), we have achieved a 35% response rate as demonstrated by clinical and CT scan improvement. Mean duration of response is approximately four months to date. Patients have been carried on this drug (monthly cycles) for up to 18 months. Our data parallel that of the Mayo Clinic, the M.D. Anderson Hospital and University of Maryland.

Progress in this area has been such that it is now possible to think in practical terms about an individualized attack on each glioma patient's tumor. This progress has led to a new SNB protocol designed to prospectively plan optimal chemotherapy for each patient based on two in vitro assay modes - aqueous microcytotoxicity testing, and an alkaline elution DNA assay.

The in vitro assays are also being utilized to develop promising new antiglioma agents, both of the traditional chemotherapy agent type as well as the newer biological growth control or "differentiation" agents such dimethylfomamide (DMF) and the various subtypes of interferon. Basic studies underway in these areas should be productive of new SNB clinical protocols. A major new protocol "The Prospective In Vitro Selection Chemotherapy Agents for Patients with Malignant Brain Tumors" is now in effect as the natural outgrowth of the basic studies.

A limitation of the microcytotoxicity assay system is the time-consuming nature of the cell-counting process required for evaluation of sensitivity and/or resistance. For an experienced human observer, counting a single plate (48 wells) takes 40-60 minutes with another 30 minutes required for calculations of cytotoxic indices,

$$C.I. = 1 - \frac{\#\ \text{cells test well}}{\#\ \text{cells control well}}$$

standard deviations, and t-values. To solve this problem we have developed an automated, image-analysis based system permitting the processing of each plate, including statistical output within 15 minutes.

The availability of this system has significantly increased our ability to study tumor cell biological properties and the responses of such cells to chemotherapeutic, immunological and biological modifying agents. Its availability is critical to the type of prospective clinical chemotherapy agent selection trial described above. To evaluate the effects of chemotherapeutic agents on glial cell metabolism, a study of cultured glioma cells treated with chemotherapy has been accomplished. The drug bischloroethylnitrosourea (BCNU), a nitrogen mustard, is a relatively effective drug for the chemotherapy of gliomas. Microcytotoxicity assays have

shown that in culture, some glioma-derived lines are sensitive to BCNU and other lines are not. The in vitro determination of sensitivity/resistance appears to predict the efficacy of BCNU treatment in patients. The mechanism of action of BCNU is still unclear; but several lines of evidence suggest a target in addition to DNA:

(1) the BCNU-mediated killing is rapid for DNA damage alone; (2) BCNU is a rather poor DNA crosslinker; (3) other nitrogen mustards which alkylate DNA have a much longer killing time.

BCNU has recently been reported to specifically and irreversibly inhibit the enzyme glutathione reductase. This is a potentially explanatory concept, since it suggests that the BCNU mediated killing is a result of peroxide-free radical damage, a process consistent with the observed time course. Furthermore, it predicts the following:
(1) Sensitive/resistant glioma lines differ in the rate of free radical/peroxide production; (2) Sensitive/resistant glioma lines differ in the mechanism of free radical/peroxide detoxification; (3) Sensitive/resistant glioma lines differ in the possible targets attacked by free radicals/peroxide and/or (4) The glutathione reductase of sensitive/resistant cell lines may be reflected in a relative sensitivity toward irreversible inhibition by BCNU.

The activities of glutathione peroxidase and glutathione reductase, and the concentrations of reduced and oxidized glutathione have been measured in selected typical sensitive and resistant cell lines (as determined by the microcytotoxicity assay). The levels of reduced and oxidized glutathione are about fourfold higher in the resistant cell lines that in the sensitive lines. The activity of glutathione peroxidase is not different between the sensitive and resistant cell lines, and this enzyme is not affected by BCNU treatment.

The activities of glutathione reductase are higher in the resistant cell lines than in the sensitive, and after BCNU treatment, this enzyme is inhibited to approximately the same degree in both sensitive and resistant cell lines. Greater residual activity is seen, however, in the resistant lines.

If the response to BCNU is mediated by inhibition of the glutathione system, modulation of endogenous levels of GSH/GSSG should alter the effect of BCNU. Studies are currently underway to test this hypothesis. Sulfoximines decrease the GSH/GSSG levels in resistant cell lines, and we will soon test the consequent effect on BCNU-mediated cell killing.

If BCNU-sensitive cell lines differ from resistant lines due to enhanced free radical/peroxide production, treatment with free radical scavengers, or lipid peroxide blockers should block the BCNU killing. These experiments are in progress.

An adequate glioma cell characterization program is, of course, much more than the few elements mentioned above. Without going into further detail about other subcomponents, the following list is a summary of the elements as they are currently used in the SNB:

1. Aqueous microtiter plate assays - Sensitivity - Resistance,

2. Antigenic expression and tumor cellular immune characteristics,

3. DNA alkaline elution assay; Interstrand cross-links; strand breaks,

4. DNA flow cytometry,

5. Bioelectrical properties,

6. Receptor analysis: protein kinase coupling,

7. Metabolic techniques, aerobic, anaerobic metabolism,

8. Peptide protein synthesis release characterization,

9. Marker expression GFA, FN S-100 Factor VIII,

10. Scanning and transmission EM preps quantitative autoradiography,

11. Image analysis quantitative morphometry.

The following chemotherapeutic agents and/or other biological probes have been used thus far in the characterization program outlined above:

	Chemotherapy Agents:	Growth-regulatory or "Differentiation-Active" Biological Agents:
1.	Nitrosoureas:	cAMP
	BCNU	cGMP
		Dimethylformamide and other polar solvents
	CCNU	Interferon (fibroblast)
2.	AZQ	Epidermal Growth Factor (EGF)
3.	Cis-platinum	Fibroblast Growth Factor (FGF)
4.	Spirohydantoin mustard	B-adrenergic agonists
5.	CBDCA	Butyrate Phenytoin
6.	Trans-hydroxy CCNU	Hexamethylene bisacetamide

This research involves both individual and collaborative work by J. Bona, R.Ph., Drs. J. Bressler, C. Kufta, J. Blacklock, P. Kornblith and W. Meyer

The major findings of these studies over the past year include:

1) The variability of glioma cell lines as defined by their responses to chemotherapy agents is clear. This has been documented in approximately 180 human glioma-derived cell lines for BCNU, 80 such lines for AZQ and some 30 lines for cis-platinum in the microcytotoxicity assays. Fifteen lines have now been evaluated with the DNA alkaline elution assay in collaboration with Drs. Kurt Kohn and Len Erikson of the National Cancer Institute with good correspondences to the microcytotoxicity assay data. It is of interest that the best correspondence is with cis-platinum. Apparently the direct effects of BCNU on the glioma cell membrane and of AZQ on the mitochondria alter responsiveness in ways other than through DNA effects and thus may have significant anti-tumor effects even with effects on DNA. Not only do glioma-derived cell lines differ from each other with respect to sensitivity and resistance to any given agent, but there are relatively sensitive and resistant sub-populations within a single tumor-derived cell line.

2) Although there are relatively sensitive and resistant cells within a given glioma cell population, the level of population sensitivity as measured by the cytotoxicity index (C.I.) is a useful indicator of population properties and appears to correlate in clinical response. For the DNA alkaline elution assay, the number of interstrand cross-links and strand breaks appear to be similarly predictive.

3) The variability of response noted for the chemotherapy agents is also seen with biological agents including cyclic AMP, dimethylformamide (DMF), glioma or fibroblast-derived interferon (GDIF, HFIF) and epidermal growth factor (EGF) and fibroblast growth factor (FGF). This variability is seen whether "response" is defined as quantitative morphological change or receptor coupling to protein kinase, interferon production, or growth kinetics and cytotoxic index.

4) It is possible to determine mechanisms of sensitivity and/or resistance to chemotherapy agents or their biological probes. Clearly established in the past have been:

   a) The selective mitochondrial toxicity of AZQ;

   b) Independence of resistance to BCNU and platinum such that for a BCNU-resistant glioma cell, platinum provides a realistic therapeutic alternative;

   c) Sulfhydryls in tumor cells inhibit platinum's action and may represent part of the mechanism of resistance to platinum. Furthermore, it appears that platinum affects glioma cell cytoskeleton.

5) The possible role of B-adrenergic agonists has been suggested by the study of human glioma-derived cell lines and the mode of phosphodiesterase induction and the effects on macromolecules phosphorylated by cyclic AMP-dependent protein kinase. Beta adrenergic receptors and the activities of adenylate cyclase, phosphodiesterase and protein kinase were examined in two human glioma cell lines. Results indicated that human glioma cell lines have functional beta-adrenergic receptors linked to adenylate cyclase. These beta receptors can also regulate phosphodiesterase activity, and cyclic AMP in human glioma cells can activate protein kinase can induce the phosphorylation of specific proteins.

C. Neuropathology

1. Histopathology

The histopathologic features of malignant astrocytes and of metastatic brain tumors may provide a basis for experimental study of these tumors.

Many adult astrocytomas originate in the hemispheric white matter, and spread extensively along white matter tracts, i.e., myelinated axons. Infiltration of grey matter is often characterized by clustering tumor cells around neurons (satellitosis). These observations suggest that glial (neoplastic astrocyte) - neuronal interactions play a role in the spread of neoplastic astrocytes through the brain.

During development, glial cell processes, which may extend from ependymal to pial surface, are used as a scaffolding for neuronal migration. Based on the principle that neoplastic cells may display, albeit in a disordered manner, fetal characteristics, one may hypothesize that the interaction of neoplastic astrocytes with neuronal cell bodies and axons involves mechanisms similar to those underlying neuronal migration. Specific areas of study relevant to this problem include: (1) the role of plasminogen activator, a protease which, if inhibited, blocks migration of rat cerebellar granule cells, and which has been demonstrated in cultured malignant astrocytes, (2) the role of laminin, an extracellular matrix protein which promotes neurite regeneration and is produced by "early" fetal rat astrocytes. In addition, laminin is felt to play an important role in the local invasion of breast and other cancers; (3) the role of these and other substances (cell membrane and myelin components, and neuropeptides) in astrocyte migration (chemotaxis).

The infiltration of astrocytomas, whether in grey or white matter, is characterized by lack of a well-defined margin from the surrounding brain. This is in contrast to the sharp margin typical of cerebral metastases. Study of carcinoma cells in parallel with neoplastic astrocytes is proposed as a way of understanding this difference in the pattern of invasion.

The manner in which primary and metastatic tumors grow within the brain parenchyma will prove to be the subject of research conducted by Dr. David Katz. He will utilize a variety of techniques including chemotaxis of cultured tumor cells. The ultimate aim of this work is to develop alternative therapeutic strategies based upon an understanding of the cell-cell, cell-stroma interactions involved in tumor invasion.

2a. Brain Tumor Protein Characterization in Tumor Dianosis

In conjunction with Dr. David Jacobowitz and his group in the Laboratoryy of Clinical Science, NIMH, Dr. Raj Narayan has analyzed protein patterns in normal human brain and in a large number of benign and malignant human brain tumors using two-dimensional gel electrophoresis with silver staining and computerized densitometry. Studies to date indicate 1) Normal human cortex as a reproducible pattern on 2DE; 2) Radiation and post-mortem changes significantly alter the quantitative densitometry of various protein spots; 3) The chemical identity of various proteins on the gels can be established using electro-immunoblotting techniques; 4) Individual tumor types manifest characteristic protein patterns which could be used for diagnostic and prognostic purposes with further refinement.

We believe that this technique can become extremely valuable in brain tumor research in the following ways: 1) As a biochemical screening procedure, to provide qualitative and quantitative data about several proteins using a single test. We are already able to use it for assessing GFAP, NSE, NNE, S-100, actin, albumin and tumulin. 2) As a diagnostic or prognostic tool, to supplement data obtained from histological studies. 3) As a research tool, to study tumor biology and immunology. We are in the process of trying to identify proteins that are specific for, or abundant in, the membranes of particular tumor types. The natural progression of such studies would involve the development of antibodies to such proteins, followed by the diagnostic and possibly therapeutic application of such antibodies.

2b. Utilization of Serum Neuron Specific Enolase Levels

This study has been initiated in collaboration with Dr. Paul Marangos, also of the Laboratory of Clinical Science, NIMH, who is an internationally recognized authority on this enzyme. This study involves the serial assessment of serum NSE levels in a variety of human brain tumor patients. NSE has already been shown to be of prognostic significance in childhood neuroblastomas. Besides extending this correlation to other tumor types, this study will also allow us to assess the effect of surgery, and other forms of brain injury, on serum NSE levels. It will allow a correlation of serum levels of the enzyme with levels in normal brain and in various brain tumor tissues obtained at surgery.

Neuropathological characterization has also continued and has been directed toward improving diagnosis of biopsies at surgery and characterizing the cells which are cultured from gliomas.

Flourescence and peroxidase staining of frozen sections for glial fibrillary acidic protein (GFAP), fibronectin, carbohydrate containing stroma and pituitary granules has been successfully employed to improve diagnosis of gliomas, nonglial neoplasms and pituitary adenomas.

Immunofluorescence for GFAP has been developed and used on biopsy material. Double immunoflourescence for anti-glial fibrillary acidic protein (anti-GFAP) and for fibronectin has been helpful distinguishing glial from

non-glial neoplasms on frozen sections with clear-cut results. Of particular relevance in astrocytomas, the neoplastic glial cells contained GFAP and not fibronectin. Sterile astrocytomas from surgery were followed with markers for GFAP and fibronectin through the process of sectioning of whole tumor, mincing, explanting and passing into culture. At initial explantation, cells containing only GFAP grew from certain fragments of tumor while cells containing only fibronectin grew from other fragments. This phenomenon would not have been noticed without examination of initial explants, since the cells become thoroughly mixed upon initial passage.

D. Ultrastructure and Cytology

Electron microscopic studies have revealed differences between the two immunologically defined cellular subpopulations cultured from gliomas. Glial cells seem to have more intermediate filaments, while divergent cells appear to have more extracellular filaments and more swollen endoplasmic reticulum. Scanning electron microscopy demonstrated the known glial cells to have more and thinner processes than the divergent cells. These morphologic impressions are being quantitated by computerized morphometry. Work on the localization of S-100 and Factor VIII (an endothelial cell marker) is also in process. Factor VIII has already been found useful in characterizing cells of vascular origin.

Time lapse cinematography studies of cis-platinum's early effects on halo formations of two established human glial cell lines is currently on-going. Since halo formation is an immune escape mechanism of glioma-derived cells, a chemotherapeutic agent that prevented or altered halo formation may prove helpful in maintaining chemosensitivity.

Another area of interest revolves around the observation of phagocytosis of lymphocytes by tumor cells. The length of survival of patients harboring primary brain tumors has been correlated with the presence and location of lymphocytic infiltration. Patients with a malignant glioblastoma multiforme containing focal perivascular infiltration, lived longer than those with no lymphocyte infiltration. This phenomena may provide potential delivery routes of cytotoxic agents such as ricin coupled to tumor selective monoclonal antibodies.

In an EM study of glioma-derived tumors affected by the differentiating agents dimethylformamide (DMF) and cyclic adenosine monophosphate (cAMP), altered distribution of mitochondria and rough endoplasmic reticulum have been noted. Additionally, more abundant Golgi apparatus were observed. These changes are suggestive of altered energy metabolism and protein synthesis of tumor cells. It can be correlated with protein electrophoretic pattern changes in the 50-90 Dalton proteins of similarly treated cells. We are documenting these effects at short and long-term exposures of 96 hours and 18 days, respectively. Longer exposure resulted in a more pronounced change in both the DMF and cAMP treated groups. The tendency was slower growth and morphologic alterations, a more differentiated state.

The EM Lab, consisting of Dr. William Meyer, Mary Ann Greenwood and Thomas Baginski, are currently examining in detail three aspects of the metabolism of gliomas: (1) glucose metabolism: (2) regulation of the expression of protease activity; and (3) chemotherapeutic drug metabolism. A

better understanding of these three important aspects of glioma metabolism will allow us to better appreciate the function of normal glia, the interrelationship of neurons and glia, the transition of normal glia to neoplastic glia, and those characteristics that are intrinsic and required for maintenance of the transformed state.

E. Biochemistry

1. Monoclonal Antibodies

The Unit of Biochemistry, headed by Dr. Richard Youle, is studying the use of monoclonal antibodies to kill disease causing cells. Monoclonal antibodies which selectively bind tumor cells can be generated, but alone are usually not cytotoxic to the tumor. Toxic proteins such as ricin and diphtheria toxin can be chemically linked to monoclonal antibodies and the new hybrid molecules will bind tumor cells via the antibody moiety and then kill the cells via the toxin moiety. These cell-type-specific reagents have immediate application ex vivo in bone marrow transplantation where T cell depletion improves allogeneic transplantation and tumor cell depletion improves autologous transplantation. The Unit of Biochemistry is supplying these reagents for clinical trials in bone marrow transplantation at the University of Minnesota.

The toxins used are enzymes that catalytically inactivate protein synthesis in target cells with only one or two molecules in the cytoplasm killing a cell. Currently, the limiting step for antibody-toxin hybrids is the entry of the toxin molecule into the cell. Thousands of molecules must bind the cell surface for one molecule to enter the cytoplasm. This low entry rate limits the log kill of these reagents in vitro and explains their frequent failure to eliminate all tumor cells in vivo. One goal of the laboratory is to improve the entry rate of antibody-toxin conjugates. The toxins will be modified chemically and genetically to increase target cell killing and block non-target cell killing. Also, the target cells can be sensitized to antibody-toxin conjugates by lysosomotropic amines. The mechanism of these drugs on toxin entry rate and their application ex vivo for bone marrow transplantation and in vivo for tumor cell killing will be studied.

The experience gained designing antibody-toxin conjugates for lymphoid tumors and bone marrow transplantation will be applied to gliomas. Monoclonal antibodies selective for glial tumors will be linked to ricin and assayed in vitro for toxicity to human glioma cell lines. These and improved reagents may eventually be used in vivo to treat brain tumors.

2. Glucose Metabolism

We have shown that there is a close correspondence between the glucose metabolic rates of glioma cell lines in culture and the ^{18}FDG-LCMRglc of the tumors from which they were derived. This suggests that: (a) despite the different techniques used to measure glucose metabolic rate, and despite the different milieux, the physiological properties regulating glucose metabolism is the same in vitro and in situ; and (b) tissue culture is an excellent model for the in situ metabolism of gliomas. Since ^{18}FDG-PET studies have demonstrated a glioma grade-dependent increase in the LCMRglc,

which is observed in the culture environment as well, the metabolism of glucose is genotypically altered in the transition of normal glia to glioma.

As is the case for most solid tumors, the glycolytic metabolism of glucose is altered in other ways as well. The levels of high energy reserves (e.g., glycogen, ATP and PCr) are maintained at set-point levels which differ from normal astrocytes. Furthermore, glycolysis is much less efficient than brain; 50% of the glucose taken up is converted to lactate and pyruvate. The glucose oxidative metabolic capacity of gliomas is decreased, and glycolysis therefore is predominantly used to generate the required ATP and PCr. This implies a genotypic lesion in the glycolytic pathway.

The enzymatic activities of the glycolytic pathway have been measured, and high grade glioma-derived cell lines only show increased hexokinase and phosphofructokinase. These are the regulatory enzymes of glycolysis, and enhanced maximum catalytic capacity is consistent with the observed increase in LCMRglc. ^{18}FDG-PET and tissue culture studies show that low grade gliomas have an LCMRglc intermediate between normal astrocytes and high grade gliomas, and the activities of hexokinase and phosphofructokinase are also intermediate. No other enzyme of glycolysis is altered in a transformation-dependent manner in high or low grade gliomas.

Increased flux into pentose phosphate pathway is also a hallmark of solid tumor metabolism. The flux through this pathway is enhanced in a grade-dependent manner in glioma-derived cell lines and in high grade glioma specimens derived from the OR. The regulatory enzyme for this pathway is glucose-6-phosphate dehydrogenase, and despite the apparent increased flux, the activity of this enzyme in high grade glioma-derived cell lines is only 10% of the activity found in normal astrocytes. This glucose-6-phosphate dehydrogenase of normal astrocytes is significantly inhibited by NADPH, but the glucose-6-phosphate dehydrogenase of gliomas is remarkably insensitive. This suggests that gliomas have an altered isozyme, and studies to determine this are underway.

The blood-brain barrier is commonly altered in gliomas, implying that these tumors may metabolize a wider range of carbon compounds than normal astrocytes. The metabolism of exogenously supplied glutamate and glutamine is rather low in surgical samples of normal cortex, but is enhanced in glioma tissue obtained from the OR. Glioma-derived cell lines also have significantly increased oxidative metabolism for glutamate and glutamine.

Taken together, these results demonstrate that the glucose metabolism of tumors is altered in three fundamental ways (1) glucose uptake is increased, to maintain glycogen, ATP and PCr largely by anaerobic glycolysis; (2) the pentose phosphate pathway flux is elevated, largely due to the alteration in the control features of the regulatory enzyme, glucose-6-phosphate dehydrogenase; and (3) greater reliance is placed on the oxidative (and thus energy yielding) metabolism of alternative fuels, such as amino acids.

3. Regulation of the expression of proteases and modification of the extracellular space.

Glioma invasiveness is primarily a function of this tumor's ability to alter the surrounding microenvironment. We have demonstrated that gliomas express and secrete plasminogen activator, and a large number of other proteases. In addition, certain other brain tumors secrete plasmin inhibitors, which block host-defense proteolysis. Lastly, high grade rapidly dividing gliomas are associated with increased plasma levels of the cellular binding protein, fibronectin.

A very sensitive technique was developed in this laboratory for the demonstration of plasminogen activator (PA) activity. This technique allows us to reliably and rapidly measure the PA activity in as little as 50 ug of cell protein (approximately 10^4 cells). Using this technique, we have been able to demonstrate that expression and secretion of PA activity is a common feature of high grade glioma-derived cell lines. Normal astrocyte cell cultures do not synthesize or secrete PA. Since this enzyme has been correlated with transformation, dedifferentiation and invasiveness in other cellular systems, we hypothesized that inhibition of growth or differentiation may result in decreased PA secretion. This is in fact the case for most glioma lines: agents which alter the differentiation state, or decrease the growth rate, decrease the expression of PA. The mechanism of this effect is currently the subject of active experimental interest. Differentiating agents appear to block the de novo synthesis of PA. At the present time, other hypothesized mechanisms for decreased PA production (such as active synthesis of a PA inhibitor) appear not to function in glioma-derived cell lines.

In collaboration with Dr. Eugene Major (IDB/NINCDS) we have begun a series of studies to elucidate the molecular basis for PA expression in JC virus-induced monkey gliomas. To date, cell lines which have the complete JC virus genome have been shown to synthesize and secrete PA. The presence of the T antigen, but not the small t antigen is required for PA production in monkey glioblastomas in vitro. Normal monkey cortical cultures do not secrete PA activity.

PA is only one of many potential proteinases which could play a role in tumor invasiveness. Chordomas are a rare tumor, but one which is characterized by invasiveness, since this notochordal remnant tumor will slowly erode bone, cartilage and a variety of other tissues. In collaboration with Dr. Bernadette Tyree (L DBA/NIDR), we are currently investigating the collagenases of chordomas. The milk protein, casein, is widely used as a substrate for the assay of proteinases. Glioma tissue, and normal cortex did not show caseinolytic activity, but the activity was significantly enhanced in chordoma tissue. Amino acids, covalently attached to carbobenzoxyl blocking groups, and conjugated with nitrophenol or nitroanilide are widely used to assay proteinases. We have applied a variety of these substrates to determine the proteinases in normal hair cortex, glioma tissue, and chordoma. For most substrates, activity is seen in homogenates of all three tissues, but the activity is extensive in chordomas, low in cortex, and intermediate in gliomas. We are in the process of identifying particular proteinase activities.

Proteases have many roles in the physiological function, such as clot formation, clot lysis, and host-defense mechanisms against tumor invasiveness. We recently examined in detail the plasmin inhibitory activity in two meningiomas, one glioma, and one sarcoma. Of the two meningiomas, one was temporal in location, and the other was extensive in size, completely occluding the sagittal sinuses. Extracts of the meningioma showed no plasmin inhibition, but the inhibitory activity was extensive in the tumor. This implies that plasmin inhibitor activity can be expressed in tumors to block host defense mechanism. Thromboembolic complications are not infrequent in patients with glioblastoma. The tissue from one glioma, removed from a individual with thromboembolic complication, was also shown to express significant levels of plasmin inhibitor. This yields some insight into the possible range of tumor-host interaction, and may explain the thromboembolysis seen in certain glioma patients.

Fibronectin (Fn) is a protein which is widely synthesized as a cellular binding protein, and high levels have been implicated in disseminated intravascular coagulopathies. Patients with various neurological disease, non-CNS solid tumors, low grade astrocytomas, or growth-arrested glioblastomas show blood Fn levels with the normal range. However, patients who have actively growing high grade gliomas have a mean Fn about twofold greater than normal volunteers. This observation has significance in its predictive value, and in a better understanding of the range of tumor interactions.

Overall, then, the characterization program is moving ahead on several fronts and the complex matrix of malignant brain tumor properties being unravelled. Progress is gratifying in this area.

F. Immunology

A focus of the cellular immunology program, under Dr. Linda Muul's supervision, has been to understand some of the mechanisms which allow gliomas to escape immune destruction by cytolytic lymphocytes and to find ways of evaluating and augmenting glioma-specific cytolytic T lymphocytes.

Over the course of this year, the major accomplishments have been: (1) established of a simple and inexpensive way to suppress the generation of anomalous non-specific cytolytic lymphocytes while still allowing glioma specific cytotoxic T lymphocytes to develop in vitro using 10^{-5}M hydrocortisone and either recombinant Interleukin-2 or irradiated third party stimulator lymphocytes; (2) providing additional evidence that non-specific cytotoxic lymphocytes represent distinct effector mechanisms; (3) establishing that glioma derived cell lines which secreted a large glycosaminoglycan coat had more cell-associated hyaluronic acid which protected the cells from attack by cytolytic T-lymphocytes.

Work has proceeded in both humoral and cellular immunology. In the serological response studies the correlation of serological immune response with malignancy and glioma patient survival has been evaluated. These studies of glioma patients' circulating antiglioma antibody tested against their own tumor cells in culture have shown diminishing effectiveness with increasing malignancy of the tumor. In general, high levels of antibody are found in younger patients and correlate with increased survival. Thus, these immune assays have prognostic value -- a first for glioma studies.

In the past, we have determined that it is possible to modify tumor cell susceptibility to antibody-induced, complement-mediated cytolysis. Treatment of malignant glial tumor cells in vitro with either dibutryl cyclic AMP or DMF has resulted in the conversion of antibody resistant glioma cells to sensitive cells.

In a potentially major new observation we have noted that there are "new" proteins demonstrable on SDS polyacrilamide gel electrophoresis after glioma cells are treated with cAMP or DMF. These "new" proteins may represent antigens induced by the differentiation agents. These proteins may possibly account for the changes in microcytotoxicity response which have been seen. It will now require two dimensional gel electrophoresis with isoelectric focussing to further identify these proteins. When these proteins are isolated, we can immunoprecipitate them and determine how they relate to the immunological responses.

Work during the past year has continued to be directed at gaining a better understanding of the mechanisms by which gliomas escape cellular immune attack. One such mechanism involves a defect in immunogenicity which can be overcome by "help" provided by soluble factors released by peripheral blood mononuclear cells in mixed lymphocyte reactions. Attempts to define the nature of this factor, as well as to elicit autologous tumor-specific responses by its use, have been hampered by the ability of mixed lymphocyte culture supernatants to elicit nonspecific "natural killer-like" cytolytic effectors in addition to augmenting specific cytolytic lymphocyte responses. Therefore several reagents were screened for their ability to inhibit the generation or action of nonspecific effectors while permitting specific cytolytic lymphocyte responses to proceed unimpeded. One such substance was identified. Hydrocortisone at concentrations of 10^{-6} M to 5 X 10^{-5} M was found to inhibit the generation of nonspecific effectors by greater than 90% while having little or no effect on specific cytolytic lymphocyte responses. The inclusion of this reagent in mixed lymphocyte-tumor cultures should thus greatly facilitate further studies on factors affecting the immunogenicity of gliomas as well as attempts to elicit autologous glioma-specific cytolytic lymphocyte responses in vitro.

A second mechanism by which glioma cells escape cellular immune attack is by the production of mucopolysaccharide cell coats. The secretion of large cell coats is stimulated by the interaction of glioma cells with a nondialyzable factor produced by some component of the blood mononuclear cell population. Initial studies suggest that T3-negative adherent cells, possibly monocytes, may be responsible for the production of this factor. Coat formation has been quantitated by use of a Bausch and Lomb image analysis system and by means of ELISA assay in which hyaluronic acid is quantitated by measuring its interaction with the hyaluronic acid-binding region of a proteoglycan from rat chondrosarcoma cells. Preliminary results with the ELISA assay have suggested that interaction of glioma cells with the blood mononuclear cell-derived factor does not increase the amount of soluble hyaluronic acid which glioma cells secrete into the culture medium but rather increases the fraction of secreted hyaluronic acid which is held in cell surface-associated form. However, problems with standardization and reproducibility of the ELISA assay have been encountered, and attempts to refine and improve this assay are in progress.

A third mechanism by which glioma cells may escape cellular immune attack is through the secretion of soluble immunosuppressive factors. It was previously reported by other laboratories that some glioma patients possessed nonspecific immunosuppressive substances in their plasma. We have obtained similar results with plasma from some of our glioma patients. The immunosuppressive substance in patients' plasma was eluted from a gel filtration column in the same fractions as marker proteins of 60,000-80,000 molecular weight. In contrast, immunosuppressive substances released by glioma cells in vitro eluted in the void volume of S-200 columns, implying a molecular weight equal to or greater than 200,000. Thus the immunosuppressive substance present in patients' plasma in vivo is not identical to that produced by glioma cells in vitro; however it cannot be excluded that the larger factor produced in vitro is a precursor of the smaller factor observed in vivo. Further biochemical characterization of these factors is in progress.

Recently, the Unit of Cellular Immunology was created in the Surgical Neurology Branch. It is headed by Dr. Elizabeth Grimm, who is initiating research devoted to developing means by which the cellular immune system can be utilized to destroy established tumors. Her recent efforts have focussed on lymphokine activation of human lymphocytes into expressing cytolytic activity that is effective in killing fresh NK resistant tumor cells. Dr. Grimm has established the basic characterization of this phenomenon and now plans to pursue experiments directed towards resolving the question of whether lymphokine activated killer cells (LAK) manifest a useful biological role. Collaborative ongoing murine models suggest that LAK do mediate the regression of established melanomas and sarcomas. Human Phase I clinical trials of LAK are in progress here at the NIH, and Phase II studies have been approved. Though collaborative research continues with these trials, studies to test the efficacy of LAK in human brain tumors patients is planned. As soon as preliminary in vitro human testing of glioma tumor cells is successfully completed, clinical trials with LAK will be pursued in the SNB.

Basic laboratory efforts will be devoted to identification of the LAK cell precursor and to characterize at the molecular level, the mechanism by which Interleukin-2 (IL-2) regulates its own receptor on the LAK cell during activation and expression of cytotoxicity.

III. BIOLOGICAL STUDIES OF HUMAN PITUITARY TUMORS

During the past year we have completed the following studies of patients with pituitary adenomas.

1. Shown that continuous infusion of CRF does not desensitize the normal corticotrophs or tumorous corticotrophs of the anterior pituitary to CRF as occurs with hormonal gonadotrophs of the pituitary gland to continuous infusion of LHRH or long acting LHRH analogs.

2. Shown that the ACTH secretion of the adenomas of Cushing's disease and Nelson's Syndrome is stimulated by corticotropin releasing factor (CRF), the hypothalamic hormone which regulates the ACTH secretion of the normal gland. This implies that these tumors have receptors for this hormone and that receptor-directed therapy may be beneficial.

3. Shown that CRF stimulation tests distinguish those patients with Cushing's Syndrome who have pituitary tumors from patients with ectopic ACTH secretion and adrenal tumors. This has been a difficult differential diagnosis in the past.

4. Shown that metabolic clearance rate of CRF in Cushing's disease and Nelson's Syndrome is similar to normal controls.

5. Established that glucocorticoids inhibit CRF-stimulated ACTH secretion in Nelson's Syndrome.

6. Shown that the growth hormone secretion of the tumors of acromegaly is stimulated by growth hormone releasing factor, a recently discovered hypothalamic hormone.

The above-mentioned studies were performed in collaboration with the Developmental Endocrinology Branch, NICHD.

IV. NEURODIAGNOSTIC STUDIES INCLUDING PET SCANNING

Another area of significant accomplishment for the Branch has been the development of a positron emission tomographic scan capability by the section of Neuroradiology and Computed Tomography under Drs. G. Di Chiro and R. Brooks. We have continued our angiographic studies of the arteriovenous malformations and vascular tumors of the spinal cord. Digital subtraction angiography (DSA), either intravenous or intraarterial, has not proven to be particularly reliable in the diagnosis of these lesions. More useful, at least for the recognition of the vascular component, has been the technique of dynamic computed tomography (DCT).

Our CT studies of such conditions as degenerative diseases of the CNS, cavities of the brain stem and spinal cord, and brain and spinal cord tumors has continued.

Our NMR imaging research has been initiated and is developing along three lines:

1) We are taking advantage of the exquisite capability of NMR to display fine anatomical detail to advance our diagnostic yield in a number of neurological lesions;

2) We are trying to learn more about the NMR signal of various tissues, starting with the signal from tumors of various types and grades and from extravasated blood, and;

3) We are comparing our clinical NMR imaging results with those of CT and PET in a variety of abnormal conditions starting with CNS tumors.

Experience with PET-FDG of CNS tumors has continued to expand. We have now studied over 200 patients and in many cases we have carried out repeat examinations. The usefulness of the PET-FDG for grading cerebral tumors is well established. We have used this technique for the prediction of the

survival rate of patients with high grade cerebral gliomas and found that PET-FDG is by far the best method to establish the prognosis in these patients.

We have initiated an analysis of the cortical glucose metabolism in the hemianopsias starting with the homonymous field defects. We found that the appropriate primary and associative visual cortices show a marked hypometabolism.

A long range research project to compare PET with NMR has begun. Preliminary observations indicate that the two techniques complement each other.

We have carried out a study of the visualization of the dilated ventricular system in our PET-FDG scans. In another study, we have evaluated the gray-white matter ratio of glucose utilization as assess by our advanced scanner, the Neuro-PET.

We have continued our evaluation of the rate constants (particularly K_1, K_2 and K_4) in our PET-FDG studies.

Finally, we have carried out our first neuro-receptor PET study in a patient with a Parkinsonian syndrome. This patient was studied twice using the ligand (^{11}C)-methylspiperone. This ligand was also used in the PET studies of three normal monkeys.

The Neuroradiology and Computed Tomography Section is also involved in the following other research projects:

Transmission Computed Tomography (CT). Our work has continued with clinical-animal/experimental research projects. These include studies of demyelinating, degenerative and atrophic processes of the brain, brain edema, hydrocephalus, postradiation cerebral necrosis, diseases of the spine and the spinal cord, surgically correctable lesions in young patients affected by chronic epilepsy, attempts at tissue characterization of normal and tumor cerebral tissue, and an experimental glioma model in primates.

Selective arteriography of the spinal cord is a diagnostic technique which has been most informative in cases of tumor, arteriovenous malformation, trauma, obstructive vascular disease, and postradiation damage of the spinal cord.

Radioisotope angiography of the spinal cord offers distinct advantages as a method of screening, and may give information not available by any other diagnostic test in certain kinds of intraspinal pathology.

Our experience with dynamic computed tomography (DCT) of the spine after injection of contrast medium shows that this methodology is helpful in the evaluation of certain vascular lesions of the spinal cord.

Our digital subtraction angiography (DSA) studies of the spine in cases of arteriovenous malformation and tumors of the spinal cord have been successful. DSA is a valuable screening and follow-up technique for the evaluation of certain vascular conditions of the spinal cord.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02367-06 SN

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Biological, Immunological and Chemotherapeutic Studies of Human Brain Tumors

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Paul L. Kornblith, Chief, Surgical Neurology Branch, NINCDS		
Maurice Gately (Departed 12/83)	Senior Staff Fellow	SN NINCDS
Paul E. McKeever (Departed 10/83)	Medical Officer	SN NINCDS
Craig Cummins (Departed 6/84)	Staff Fellow	SN NINCDS
Joseph Bressler	Senior Staff Fellow	SN NINCDS
Conrad Kufta	Senior Staff Fellow	SN NINCDS
Edward Oldfield	Senior Staff Fellow	SN NINCDS

OTHER*

COOPERATING UNITS *(if any)*
Radiation Oncology, NCI; Medical Oncology, NCI; BEIB, DRS, NIH

LAB/BRANCH
Surgical Neurology Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
7.0	6.0	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Human brain tumors are evaluated in a tissue culture environment as to their basic biological behavior, their response to chemotherapeutic agents and the detailed immunological interactions between the host and the tumor. A primary goal is to improve the therapy of patients by understanding the basic cellular biology of malignant human brain tumors.

SNB has continued the biological characterization program with the inclusion of flow cytometry, karyotyping, glial fibrillary acid protein, fibronectin, S-100 and Factor VIII assays, DNA repair, adrenergic and other receptor assays, ganglioside and glycoprotein assays, cloning techniques, in-depth neuropathological studies, and automatic image analysis; utilized both aqueous and surface chemotherapy assays to test several new potential antiglioma agents and initiated a prospective in vitro selection of clinical trials with these agents; carried out protocols with AZQ, spiromustine and platinum derivatives; defined the basis of cellular sensitivity or resistance to nitrosoureas; characterized the humoral cellular immunological response to gliomas; and carried out correlative cellular and PET scan glucose metabolic studies.

*OTHER (Cont'd)

Linda M. Muul (Departed 7/84)	Special Expert	SN NINCDS
Raj K. Narayan	Special Expert	SN NINCDS
Donald C. Wright	Senior Staff Fellow	SN NINCDS
David Katz (Departed 7/84)	Senior Staff Fellow	SN NINCDS
William Meyer	Special Expert	SN NINCDS
James Bona	Staff Pharmacist	SN NINCDS
J. Bob Blacklock	Guest Researcher	SN NINCDS
Steven Jacobs	Senior Staff Fellow	SN NINCDS
Jeffrey Bruce	Medical Staff Fellow	SN NINCDS
Elizabeth A. Grimm	Senior Staff Fellow	SN NINCDS
Richard Youle	Research Chemist	SN NINCDS

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02454-04 SN

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biological Studies of Human Pituitary Tumors

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Edward H. Oldfield, Senior Staff Fellow, Surgical Neurology Branch, NINCDS

Paul E. McKeever (Departed 10/83)	Medical Officer	SN NINCDS
Paul L. Kornblith	Chief	SN NINCDS
Craig Cummins (Departed 6/84)	Staff Fellow	SN NINCDS

COOPERATING UNITS *(if any)*

Developmental Endocrinology Branch, NICHD; Diagnostic Radiology, CC

LAB/BRANCH

Surgical Neurology Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The influence of the hypothalamic releasing factors CRF and GRF on the hormonal secretion of pituitary adenomas has been determined in vitro and correlated with the patients' response in vivo. These studies indicate that the pituitary tumors causing Cushing's disease, Nelson's Syndrome and acromegaly are responsive to their appropriate releasing factor. We are also investigating the influence of the releasing factors on the rate of growth of pituitary tumors in vitro. By understanding the mechanism of the secretory responses of these tumors to the releasing factors, new therapeutic methods may evolve.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-NS 01195-20 SN

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Radiographic and Radioisotopic Angiography of the Spinal Cord

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

G. Di Chiro, Chief, Neuroradiology and Computed Tomography Section — SN NINCDS

OTHER:

J.L. Doppman	Chief	DR CC
E.H. Oldfield	Senior Staff Physician	SN NINCDS
S.M. Larson	Chief	CC NM

COOPERATING UNITS *(if any)*

Diagnostic radiology and Nuclear Medicine Departments, Clinical Center, NIH; Medical Examiner's Office, Department of Public Health, Philadelphia, PA

LAB/BRANCH

Surgical Neurology Branch

SECTION

Neuroradiology and Computed Tomography Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Selective arteriography (radiographic) of the spinal cord is a diagnostic technique which has proven to be very informative in cases of arteriovenous malformation, tumor, obstructive vascular disease, trauma, and postradiation damage of the spinal cord.

Radioisotope angiography of the spinal cord offers distinct advantages as a screening method, and in certain types of intrapsinal pathology may give information not available by any other diagnostic test.

Preliminary experience with new techniques, dynamic computed tomography, (DCT), digital subtraction angiography (DSA), positron emission tomography (PET) using ^{18}F-2-deoxyglucose and nuclear magnetic resoance imaging (MRI) of the spine indicates that these methods may be useful screening and follow-up procedures in the evaluation of certain vascular lesions and tumors of the spinal cord.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02073-11 SN

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Computed Tomography (Transmission) and Nuclear Magnetic Resonance (NMR)

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

G. Di Chiro,	Chief, Neuroradiology and Computed Tomography Section	SN NINCDS
OTHER:		
R.A. Brooks	Staff Physicist	SN NINCDS
R.F. Wayner	Staff Fellow	SN NINCDS
D.S. Fishbein	Staff Fellow	SN NINCDS
J.D. Doppman	Chief	DR CC
S.M. Larson	Chief	CC NM
A.M. Cormack	Physicist	Tufts University

COOPERATING UNITS *(if any)*

Diagnostic Radiology, Nuclear Medicine Department, CC, NIH; Physics Department, Tufts University, Boston, MA

LAB/BRANCH

Surgical Neurology Branch

SECTION

Neuroradiology and Computed Tomography Section

INSTITUTE AND LOCATION

NINCDS/NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.9	0.9	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Computed Tomography in its transmission (CT), emission (PET, SPECT), and soon Nuclear Magnetic Resonance (NMR) modalities, represents the main research area of the Neuroradiology and Computed Tomography Section.

CT: Ongoing clinical - animal/experimental research projects in transmission CT include studies of degenerative, demyelinating and atrophic processes of the brain, hydrorocephalus, brain edema, postradiation cerebral necrosis, surgically correctable lesions in young patients affected by chronic epilepsy, diseases and abnormal (e.g. tumoral) cerebral tissue, and an experimental glioma model in primates. Physics projects: Improved dual-energy CT scanning using both a split-detector and a dual kVp method; analysis of aliasing effects and development of methods for their elimination; phantom studies for the evaluation of artifacts and calibration of CT machines; feasibility tests for a new type of CT device which used protons instead of x-rays.

NMR: Our NMR imaging research is developing along three main lines: 1) we are taking advantage of the exquisite capability of NMR to display fine anatomical detail to advance our diagnostic yield in a number of neurological lesions; 2) we are trying to learn more about the NMR signal of various tissues, starting with the signal from extravasated blood (various types of CNS hemorrhages); and 3) we are comparing out clinical NMR imaging results with those of CT and particularly PET in a variety of abnormal conditions starting with CNS tumors.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02315-07 SN

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Positron Emission Tomography

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

G. Di Chiro, Chief, Neuroradiology and Computed Tomography		SN NINCDS	
OTHER:			
R.A. Brooks	Staff Physicist	SN NINCDS	
R.F. Wayner	Staff Fellow	SN NINCDS	
D.S. Fishbein	Staff Fellow	SN NINCDS	
E.J. Finn	Staff Physicist	SN NINCDS	OTHER (Con't)*
D. Bairamian	Guest Worker	SN NINCDS	

COOPERATING UNITS *(if any)* BIEB, NIH; Naval Res. Lab., Wash. DC; Lab of Cerebral Metabolism, NIMH, NIH; ODIR, NINCDS; ETB, NINCDS; LPC, NCI; Brookhaven National Lab., Upton, NY; Div. of Nucl. Med., Dept. of Rad. Science, UCLA, Los Angeles, CA

LAB/BRANCH
Surgical Neurology Branch

SECTION
Neuroradiology and Computed Tomography Section

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.8	1.8	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Positron Emission Tomography (PET) with (^{18}F)-fluorodeoxyglucose (FDG) allows us to obtain anatomical data (e.g., axial transverse or coronal images of the brain) as well as dynamic functional data (such as regional cerebral glucose consumption rate; measurements of the storage, degradation and turnover of tagged metabolites; follow-through of the movement of the CSF in the deep intracranial cavities.) The unique property of PET is that it provides physiologic information not available with any other imaging procedure.

Since June 1982 we have been using the new high-resolution, high-sensitivity scanner built in our section, the Neuro-PET. The performance of this scanner has exceeded all our expectations. This device has allowed new applications of the PET technique.

OTHER
*Cont'd

N.J. Patronas	Staff Physician	DR CC
S.M. Larson	Supervisory Med. Ofcr.	CC NM
R.E. Carson	Staff Physicist	CC NM
W.C. Eckelman	Staff Chemist	CC NM
M. Dalakas	Senior Staff Fellow	ID NINCDS
W.H. Theodore	Neurologist	EB NINCDS
T.N. Chase	Chief	ETB NINCDS
A.G. Blasberg	Medical Officer	LCP NCI
A.P. Wolf	Senior Chemist	Brookhaven
L. Sokoloff	Chief	LCM NIMH
D.F. Kuhl		UCLA